THESIS APPROVAL

GRADUATE SCHOOL, KASETSART UNIVERSITY

Doctor of Philosophy (Food Science)

DEGREE

Food Science | Food Science and Technology

FIELD | **DEPARTMENT**

TITLE: Characterization of *Lactobacillus johnsonii* KUNN19-2 and *Pediococcus pentosaceus* KUNNE6-1 in Respect of the Probiotic Properties and Application in Fermented Chicken Cartilage (Nham Kor Khai)

NAME: Miss Panward Prommadee

THIS THESIS HAS BEEN ACCEPTED BY

THESIS ADVISOR

(Associate Professor Wunwiboon Garnjanagoonchorn, Ph.D.)

COMMITTEE MEMBER

(Associate Professor Sunee Nitisinprasert, D.Sc.)

COMMITTEE MEMBER

(Assistant Professor Wannee Jirapakkul, Ph.D.)

DEPARTMENT HEAD

(Assistant Professor Wannee Jirapakkul, Ph.D.)

APPROVED BY THE GRADUATE SCHOOL ON _____

DEAN

(Associate Professor Gunjana Theeragool, D.Agr.)

THESIS

CHARACTERIZATION OF *Lactobacillus johnsonii* KUNN19-2 AND *Pediococcus pentosaceus* KUNNE6-1 IN RESPECT OF THE PROBIOTIC PROPERTIES AND APPLICATION IN FERMENTED CHICKEN CARTILAGE (NHAM KOR KHAI)

PANWARD PROMMADEE

A Thesis Submitted in Partial Fulfillment of
the Requirements for the Degree of
Doctor of Philosophy (Food Science)
Graduate School, Kasetsart University
2014

Panward Prommadee 2014: Characterization of *Lactobacillus johnsonii* KUNN19-2 and *Pediococcus pentosaceus* KUNNE6-1 in Respect of the Probiotic Properties and Application in Fermented Chicken Cartilage (Nham Kor Khai). Doctor of Philosophy (Food Science), Major Field: Food Science, Department of Food Science and Technology. Thesis Advisor: Associate Professor Wunwiboon Garnjanagoonchorn, Ph.D. 242 pages.

Lactobacillus johnsonii KUNN 19-2 and *Pediococcus pentosaceus* KUNNE 6-1 were shown to be resistant to some antibiotics and inhibit growth of certain pathogens. Both strains were found as starter cultures that provide firmness, color and flavor to fermented meat products. In this study, both strains showed faster pH reduction and increasing number of lactic acid bacteria (TPC on MRS agar) during cartilage fermentation compared to natural fermentation. Survival of *Lb. johnsonii* KUNN19-2 and *P. pentosaceus* KUNNE6-1 at gastric condition with low pH of 1.5 were 60.40%, 40.51%, and in the presence of pepsin for 2 h were 70.97%, 58.80%, respectively. In addition, their survival in the intestinal conditions by the presence of pancreatin and bile salt for up to 4 h were at 57.14% and 54.47%. However, when *Lb. johnsonii* KUNN19-2 and *P. pentosaceus* KUNNE6-1 exposed to gastric phase and then continued to intestinal phase, their survival were reduced to 30.84% and 19.40%, respectively. Heat-treated cells of *Lb. johnsonii* KUNN 19-2 was able to induce both T and B cells proliferation and modulate IgG production in BALB/c mice spleen cells by *ex vivo* test for 3.66 and 4.239 µg/ml, respectively whereas *P. pentosaceus* KUNNE 6-1 induced only B cells proliferation and stimulated IgG production for 3.83 µg/ml. The results illustrated that both strains have potential as probiotic candidate. The microbial diversity of *Lb. johnsonii* KUNN19-2 inoculated sample showed in DGGE is sharply reduced compared to control. They were divided into 3 groups which indicated 1-2 dominated strains through the process. Although this study failed to monitor *Lb. johnsonii* KUNN19-2 in fermented chicken cartilage but the validated extraction method for suitable DNA is suggested for future study.

_____ _____ __/__/__

Student's signature Thesis Advisor's signature

ACKNOWLEDGEMENTS

First and foremost I offer my sincerest gratitude to Assoc. Prof. Wunwiboon Garnjanagoonchorn for her confidence on me. She has enormous impact on my life as my ´Wonderful Ph.D thesis advisor´, guiding me to the world of probiotic. I greatly appreciate her critical reading of the manuscript, the valuable comments and suggestions. My gratefully thanks to Assoc. Prof Sunee Nitisinprasert, who has supported me throughout my thesis with her patience and knowledge whilst allowing me to work in my own way. Ajarn Sunee is also taking care, teach me to real life university to learn and walking. My very special thanks goes out to Prof. Kees De Lange and Dr. Stewart Nivens, from Department of Animal and poultry science, University of Guelph, both are the professor/teacher who truly made a difference in my life, provided me with direction, technical support and became more of a mentor and friend, than a professor. It was though their persistence, understanding and kindness that I completed my research on Immunology and gastrointestinal tract. I would also wish to thank Assoc. Prof. Wannee Jirabhakkul. for the continuous support of my Ph.D study and research, for her kindness, suggest and offer me many valuable comments and words of encouragement. I could not have imagined having a better advisor for my study. My dearest thanks I owe to my family: mom and P´ Jom, who have expressed their loves, encouraged and understood me during this time.I attribute the level of my Doctoral degree to the encouragements and efforts of my thesis committee, beloved family, Assoc.Prof. Thongchai Suwansichon and friends, without them this thesis, too, would not have been completed or written. I doubt that I will ever be able to convey my appreciation fully, but I owe them my eternal gratitude. This work was funded by The Commission on Higher education, Thailand under the Faculty development grant 2004 -2006. I am also grateful thank to Assoc. Prof. Vichien Leelawatcharamas and Assoc. Prof. Pinmanee Kwanmuang, who have kindly given the stock cultures of *L. johnsonii* KUNN 19-2 and *P. pentosaceus* KUNNE 6-1 for using in this study.

Panward Prommadee

July, 2014

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

Page

LIST OF TABLES

LIST OF TABLES (Continued)

LIST OF FIGURES

LIST OF FIGURES (Continued)

CHARACTERIZATION OF *Lactobacillus johnsonii* KUNN19-2 AND *Pediococcus pentosaceus* KUNNE6-1 IN RESPECT OF THE PROBIOTIC PROPERTIES AND APPLICATION IN FERMENTED CHICKEN CARTILAGE (NHAM KOR KHAI)

INTRODUCTION

In the last decades consumer demanding in food products has changed, they considerably believe that their good health and well being could be contribute directly by food. Functional foods play an outstanding role to meet the consumer demanding as nowadays foods are not expected to only satisfy hunger and to provide necessary nutrients but to prevent nutrition- related diseases and improve physical and mental well-being of consumers. Food products can strengthen health benefit aspects by the addition of probiotics as well as by certain types of soluble fibers known as prebiotics. The administration of probiotic bacteria as nutraceuticals or functional food is an area that has rapidly expanded in recent years, with a global market worth $32.6 billion predicted by 2014 (Cook *et al*., 2012). An increase in knowledges and also consumer interests of food nutrition has led to the development of foods which have health benefits beyond adequate nutrition. The health effects were scientifically documented. Some dairy products especially yoghurt are produced by viable probiotic lactic acid bacteria (LAB) with scientifically proven for health benefits and safety (Salminen *et al*., 1998). Fermented sausages are non-heated meat products, which may be suitable carriers for probiotics into the human gastrointestinal (GI) tract. The idea of using probiotic bacteria as the fermenting agents or starter culture in meat products is beginning to develop and the idea of using antimicrobial peptides, bacteriocins, or other antimicrobial compounds as a hurdle for dry fermented sausage safety has been introduced and being developed.

Fermentation is a traditional way to preserve quality and ensure safety of food. Fermented foods quality depend on the metabolic activities of many microorganisms especially lactic acid bacteria. The low pH and high concentration of salt in fermented foods can inactivate or inhibit the growth of pathogens. Therefore, fermented foods are generally considered as safe foods. However, pathogenic bacteria and their toxins could appear if the process does not result in proper acid production.

Nham is a traditional Thai fermented pork sausage product. Its ingredients, processing, physical characteristics, and consumption manners give the uniqueness to the product. The Thai identity of Nham is reflected on the use of pork rind, garlic and chili which give Nham characteristics not found in other fermented pork sausages in other countries. Moreover, Nham is usually served raw and consumed with garlic, chili, ginger and peanut. Nowadays there are many types of Nham from other meats (pork spare rib, beef, fish, chicken and chicken cartilage etc.) which are well known and consumed in Thailand. The manner and specific way of which Nham is eaten can be represented as the uniqueness style of Thai food. One of the factors which determine the special characteristic of Nham is the microorganisms involved in the fermentation. The present study provides an insight into the technology and microbiology of fermented chicken cartilage sausage with both probiotic and bioprotective LAB.

Probiotics are live, non-pathogenic bacterial preparations that beneficially exert health effects to their host when ingested in adequate amounts (FAO/WHO, 2006). The selection of the potentially probiotic strains that would be capable of effectively performing in the gastrointestinal (GI) tract is a significant challenge. Acid and bile tolerance are two fundamental properties indicating the ability of micro-organism to survive through the host GI tract, particularly the acidic condition in the stomach and the presence of bile salt in the small intestine (Erkkilä and Petäjä, 2000). The probiotic effects on the host immune modulation are also claimed to be investigated and reported (MacFarlane and Cummings, 2002; Tannock, 2002; Vaarala, 2003; Lee *et al*., 2004). The majority of evidence from those *in vitro* systems

of both animal and humans models suggested that probiotics can enhance both specific and non-specific immune system responses.

Lactobacillus johnsonii KUNN19-2 and *Pediococcus pentosaceus* KUNNE6-1 used in the current study are two lactic acid bacteria (LAB) which isolated from the commercial Thai style fermented pork sausages (Nham) produced in Thailand. Both LAB types in the previous study of Kwanmuang (2003) showed resistance to some antibiotics (streptomycin, chloramphenicol and tetracycline) and could produce some anti-bacterial substances that inhibit the growth of food borne pathogenic *Bacillus cereus*, *Staphylococcus aureus* and food spoilage *Leuconostoc mesenteroides*. Furthermore, Kwanmuang (2003) found that inoculation of both strains during Nham fermentation resulted in good quality product with respect to faster acid production and better test panels preference scores compared to natural fermentation. However, their probiotic benefits from a health aspect have not yet been determined.

The beneficial effects of food with added live microbes (probiotics) on human health, and in particular on children and other high-risk populations, are being increasingly promoted by health professionals. It has been reported that these probiotics can play an important role in immunological, digestive and respiratory functions and could have a significant effect in alleviating infectious diseases in children. Therefore, the aims of the current study were to study the fermentation of chicken cartilage using both isolated strains meanwhile determine the capability of these two strains to survive along the passage of the GI tract and their response to the immune modulation to pose their future probiotic potential for human health.

OBJECTIVES

The objectives of this study are:

1. To characterize *Lactobacillus johnsonii* KUNN19-2 and *Pediococcus pentosaceus* KUNNE6-1 in respect of their probiotic properties.

2. To study the technological characteristics of *Lactobacillus johnsonii* KUNN19-2 and *Pediococcus pentosaceus* KUNNE6-1 in fermented chicken cartilage (Nham Kor Khai) application.

3. Selection of the most suitable strain according to their technological characteristics and probiotic properties to be used as a starter culture in fermented chicken cartilage.

Hypothesis

The addition of the selected strains; *Lactobacillus johnsonii* KUNN19-2 and *Pediococcus pentosaceus* KUNNE6-1 to fermented chicken cartilage may improve safety and stability of the product and provides health benefits from their probiotic characteristics.

LITERATURE REVIEW

Lactic acid bacteria

Lactic acid bacteria (LAB) are gram-positive usually non-motile, non-spore-forming rods and cocci. They lack the ability to synthesize cytochromes and porphyrins (components of respiratory chains) and therefore cannot generate ATP by a proton gradient creation. The LAB can only obtain ATP by fermentation, usually of sugars. Since they do not use oxygen in their energy production, lactic acid bacteria happily grow under anaerobic conditions, but they can also grow in the presence of oxygen. They are protected from oxygen byproducts (e.g. H_2O_2) because they have peroxidases. These organisms are aerotolerant anaerobes (Michaela *et al*., 2009). Two main sugar fermentation pathways can be distinguished among lactic acid bacteria. Glycolysis (Embden-Meyerhof pathway) results in almost exclusively lactic acid as end product under standard conditions, and the metabolism is referred to as homolactic fermentation (Derek *et al*., 2009).

The 6-phosphogluconate/phosphoketolase pathway results in significant amounts of other end products, such as ethanol, acetate, and CO_2 in addition to lactic acid and the metabolism is referred to as heterolactic fermentation. Various growth conditions may significantly alter the end-product formation by some lactic acid bacteria. These changes can be attributed to an altered pyruvate metabolism and/or the use of external electron acceptors such as oxygen or organic compounds.

LAB form a taxonomically diverse group of microorganisms that can convert fermentable carbohydrates into lactic acids. A large number of LAB species are involved in the production and consumption of fermented foods and beverages. Most LAB are members of the intestinal flora. Bacteria in the human intestine play an important role in human physiology, most of which are beneficial or neutral for the host.

Classification of LAB genera was based on morphology, mode of glucose fermentation, growth at certain temperatures, and range of sugar utilization. LAB constitute a group of bacteria that have morphological, metabolic and physiological similarities, and they are also relatively closely related phylogenetically.

The general description of the bacteria within the group is gram-positive, non-sporulating, non-respiring cocci or rods, which do, through the fermentation of carbohydrates, produce lactic acid as their major end product. There is a core group consisting of four genera; *Lactobacillus, Leuconostoc, Pediococcus* and *Streptococcus*. Recent taxonomic revisions have proposed several new genera and the remaining group now comprises the following: *Aerococcus, Alloiococcus, Carnobacterium, Dolosigranulum, Enterococcus, Globicatella, Lactococcus, Oenococcus, Tetragenococcus, Vagococcus, and Weissella. Lactobacilli, Carnobacteria* and some *Weissella* are rods while the remaining genera are cocci (Jin *et al*., 2009). For LAB identification, phenotypic methods have been most commonly used (Corsetti *et. al*., 2001). More recently, genetic techniques, such as 16S rDNA sequencing have been developed which allows a more consistent and accurate identification of individual strains (Buddhiman *et al*., 2008). Determination of short sequences of 16S rDNA is nowadays used as a simple way for species determination of isolates of lactic acid bacteria (Schleifer and Ludwig, 1995).

The taxonomy of LAB has been based on the gram reaction and lactic acid production from various fermentable carbohydrates. The classification of lactic acid bacteria into different genera is largely based on morphology, mode of glucose fermentation, growth at different temperatures, and configuration of the lactic acid produced, ability to grow at high salt concentrations, and alkaline or acid tolerance. For some of the newly described genera (Pilar *et. al*., 2008), additional characteristics such as fatty acid composition and motility are used in classification. The measurements of true phylogenetic relationship with rRNA sequencing have aided the classification of lactic acid bacteria and clarified the phylogeny of the group. Most genera in the group form phylogenetically distict group, but some, in particular *Lactobacillus* and *Leuconostoc* are very heterogeneous and the phylogenetic cluster do not correlate

with the current classification based on phenotypic characters. New tools for classification and identification of lactic acid bacteria are underway (Sascha and Magdalena, 2010). The most promising for routine used are nucleic acid probing techniques, partial rRNA gene sequencing using the polymerase chain reaction, and soluble protein patterns. The growth is optimum at pH 5.5-5.8 and the organisms have complex nutritional requirements for amino acids, peptides, nucleotide bases, vitamins, minerals, fatty acids and carbohydrates**.**

Metabolism of lactic acid bacteria

LAB are chemotrophic, they find the energy required for their entire metabolism from the oxidation of chemical compounds. The oxidation of sugars constitutes the principle energy producing pathway. Lactic acid bacteria of the genera *Lactobacillus, Leuconostoc* and *Pediococcus*, assimilate sugars by either a homofermentative or heterofermentative pathway.

Homofermentative metabolism of hexoses

Homofermentative bacteria transform nearly all of the sugars they use, especially glucose into lactic acid. The homofermentative pathway includes a first phase of all the glycolysis reactions that lead from hexose to pyruvate. The terminal electron acceptor in this pathway is pyruvate which is reduced to lactic acid. In fermentation, pyruvate is decarboxylated to ethanal, which is the terminal electron acceptor, being reduced to ethanol.

Heterofermentative metabolism of hexoses

Bacteria using the heterofermentative pathway, which includes *Leuconostoc* (the most important bacterium in enology) use the pentose phosphate pathway. In this pathway, NADPH is generated as glucose is oxidized to ribose 5-phosphate. This five-carbon sugar and its derivatives are components of important biomolecules such as ATP, CoA, NAD+, FAD, RNA and DNA. NADPH is the currency of readily

available reducing power in cells (NADH is used in the respiratory chain). This pathway occurs in the cytosol. After being transported into the cell, a glucokinase phosphorylates the glucose into glucose 6-P (glucose 6-phosphate). Its destination is completely different from the glucose 6-P in the homofermentative pathway.

Two oxidation reactions occur: the first leads to gluconate 6-P and the second, accom-panied by a decarboxylation, forms ribulose 5-P. In each of these reactions a molecule of NADP+ is reduced. Ribulose 5-P can then be epimerized either to ribose 5-P or to xylulose 5-P. Xylulose 5-P is then cleaved into acetyl-phosphate and glyceraldehydes 3-phosphate . The glyceraldehyde 3-phosphate is then metabolized into lactic acid by following the same pathway as in the homofermentative pathway. The acetylphosphate has two possible destinations, depending on environmental conditions. This molecule can be successively reduced into ethanal and ethanol, in which case the molecules of the coenzyme NADPH formed during the two oxidation reactions of glucose at the beginning of the hetero-fermentative pathway, are reoxidized. This reoxidation is essential for regenerating the coenzymes necessary for this pathway. The final products are then lactate and ethanol. Or the acetyl-phosphate can produce acetate (acetic acid) through the enzyme acetate kinase. This reaction also yields a molecule of ATP. The final products of this pathway are then lactate and acetate. Bacteria of the genus *Leuconostoc* preferentially produce lactate and ethanol in a slightly aerated environment and lactate and acetate in an aerated environment.

LAB strains are the major bacteria for food fermentation, being responsible for the fermentation of sour dough bread, sorghum beer, fermented milks, cassava and pickled (fermented) vegetables (Battcock and Azam-Ali, 1998). Some LAB strains can be used as probiotics for human and animals (Chou and Weimer, 1999) and in general, the probiotic properties of LAB are their resistance to host GI conditions, adhesion to host intestinal epithelium and the prevention of the growth or invasion of pathogenic bacteria, such as *Samonella* spp. and *Escherichia coli*, in the animal intestine (Jin *et al*., 1998; Chou and Weimer, 1999).

The adherence ability to host intestinal epithelium for LAB cells survived from gastric and bile conditions may confer a competitive advantage and is important for bacterial maintenance in the human GI tract (Naidu *et al*., 1999). Such properties may play some role in the competitive exclusion of some pathogenic bacteria (Chou and Weimer, 1999; Naidu *et al*., 1999). Some LAB strains have been used to improve the gut performance and reduce the pathogen invasion (La Ragione *et al*. 2004; Tzortzis *et al*., 2004; Tsai *et al*., 2005). Furthermore, certain LAB strains have been postulated to have other interesting health beneficial effects, such as stimulation of the immune system of the human hosts (Schiffrin *et al*., 1997; Choi *et al*., 2003; Adams, 2010).

LAB, as a rule, are facultatively heterofermentative strains, which produce lactic acid from hexoses, such as glucose and lactose, as their only metabolic product (glycolysis pathway). As there is no sufficient glucose in meat to markedly reduce the pH, glucose is added at 0.4-0.7% (w/w) to the sausage matrix. For lactose fermenting LAB, such as *Lactobacillus sakei*, lactose may also be used (0.5-1.0%) (Tyopponen, 2003). However,not all LAB can easily ferment lactose and especially some probiotic, such as *Lb. rhamnosus* GG, are not able to utilize lactose. Thus, the starter culture properties have to be taken into account prior to planning the new applications. As indicated in commercial catalogues LAB strains currently most employed in meat starter cultures are *Lb. casei, Lb. curvatus, Lb. pentosus, Lb. plantarum, Lb. sakei, Pediococcus acidilactici* and *P. pentosaceus.*

Starter cultures

According to the definition of Hammes (1998), meat starter cultures are ‘‘preparations which contain living or resting microorganisms that develop the desired metabolic activity in the meat’’.

Fermentation is a method of preservation and prolongation of the shelf life of meat.During fermentation, ripening and drying of fermented sausages, many complex microbial,biochemical and physicochemical processes take place and influence the

quality and safety of the final products. Different microorganisms, derived from raw materials and the environment, naturally contaminate dry sausage mixtures. Among them, LAB are found to be the most active microorganisms in fermented sausages (Zeljka *et al.,* 2012). They ferment the sugars to acid and thus lower the pH, improving the texture of the products, providing prolonged stability against the proliferation of food pathogens and producing some aromatic compounds. The most common isolated LAB in fermented sausages are lactobacilli such as *L. sakei*, *L. plantarum* and *L. curvatus* (Parente *et al.,* 2001; Ammor *et al*., 2005; Drosinos *et al*., 2005; Cocolin *et al*., 2009).

From a hygienic and technological point of view, it is important to determine their influence on the natural microbial flora, and physicochemical and sensorial changes during the ripening of the sausages. Those strains of LAB found in fermented food complex may play significant roles in the ripening process resulting in unique sensory characteristics in the end products. They were naturally selected from the product microbiota by the ability to best compete under the prevailing conditions of the ecological niche. The LAB isolated and identified could then be used as starters culture in fermented food production to obtain a more uniform sensory quality in the end products without undesirable flavors and microorganisms that may often be found in a natural fermentation and the products could also be safety and pleasantly consumed.

Lactic acid bacteria (LAB) as well as *Micrococcaceae* strains are important microorganisms used as starter cultures in meat fermentation process in order to improve safety and stability, extending the shelf life of the product and provides diversity resulting in new sensory properties as well as health benefits by probiotic characteristic (Lucke, 2000). The use of bacteriocin-producing bacterial strains as starter cultures or protective co-cultures for control of food pathogens is one of the interesting and acceptable ways to improve food safety (Kim, 1993; Stiles, 1996; Hugas, 1997; Noonpakdee *et al*., 2003).

LAB are natural inhabitants of the GI tract and are the most frequently used probiotics. Some Lactobacillus and Bifidobacterium strains have been used due to their beneficial effects on health (Lee and Salminen, 1995), although probiotic characteristics and resistance to biological barriers vary widely between species and among strains of the same species (Vinderola and Reinheimer, 2003).The use of native lactic acid bacteria as bioprotective cultures is important for cooked meat products. Contrary to dry meat products where the starter culture become dominant changing the environment to ensure microbiological quality, cooked meat products need a thermal processing to develop texture and destroy vegetative forms in order to ensure the adequate shelf life.

There are a wide variety of typical sausage preparations (Ward *et al*., 1998). Many typical fermented meat products are still produced with traditional technologies without selected starters. However, the use of starter cultures for sausage production is becoming increasingly necessary to guarantee product safety aspect and to standardize product properties, including consistent flavor and color and shorter ripening time. (Cocolin *et al*., 2001)

Pediococci are used as starter cultures in the commercial fermentation of meats and vegetables. They can also be used to sour wheat flour with no added sugar. The pediococci were tested as biopreservatives of refrigerated foods, but the refrigeration temperature did not favor their fermentative activity. *P. acidilactici* NCIB 6990 is highly sensitive to pantothenic acid and can be used for the bioassay of this vitamin. The pediococci are also associated with the spoilage of beer, wine and juices.

The initial concentrations of starter cultures may vary in accordance with the type of sausage and species of bacteria. For example, *Lb. plantarum, P. cerevisae* and *M. varians* amounts of 10^3, 10^6 and 10^3 CFU/g of meat system respectively were supposed to be in Nham (Boonthanom *et al*., 1994).There were some reports about fermentation time for various sausages using starter cultures, among them were 2 days at 30°C for Nham (Wiriyacharee *et al*., 1993), 3 days at 14±1°C for fermented pork sausage (Ibanez *et al*., 1996) and 4 days at 20-22°C for salami (Bohme *et al*., 1996).

Lactic acid bacteria (LAB) as Probiotic

There are beneficial and potentially harmful microorganisms in the intestine and that the one could be used to influence the activities of the other. These findings led to the concept of probiotic, originally used to describe microbial feed supplements which stimulate the growth of farm animals and recently the use of these live micro-organisms as the dietary supplements has been extended to humans. Many definitions of probiotics have been issued, starting from Fuller (1991), who defined a probiotic as "a live microbial feed supplement which beneficially affects the host by improving its intestinal microbial balance". And the more recent one from FAO/WHO (2006) is the following: "live microorganisms that when being administered in appropriate dose, they confer a benefit of health to the receiver".

Probiotics

Probiotics are live, non-pathogenic bacterial preparations that beneficially exert health effects to their host when ingested in adequate amounts (FAO/WHO, 2006). Probiotics are lactic acid bacteria (LAB) or bifidobacteria, currently mainly of Lactobacillus species. Other species have been introduced including enterococci, pro-pionibacteria and even clostridia (Sanders and Huis in't Veld, 1999; von Wright and Salminen, 1999). The ability to produce different antimicrobial compounds, such as bacteriocins and/or low-molecular mass anti-microbial compounds, may be one of the critical characteristics for effective competitive exclusion of pathogens and survival in the intestine to express probiotic effect to the host (Ouwehand, 1998; Salminen *et al*., 1998). The acidic conditions in the stomach may even enhance the activity of these antimicrobial compounds (Ganzle *et al*., 1999a). Furthermore, the probiotic effect of LAB may partly be based on the production of lactic acid in the microenvironment, which in combination with detergent like bile salts inhibit the growth of Gram-negative pathogenic bacteria (Alakomi *et al*., 2000). In fact, the exact mechanism by which a probiotic strains interacts with other bacteria in the GI tract or with the mucus of GI tract itself is still not known (Havenaar *et al*., 1992; Fuller, 1991; von Wright and Salminen, 1999). However, several clinical studies concerning GI disorders, food

allergies and inflammatory bowel diseases have proofed for the claimed health effects (Isolauri *et al*., 1999; von Wright and Salminen, 1999). During the past years, probiotic have been used for alleviation of symptoms of food allergy in infants and adults as well as prevention of atopic diseases in infants (Isolauri *et al*., 1999; Ouwehand *et al*., 1999)

Desirable characteristics of organisms to be used as probiotics

There are several desirable characteristics for organisms to be used as dietary adjuncts as summarized in Table 1. Organisms should be a normal inhabitant of the human intestinal tract, non pathogenic, non toxic and be capable of surviving passage through the gastrointestinal tract. Within the gut it must produce the desired effects. Furthermore, it must maintain viability and activity in the carrier food before the consumption. Lastly, the organism should be sensitive to antibiotics used to treat the infection and should not harbor plasmids resulting in antibiotic resistance. It is also important to know the number of organisms needed to colonize human subjects to estimate the effective therapeutic dose.

Table 1 Key selection and safety criteria for probiotic bacteria

Desired properties	Desired effects
- Human origin	Ablility to maintain viability, species specific effects on health.
- Acid and bile stability	Maintenance of viability in the intestine
- Adherence to human intestinal cells	Maintenance of mild acidity in intestine, antagonism against pathogens,competitive exclusion, immune effects
- Colonization of the human gut	Maintenance of colonizing properties, antagonism against pathogens, competitive exclusion
- Production of antimicrobial substances	Antagonistic against pathogens, competitive exclusion (in GI tract and oral cavity)
- Safety in human use	Tested safety in animal models and human use, accurate strain identification (genus, species)

Source: Modifiied from Klaenhammer and Kullen (1999)

The genera most commonly used in the probiotic preparations in foods or supple-ments are *Lactobacillus* spp., *Bifidobacterium* spp., *Streptococcus* spp. and *Lactococcus* spp. and also some fungal strains. The organisms used as probiotics showed in Table 2 (O'May and Macfarlane, 2005). Single and mixed cultures of live microorganisms are used in probiotic preparations in foods for human consumption that containing mainly lactic acid bacteria include fermented milks, cheeses, fruit juices, wine, and sausages.

Table 2 Organisms used as probiotics

Lactobacilli	Bifidobacteria	Other species
L. actdophilus	*B. adolescentis*	*Bacillus subtilis*
L. casei	*B. animalis*	*Enterococcus faecalis*
L. crispatus	*B. bifidum*	*Enterococcus faecalis*
L. delbruckii	*B. breve*	*Escherichia coli*
L. gallinarum	*B. infantis*	*Lactococcus lactis*
L. gasseri	*B. lactis*	*Leucnostoc mesenteroides*
L. johnsonii	*B. longum*	*Pediococcus acidilactici*
L. paracasei		*Pediococcus pentosaceus*
L. plantarum		*Saccharomyces boulardii*
L. reuteri		*Sporolactobacillus inulinus*
L. rhamosus		*Streptococcus thermophilis*

Source: O'May and Macfarlane (2005)

The most common use of probiotic microorganisms is in fermented dairy products (Ouwehand *et al*., 2002), although recently a new idea of developing to use probiotic strains in other foods such as fermented meat products have been afforded (Hugas and Monfort, 1997; Hammes and Hertel, 1998; Tyopponen *et al*., 2003).The development of new fermented meat products presupposes the new application of probiotic lactic acid bacteria to be capable of activating and growing quickly during the fermentation and ripening, acid and bile resistant and able to colonize the human intestinal tract by mean of some mechanisms for adhering to the intestinal cells (Salminen *et al*., 1998; Sameshima *et al.*, 1998).Nowadays, the meat industry has begun to find starter cultures with additional value (Erkkila *et al*., 2001). Many scientists have recently reported the use of probiotic meat starter cultures for dry fermented sausage manufacture (Hugas and Monfort, 1997; Incze, 1998). It has been shown that it is possible to successfully use probiotics as starter cultures or even bio-protective co-cultures for sausage fermentation processes, since there are no signi-

ficant technological and sensorial differences between the sausages fermented by probiotic or traditional starter cultures were claimed (Erkkila *et al*., 2001).

Some studies described the effect of selected probiotic strains for their use in the fermented sausages production or the potential probiotic use of some selected strains present in commercial meat product starter cultures (Erkkila *et al*., 2001). Strains which are interested to use as starters are usually selected from the food products they are going to be employed for. Therefore, to determine the potential to use probiotics in meat products a screening procedure was performed to select potentially probiotics directly from fermented meat products. The International Dairy Federation has recommended that the bacteria be active and abundant in the product, and be present at least 10^7 CFU/g to the date of minimum durability (Ouwehand and Salminen, 1998). It is generally accepted that successful delivery and colonization of viable cells in the intestine tract is essential for probiotic to be effective (Conway, 1996). However, studies indicate that bacteria may not survive in sufficient numbers when incorporated into dairy products and also during their passage through the gastrointestinal tract (Hamilton-Miller and Shah, 1999). Several factors influence the survival and colonization of these bacteria, including the tolerance to low pH, bile acids and digestive enzymes (Conway, 1996). However, low pH and bile tolerance were shown to be dependent on both the species, identified by phenotypic and molecular methods, and the strain tested. Acid-tolerance is a fundamental property that indicates the ability of probiotic microorganisms to survive passage through the stomach (Prasad *et al*., 1998; Park *et al*., 2002), bile plays a fundamental role in defense mechanism of the gut and the magnitude of its inhibitory effect is determined primarily by the bile salts concentration (Charteris *et al*., 2000). In the human gastrointestinal tract, the mean bile concentration is believed to be 0.3% w/v while the mean pH is 1.5 and it is considered as critical and high enough to screen for resistant strains (Gilliland *et al*., 1984; Pennacchia *et al*., 2004).

Although the overall quality of the final product is important, the microbes are mainly selected on the basis of their potential health supporting properties. It is preferable, nevertheless, that the microbes can grow in expecting food complex and essentially survive in that condition of food environment to provide a suitable shelf life period for the product. The isolation and selection of lactic acid bacteria which can be used as starter cultures in meat fermentation present a considerable challenge to standardization and for the quality management of fermented sausage. Lactic acid bacteria originating from fermented meats are specially adapted to the ecology of meat fermentation (Hugas and Monfort, 1997). Probiotic candidate culture to be used as meat starter culture is then should be the strain that isolated from the meat itself which is the food source that they are going to be employed. Combining probiotic potential and technological productions of Lactobacillus strains are interesting to use as a starter cultures in dry fermented sausages. Arihara *et al*. (1998) showed that the potentially probiotic strain *Lactobacillus gasseri* JCM1131 can be used for meat fermentation to enhance product safety, and Kwanmuang (2003) has screened LAB from Nham which were collected from around Thailand to find the most suitable strain for use as Nham starter cultures and found that *Lactobacillus johnsonii* KUNN 19-2 and *Pediococcus pentosaceus* KUNNE6-1 gave the high sensory scores for texture, color, flavor and consumer acceptance of Nham and also showed their potential for use as probiotics since both strains were resisted to antibiotics, Chloramphenicol and Tetracycline at 50 µg/mL and Streptomycin at 100 µg/mL and could produced anti-bacterial substances to inhibit the growth of foodborne toxigenic bacteria , *Bacillus cereus*, *Leuconostoc mesenteroides* and *Staphylococcus aureus* .

The minimum dose of daily ingestion of probiotic bacteria has much been the subject of discussion, but it is estimated to be 10^9-10^{10} viable microbes in order to express a health effect and temporary colonization as measured by levels of 10^6-10^8 viable microbes/g feces. For a dry sausage containing 10^8 viable microbes/g the minimum dose therefore could be 10-100 g sausage per day. However, the minimum dose depends on several factors, such as individual person, bacterial strains and food product. Since the exact mechanism by which a probiotic strain interact with other bacteria in the gastrointestinal tract or with the mucus of gastrointestinal tract in itself

is not known (Havenaar *et al*., 1992; Fuller, 1992; Playne, 1995; Berg, 1998), no direct extrapolations of how a strain would affect a host can be made (Mattila-Sand holm *et al*., 1999). Finally, the food product itself is important. The number of viable probiotic in the product is effected by several factors, such as cooking and storage temperature, food compositions, and at expiration date the product is also expected to contain those minimal numbers of living microbial cells.

Survival in the gastrointestinal tract of probiotics

Many of the health promoting claims attributed to probiotic bacteria are dependent on the cells being both viable and sufficiently numerous in the intestinal tract. In order to perform functionally and efficiently, probiotic bacteria must survive during passage through the GI tract and still be alive at their functional site. In the upper part of the GI tract, bacteria will be subjected to several stress factors. In the stomach, they are stressed by very low pH within the range 1.5-3.0 and by the proteolytic enzyme, pepsin.

High viability of probiotic strains is generally required in order to maintain functionality in the gut. Probiotics must overcome the two main biological barriers of stomach acidity and bile secretion in the small intestine to reach the lower part of the gastrointestinal tract in a viable state. Probiotic bacteria vary considerably in their tolerance to low pH and bile salts; this can be evaluated *in vitro* by controlled incubation in gastric juice followed by bile treatment to reflected the physiological conditions found in the upper part of the gastrointestinal tract (Dunne *et al*., 2001) Tolerance to gastrointestinal stress is generally low and it is important to find new strategies to improve probiotic strain resistance (Noriega *et al*., 2004). Underlying bacterial mechanisms should be systematically investigated to design future generations of probiotics (Whitehead *et al*., 2008; Gueimonde *et al*., 2009).

Probiotics for human consumption, are of increasing interest due to the growing evidence of health benefits associated with their use. Probiotic bacteria that are delivered through food systems have to firstly survive during the transit through the upper GI tract, and then persist in the gut to provide beneficial effects for the host (Huang and Adams, 2004). In order to be used as potential probiotics, bacterial strains need to be screened for their capacity of transit tolerance to the upper gastrointestinal tract conditions. The low pH of the stomach and the antimicrobial action of pepsin are known to provide an effective barrier against entry of bacteria into the intestinal tract (Holzapfel *et al*, 1998). The pH of the stomach could be as low pH 1.5 or as high as pH 6.0 or above after food intake (Jonhson, 1977), but generally ranges from pH 2.5 to pH 3.5 (Holzapfel *et al*, 1998).There are no agreed rules for the screening of acid tolerance of potential probiotic strains. A range of pH values, from pH 1.0 to pH 5.0 has been used to screen in vitro acid tolerance of *Lactobacillus* and *Bifidobacterium* (Chung *et al,* 199; Zarate *et al*, 2000). However food and food ingredients have been shown to protect probiotic bacteria from acid conditions and enhance gastric survival. Milk has been reported to increase the viability of acid sensitive *Lacto-bacillus* and *Bifidobacterium* strains during simulated gastric tract transit (Huang and Adams, 2004). The protective effect may be due to the increase of gut pH after milk addition. Maize starch granules at pH 3.5 have also been found to increase the viability of the more sensitive Bifidobacterium strains (Wang *et al,* 1999). Currently, orally ingested probiotic bacteria for humans are mainly prepared as food supple-ments or in conjunction with food especially dairy products. Although pH could be used as, a suitable direct measure for selection of probiotic strains, most probiotics are consumed in food products. The presence of food and food ingredients has been reported to improve viability of microorganisms during gastric transit (Huang and Adams, 2004). The suggested mechanism for the beneficial effect of food ingredients is the pH increase of the gastric contents resulting from the addition of the food (Zarate *et al*, 2000). According to a large loss of viability of most bacteria associated with passage through the stomach after the oral administration which is attributed to the high acid and bile salt concentrations present. This loss of viability effectively lowers the efficacy of the administered supplement. The formulation of these pro-biotics into microcapsules is an emerging method to reduce cell death during GI

passage, as well as an opportunity to control release of these cells across the intestinal tract. The majority of this technology is based on the immobilization of bacteria into a polymer matrix, which retains its structure in the stomach before degrading and dissolving in the intestine. Kawther *et al*. (2011) reported that the simulated gastric transit tolerance of *L.johnsonii* B-2178, *L. gasseri* B-14168 and *L. salivarius* B-1950 was pH dependent and correspondingly showed lower viability at pH 2.0 after 180 min compared with pH 3.0 and pH 4.0 and the presence of milk proteins, singly and in combination with starch at pH 2.0 and pH 3.0 exerted a major effect on the gastric tolerance of all tested strains in yoghurt during storage as they were capable of undiminished survival during simulated gastric transit in the presence of sodium caseinate, whey protein and their combination with starch

Bacteria are generally sensitive to the stomach's low pH values, however, some LAB can survive and grow at relatively low pH because they have a system that simultaneously transports lactic acid and protons to the cell's exterior (Conway *et al*., 1987). In the study of Goldin *et al*. (1992) Lactobacillus GG survived pH 3 for 4 h, while Charteris *et al*. (1998) reported from their study that *Lb. casei* 212 and Lactobacillus GG exhibited good growth at pH 2.3 for 3 h and classified the strains as intrinsically tolerant, when ingested under fasting conditions they were intrinsically resistant to transit through the human GI tract, meaning a large amount of micro-organisms could therefore arrive at the upper intestine. Under the same conditions, *L. casei* F19 was reported as intrinsically sensitive, intolerant of GI tract conditions as its viability decreased from 10^9 to 10^3 CFU/ml. In another study, 99 *Lb casei* strains were shown to resist pH 3 for 3 h, but the strains *Lb. casei* NCDC 17 and *Lb. casei* C1 were able to resist pH 2 for 3 h (Mishra and Prasad, 2005).

The acid tolerance of lactobacilli is attributed to the presence of a constant gradient between extracellular and cytoplasmic pH. The F_0F_1-ATPase is a known mechanism that gram-positive organisms use for protection against acidic conditions (Cotter and Hill, 2003). The F_0F_1-ATPase is a multiple-subunit enzyme consisting of a catalytic portion (F_1) incorporating the α, ß, γ, δ, and ε subunits for ATP hydrolysis and an integral membrane portion (F_0) including the a, b, and c subunits, which

function as a membranous channel for proton translocation. The role of the F_0F_1-ATPase in organisms devoid of a respiratory chain is to generate a proton motive force, via proton expulsion. It is assumed that the F_0F_1-ATPase can increase the intracellular pH at a low extracellular pH as F_0F_1-ATPase is induced at low pH, and regulation appears to occur at the transcriptional level (Fortier *et al*., 2003).

Normally bacteria can be introduced to the stomach and reside there under acidic gastric conditions for 1–4 h, depending on the diet, which is long enough to kill the majority of bacteria (Olejnik *et al*., 2005). The possible acid resistance mechanism was explained by Matsumoto *et al*.(2004) as it resulting from the bacteria perhaps trying to maintain pH homeostasis by discharging H+ from cells by H^+-ATPase. Furthermore, an increase of *atp* BEF genes of the *atp* operon, whose genes encode the various subunits of the F_1F_0-ATPase, may function in the ATP-dependent extrusion of protons and maintenance of cytoplasmic pH which is involved in stress responses relating to the enhanced acid tolerance properties of LAB reported by Klaenhammer and Kullen (1999).

Corcoran *et al*., 2005 found that survival rate of probiotic lactobacilli in acidic conditions was increased in the presence of glucose the presence of 19.4 mM glucose resulted in up to 6-$\log_{10}$-enhanced survival of *Lactobacillus rhamnosus* GG at pH 2.0 following 90 min of exposure, this similar results has also been previously reported by Charalampopoulos *et al*. (2003) indicated that glucose provides ATP to F_0F_1-ATPase via glycolysis, enabling proton exclusion and thereby enhancing survival during gastric transit. They stated that, F_0F_1-ATPase requires ATP for expulsion of H^+ from the cell, thereby maintaining pH homeostasis and cell viability. The accumulation of ATP is as a result of energy-generating factories, such as the glycolytic system. Glucose in acid conditions can therefore enhance probiotic survival by providing the ATP pool required, permitting optimal H^+ extrusion by F_0F_1-ATPase. Such a mechanism can provide more effective delivery of viable probiotic lactobacilli to the human GIT. It is therefore not unreasonable to assume that the beneficial effect of glucose occurs for each *Lactobacillus* at a critical pH value. The survival of *Lb. rhamnosus* GG in simulated gastric juice in the presence of glucose also appeared to

be dependent on glucose concentrations as low as 1 mM. The F_0F_1-ATPase is importantly linked to the survival effect observed in simulated gastric juice in the presence of glucose, and it was up regulated as a result of acid stress in lactobacilli (Kullen and Klaenhammer,1999; Corcoran *et al*., 2005).

Bile tolerance

Survival during passage through the small intestine in the presence of bile salts and pancreatin is another barrier for bacteria to overcome in order to have a positive effect on their host. The pH of the human small intestine is around 8.0 with the concentration of bile acids between 5,000 and 20,000 ìM (Hofmann, 1991)and the general transit time of food is between 1and 4 h (Huang and Adam, 2004). Several previous studies (Goldin and Gorbach 1992; Casey *et al*., 2004) proposed that 0.3% bile salt (equivalent to approximately 7,353 μM) was considered as a critical bile concentration, which is high enough to screen for the resistant strains. In this study *Lb. johnsonii* KUNN19-2 exhibited higher bile salt toleration compared to *P. pentosaceus* KUNNE6-1 which might have been due to bile salt hydrolase (BSH) enzyme activity (Erkkilä and Petäjä, 2000; Haller *at al*., 2001; Begley *et al*., 2006).

Bile is a steroid produced by the liver and secreted through the bile duct in the form of bile salts. These salts can occur as conjugates between cholic acid and the amino acids glycine or taurine (forming glycholic or taurocholic acid), or as deconjugates such as sodium desoxycholate (Begley *et al*., 2006).

Some LAB strains are capable of surviving high bile concentrations (0.3%) and can therefore adapt to GI tract conditions (Gilliland *et al*., 1984). *Pediococcus pentosaceus* 12, *P. pentosaceus* 15L, *P. pentosaceus* 22 and *P. pentosaceus* 15a were reported by Ramirez-Chavarin *et al*. (2013) that they can grow effectively at up to 2.0% bile concentrations (0.1, 0.3, 0.5, 1.0 and 2.0%) and they also grew well in 7 mM taurocholic acid compared to the control. However, in another study, most probiotic bacteria were also reported to grow in MRS medium supplemented with more than 0.5% conjugated bile salts (Noriega *et al*., 2006).

Pediococcus pentosaceus MP12 and *Lactobacillus plantarum* LAP6, isolated from pickled vegetables were assayed for their acid and bile tolerance and activities against Salmonella invasion in mouse liver and spleen. Both strains showed their acid and bile tolerance (pH 2- 3.2 at 37ºC for 3 h and 0.3% (w⁄v) oxgall bile at 37 ºC for 24 h respectively) and were found to be able to inhibit the Salmonella invasion in host (Chiu *et al*., 2008), while the cells of *L. acidophilus* in the study of Lankapurtha and Shah (1995) are able to survive the acidity of the stomach which can reach a pH as low as 1.5, and then bile salt up to 2.0% concentrations in the small intestine. This was in agreement with previous studies by Haller *et al*. (2001) and Visozo Pinto *et al*. (2006) who concluded that *Lb. johnsonii* spp. had good survival rates under acidic condition.

Pediococcus pentosaceus and *Lactobacillus plantarum* which have been previously studied as a biopreservative culture in cooked meat products, inoculated as free cells or spray-dry encapsulated and were found to improve the texture of low fat sausages (Pérez-Chabela *et al*., 2012). Both strains were then isolated from cooked meat products by Ramirez-Chavarin *et al*. (2012) to identify their probiotic properties, including tolerance to low pH, tolerance to taurocholic acid and bile, coaggregation, autoaggregation and adherence to HEp-2 epithelial cells. Although only *Lb. plantarum* was found to exhibit good growth values at different pH values (0.5, 1, 2, 3, 4 and 5) for 1 h but both *P. pentosaceus* , *Lb. plantarum* grew in taurocholic acid and bile concentrations greater than 0.3% after 90 min incubated at 35 ± 2°C this indicating the good probiotic candidates.

In the study of Buntin *et al*. (2008) *P. pentosaceus* APa4, *P. pentosaceus* AIa1 were reported as the potential probiotic candidates based on their survival at low pH (pH 1, 2, 2.5 and 3 incubated at 37^{o}C for 4 hr) and high concentration of bile salt (2000, 3000 and 4000 ppm bile salt concentrations incubated at 37^{o}C for 48 hr) which was in agreement to the result obtained by Erkkila and Petaja (2000) that the strains of *P. acidilactici* P2, *Lb. curvatus* RM 10 and *Lb. sake* L2 were the most resistant to 3000 ppm bile salt at pH 6, indicating their high potential to be able to survive in the human upper gastrointestinal tract. Lactobacillus strains in general could not survive

conjugated bile salts, probably due to an absence of the enzymes needed to hydrolyze them, but achieving growth in the presence of deconjugated bile salts (Suskovic *et al*., 1997). Similar results were reported by De Boever *et al*. (2000) in an *in vitro* study of *Lb. reuteri* strains with hydrolytic activity for bile salts in which the bile salts had no negative effect on strain growth probably because the deconjugated salts were adsorbed onto the lactobacillus cell surface, thus diminishing their bioavailability.

In the human GI tract, the mean bile salt concentration is believed to be 3000 ppm, which is considered as critical and high enough to screen for resistant strains (Gilliland *et al*., 1984; Goldin and Gorbach, 1992). However, *Lb. rhamnosus* strains isolated from Parmigiano Reggiano cheese were able to survive at bile salt concentration of 10,000, 15,000 and 20,000 ppm after 48 hour of incubation at 37^{o}C (Succi *et al*., 2005) while Pennacchia *et al*. (2004) reported that the bile salt tolerance of the *Lacto-bacillus* strains were able to grow in MRS agar supplemented with 3000 ppm bile salt. It has been reported that certain strains of *Lactobacillus* are able to reduce this detergent effect by their ability to hydrolyze bile salt by bile salt hydrolase enzyme (BSH) (Erkkila and Petaja, 2000), which are then readily excreted from the GI tract (Maragkoudakis *et al*., 2006). This particular enzyme decreases bile solubility and thus weakening its detergent effect.

In addition, another protective mechanism in the host GI tract might possibly occur from the protein layer on the outside of the bacteria cell wall, known as s-layer (Sleytr and Beveridge, 1999). These proteins are hypothesized to be involved in cell protection, adhesion and trapping of some molecules including ions (Avall-Jaaskelainen and Palva, 2005). Frece *et al.* (2005) reported the functional role of the s-layer of *Lb. acidophilus* M92 which is tolerant to the pancreatin and pepsin existing in the small intestine and in gastric juice**,** respectively. The viability of *Lb. acidophilus* M92 cells without an S-layer was reduced in simulated gastric juice at low pH (2–3) and lost all viability within 1 h in simulated pancreatic juice with bile salts (3 g.L^{-1}).

Bacterial surface (S) layers

S-layers are crystalline arrays of proteinaceous subunits located at the outermost part of the cell wall in several species of the genus *Lactobacillus*, as well as in many other bacteria and *Archaea*. Lactobacillar S-layers are relatively small, 25 kDa to 71 kDa in size (Åvall-Jääskeläinen and Palva, 2005), whereas the molecular masses of S-layers in other bacterial species range up to 200 kDa (Sara and Sleytr, 2000). S-layers are normally 5-15 nm thick possessing a smoother outer surface compared with a more structured inner surface. Each S-layer forms a highly porous structure with pores of an identical size and morphology. Based on electron microscopy, the S-layer subunits are composed of lattices with oblique, square or hexagonal symmetry (Sara and Sleytr, 2000). The oblique lattice type was identified in the S-layers of *L. acidophilus* (Smit *et al*., 2001), *L. brevis* (Jakava-Viljanen *et al*., 2002) and *L. helveticus* (Lortal *et al*., 1992) and the hexagonal lattice type in *L. casei* and *L. buchneri* (Masuda and Kawata, 1985).

The S-layer subunits are non-covalently linked to each other and to the supporting cell envelope, and can be disintegrated into monomers by denaturing agents such as urea or GHCl, by metal-chelating agents or by cation substitution (Sara, 2001). In addition to peptidoglycan, the rigid cell envelope of lactobacilli is composed of secondary cell wall polymers (SCWP) such as teichoic acid, lipoteichoic acids, lipoglycans or neutral or acidic glygans (Navarre and Schneewind, 1999; Neuhaus and Baddiley, 2003).

S-layer protein encoding genes have been cloned and sequenced from two *L. brevis* strains (Vidgrén et al., 1992; Jakava-Viljanen *et al.,* 2002), two *L. acidophilus* strains (Boot *et al*., 1993; Altermann *et al*., 2005), one *L. helveticus* strain (Callegari *et al*., 1998), one *L. crispatus* strain (Sillanpää *et al*., 2000) and seven *L. gallinarum* strains (Hagen *et al*., 2005). Additionally, strains of *L. amylovorus, L. buchneri, L. kefir* and *L. parakefir* have also been shown to possess an S-layer (Boot *et al*., 1996b; Garrote *et al*., 2004), but their S-layer protein genes have not yet been sequenced. The presence of multiple S-layer protein genes seems to be quite common for lactobacilli.

Formerly, *L. johnsonii* and *L. gasseri* were proposed to lack an S-layer (Boot *et al*., 1996a), but Ventura *et al.* (2002) identified the protein called aggregation-promoting factor from these species as an S-layer-like protein, having amino acid composition and physical properties similar to lactobacillar S-layers. However, the formation of a regular lattice structure has not been demonstrated. Several different strains of these species were shown to possess two genes encoding surface proteins, one silent and one actively transcribed (Boot *et al*., 1995; Sillanpää *et al*., 2000). These bacteria may express alternative S-layer protein genes, most likely to adapt to different stress factors such as drastic changes in the environmental conditions for its cell protective function. In most of the characterized cases, the mechanism of S-layer variation is based on DNA rearrangements, but in *L. brevis* ATCC 14869, the variation of S-layer protein content took place by a unique mechanism involving activation of transcription by a soluble factor as a result of an environmental change (Jakava-Viljanen *et al*., 2002).

As S-layer proteins represent 10-15% of the total amount of proteins in *Lacto-bacillus* cells (Boot and Pouwels, 1996), their transcription and secretion mechanisms must be efficient and tightly regulated. Multiple promoters precede several S-layer genes (Boot and Pouwels, 1996), including S-layer genes of *L. acidophilus* (Boot *et al*., 1996b) and *L. brevis* (Vidgrén *et al*., 1992; Kahala *et al*., 1997; Jakava-Viljanen *et al*., 2002) and are likely to ensure efficient transcription of these genes. Despite their similar amino acid composition, such as a low content of cysteine and methionine as well as a high content of hydrophobic amino acids and hydroxyl amino acids, sequence similarity between the S-layer protein genes can only be found between genes of related species (Åvall-Jääskeläinen and Palva, 2005). This has also been demonstrated for the S-layer protein genes of lactobacilli by DNA–DNA hybridizations.

The gene and protein sequences of 10 S-layer proteins belonging to group A of the *L. acidophilus* complex are currently available in GeneBank (Hagen *et al*., 2005). A comparison of the amino acid sequences of these proteins has revealed two conserved regions, namely, an N-terminal signal sequence of ca. 30 amino acids, which directs secretion of the protein by the general secretory pathway and a C-terminal domain of approximately 123 amino acids that anchors the protein to the cell surface. A variable domain is located between the two conserved regions.

The functions of Slps are not yet completely revealed but it has been proposed that these structures protect the microbe from hostile environmental agents and aid in maintaining cellular integrity (Åvall-Jääskeläinen and Palva, 2005). Several lacto-bacillar S-layers have been identified as putative adhesins with an affinity for various tissue compartments or molecules. Slps protein of lactobacilli have been shown to confer tissue adherence, including *L. crispatus* and *L. acidophilus,* whose ability to bind to host epithelial cells is decreased after removal or disruption of the S-layer proteins (Sillanpää *et al*., 2000; Buck *et al*., 2005; Frece *et al*., 2005). The *L. brevis* ATCC 8287 SlpA protein has been shown to possess affinity for human intestinal epithelial cell lines, urinary bladder, endothelial cells and fibronectin (Hynönen *et al*., 2002). The ability of the receptor-binding region of SlpA to adhere to fibronectin was also confirmed with a lactococcal surface display system from the study of Åvall-Jääskeläinen *et al*. (2003).

Recently, by using surface plasmon resonance, SlpA was found to interact with fibronectin and laminin whereas its interaction with collagen and fibrinogen was found to be of much lower affinity (de Leeuw *et al*., 2006). In addition, the S-layer protein which was extracted from *L. helveticus* have also been shown to inhibit enterohaemorrhagic *E. coli* adhesion to host epithelial cells (Johnson-Henry *et al*., 2007).

Probiotic and the gastrointestinal (GI) immune system

All warm blooded vertebrates live in symbiotic association with a complex population of microorganism which inhabits their gastrointestinal (GI) tract. One of the benefits which the host obtains from this relationship is an enhanced resistance to infectious diseases (Fuller,1991). Thus conventional animals with a complete intestinal microflora are more resistant to infection than are germfree animals. The detailed basis for this difference is not known yet but it seemed certainly that changes in immunity are likely to be involved. The intestinal microflora stimulates mainly a local response at the intestinal wall. Mucosal immunity is an important component of the animal's immune status because it is responsible for the control of infections as well as inducing tolerance to environmental and dietary antigens.

Under natural conditions the level of immunity is adequate, but under domesticated conditions, stress factors cause deficiencies to occur which render the animal vulnerable infection. Under these circumstances, the supplementation with the live microorganisms to repair the deficiencies in the composition of the intestinal microflora can stimulate an immune response and restore the host resistance to infection. These supplements known as probiotics have been originally defined by Fuller (1989) as "live microbial food supplement which beneficially affects the host animal by improving its intestinal microbial balance". This definition includes not only preparations specifically designed to act as probiotics but also the traditional fermented products where the benefits conferred on the consumer may be incidental to their primary role as a tasty and nutritious food. By far the most commonly used microorganisms in probiotic products are the LAB and it is important to know how these LAB affect the immune status of the consumer. The probiotics approach is attractive because it is a reconstitution of the natural condition; it is the means of repairing a deficiency rather than the addition of any foreign substances to the body which may have the toxic consequences or, as in the case of antibiotics induce the resistance and compromise subsequent therapy.

Besides its role as a barrier to potential pathogens, the intestinal flora is also assumed to protect the host by initiating the immunological defense mechanisms. The scientific attention is increasingly being focused on the mechanism(s) of the innate immune response of the host to various components of the autochthonous microbiota, including lactobacilli and bifidobacteria. In the selection of LAB by their immune modulation capacity, helps to know not only the effect which they have on the muco-sal immune system, but the specific use to which these oral vaccine vectors are being put. Although there are some reports of the protection of humans and animals against diseases such as microbial infections and cancer, more works need to be done on the use of LAB for therapeutic purpose.

Recent trends in the marketing of food products have clearly demonstrated a tendency towards claiming specific or general health benefits from the consumption of special food formulations, probiotic bacteria or medical plants. Although there are some reports of the protection of humans and animals against diseases or infections, but still more works need to be done on the use of probiotics for therapeutic purpose. This new field of nutraceutical and health benefits that may ensure from their consumption can now be subjected to strict immunological analysis. It may now be the time to determine whether immunological parameters can be used as the indicators of healthy and well-being. Certainly, there is available a range of immunological tools and techniques, as well as animal models, to study the effects of various dietary supple-ments on the immune response. The discovery that probiotics can stimulate an immune response (Perdigon *et al*., 2001) provides a scientific basis for some of the observed probiotic effects. This is an important function of probiotic preparations and a rapidly developing area of research. However, the scientific results and their practical implications should be carefully reviewed.

Immunological structures and functionalities of the gastrointestinal tract (Kagnoff,1993)

The immune system, made up of special cells, proteins, tissues, and organs, defends human or host against germs and microorganisms every day. In most cases, the immune system does a great job of keeping the host healthy and preventing infections. But sometimes problems with the immune system can lead to illness and infection. The immune system is the body's defense against infectious organisms and other invaders. Through a series of steps called the immune response, the immune system attacks organisms and foreign substances that invade host body systems and cause disease. The immune system is made up of a network of cells, tissues, and organs that work together to protect the body. The cells that are part of this defense system are white blood cells, or leukocytes. They come in two basic types, which combine to seek out and destroy the organisms or foreign substances that cause disease.

Leukocytes are produced or stored in many locations throughout the body, including the thymus, spleen, and bone marrow. For this reason, they are called the lymphoid organs. There are also clumps of lymphoid tissue throughout the body, primarily in the form of lymph nodes,that house the leukocytes. The leukocytes circulate through the body between the organs and nodes by means of the lymphatic vessels. Leukocytes can also circulate through the blood vessels. In this way, the immune system works in a coordinated manner to monitor the body for germs or substances that might cause problems.

The two basic types of leukocytes are:

1. Phagocytes: cells that chew up invading organisms

2. Lymphocytes: cells that allow the body to remember and recognize previous invaders and help the body destroy them

A number of different cells are considered phagocytes. The most common type is the neutrophil, which primarily fights bacteria. If doctors are worried about a bacterial infection, they might order a blood test to see if a patient has an increased number of neutrophils triggered by the infection. Other types of phagocytes have their own jobs to make sure that the body responds appropriately to a specific type of invader.

There are two kinds of lymphocytes: the B lymphocytes and the T lymphocytes. Lymphocytes start out in the bone marrow and either stay there and mature into B cells, or they leave for the thymus gland, where they mature into T cells.

B lymphocytes and T lymphocytes have separate jobs to do: B lymphocytes are like the body's military intelligence system, seeking out their targets and sending defenses to lock onto them, whereas T cells are like the soldiers, destroying the invaders that the intelligence system has identified.

Antigens are foreign substances that invade the body.When an antigen is detected, several types of cells work together to recognize and respond to it. These cells trigger the B lymphocytes to produce antibodies, specialized proteins that lock onto specific antigens. Antibodies and antigens fit together like a key and a lock.

Once the B lymphocytes have produced antibodies, these antibodies continue to exist in the host's body, so that if the same antigen is presented to the immune system again, the anti-bodies are already there to do their job and recognize the antigen by the memory B cells. So we can use the immunizations to prevent getting certain diseases. The immunization introduces the body to the antigen in a way that doesn't make a person sick, but it does allow the body to produce antibodies that will then protect that person from future attack by the germ or substance that produces that particular disease.

Although antibodies can recognize an antigen and lock onto it, they are not capable of destroying it without help. That is the job of the T cells. The T cells are part of the system that destroys antigens that have been tagged by antibodies or cells that have been infected or somehow changed. (There are actually T cells that are called "killer T cells.") T cells are also involved in helping signal other cells (like phagocytes) to do their jobs. Antibodies can also neutralize toxins (poisonous or damaging substances) produced by different organisms. Lastly, antibodies can activate a group of proteins called complement that are also part of the immune system. Complement assists in killing bacteria, viruses, or infected cells.

Innate Immunity

Innate (or natural) immunity, a type of general protection that humans have and naturally get from mother. Many of the germs that affect other species don't harm us. For example, the viruses that cause leukemia in cats or distemper in dogs don't affect humans. Innate immunity works both ways because some viruses that make humans ill such as the virus that causes HIV/AIDS don't make cats or dogs sick either.Innate immunity also includes the external barriers of the body, like the skin and mucous membranes (like those that line the nose, throat, and gastrointestinal tract), which are our first line of defense in preventing diseases from entering the body. If this outer defensive wall is broken (like when we get a cut in our body), the skin attempts to heal the break quickly and special immune cells on the skin attack invading germs.

Adaptive Immunity

Second kind of protection called adaptive (or active) immunity. This type of immunity develops throughout our lives. Adaptive immunity involves the lymphocytes and develops as children and adults are exposed to diseases or immunized against diseases through vaccination.

Passive Immunity

Passive immunity is "borrowed" from another source and it lasts for a short time. For example, antibodies in a mother's breast milk provide an infant with temporary immunity to diseases that the mother has been exposed to. This can help protect the infant against infection during the early years of childhood

Role of intestinal immunity

The intestinal or mucosal immunity is the local immunity which has the intestine as the largest immunological organ in the body. It contains 70-80% of all the Immunoglobulin A (IgA) producing cells which exceeds the total production of all other immunoglobulin classes in the body.The most important factor for local immunity is the cellular migration of specific activated B and T cells from the Peyer's patches (PP) which are the inductive sites to the distant mucosal sites such as respiratory, genitourinary tract and various secretory glands. They also return to the intestinal lamina propria and epithelium (Phillips-Quagliata and Lamm, 1990). The specific migration of these immune cells is the basis of the common mucosal immune system (Mestecky *et al*., 1994).

The structures and functions of the intestinal mucosa are designed to supply different biologically active molecules such as gastrointestinal peptides, enteroglucan, peptides, hormones, prostaglandins, growth factors and mucus produced for the goblet cells. And the functioning of the gut mucosal immune system requires a complex network of signals with multiple interactions between commensal and foreign antigens and the eukaryotic cells. These include epithelial cells, macrophages, dendritic cells, and other cells that belong to the nonspecific barriers, mucus-producing cells such as goblet cells, and Paneth cells, which secrete antimicrobial peptides and produce cryptidins or defensins (Levis *et al*., 1992; Wright, 1995)

Intestinal peristalsis is another important mucosal mechanism of defense for the host preventing the colonization of the intestinal by foreign microorganisms ingested with the food. The intestinal epithelium is also a barrier for the antigents present in the daily diet. This barrier is maintained by the tight junction at the apical site of the epithelial cell, by phospholipids and proteins that cover the microvillus of these cells. The lamina propria of the intestine is rich in immune cells such as lymphocytes, plasma cells, neutrophils, eosinophils, macrophages and mast cells. The lymphocytes are associated not only with the lamina propria but also with the intestinal epithelium: intraepithelial lymphocytes (IEL). These cells are active in maintaining the hypo-responsiveness at the intestinal level.

The mucosal epithelial cells are crucial in coordinating the defense mechanisms. They respond to environmental signals by releasing chemokines and cytokines that recruit the immune cells from both the innate and adaptive immune responses. These recruited immune cells can in turn act upon the epithelial cells, stimulating the release of cytokines. This response must not be triggered by harmless intestinal commensal bacteria, and the inflammatory response must be controlled. The particular characteristics of soluble, particulate antigens and pathogens will affect the gut immune response in relation to the way that they initiate the interaction with the immune system. At least three different routes exist for the uptake of luminal antigens dendritic cells, specialized M cells from the Peyer's patches, and individual M cells found in the villous epithelium (Jang *et al*., 2004.). The anatomical location of the immune cells from the innate response (macrophages and dendritic cells) and the way by which these cells acquire antigens are crucial in determining the nature of the subsequent responses. Thus, the immune response induced can be the result of uptake of antigens by transepithelial sampling involving dendritic cells or by dendritic cells present in the lamina propria of the intestine or by M cells from Peyer's patches or from the intestinal villous. (Kagnoff, 1993)

When an antigen is orally administered the main immune response induced is in the form of hypo-responsiveness. It is known as oral tolerance and increase in the inflammatory immune response that can lead to an enhancement in intestinal permeability.In general, soluble antigens give a strong oral tolerance. Particulate antigens, especially bacteria or virus, favor the induction of the immune response. The hypo-responsiveness is related to the dose of the antigen administered. Lower or higher doses of antigens can induce oral tolerance through suppression of cytokines such as interleukin 10 (IL-10) or transforming growth factor β (TGF-β) or by clonally deletion (Salminen *et al*., 1996). However, oral tolerance can be abrogated and an immune response induced.

The entry of the antigens by the oral route is essential to induce a mucosal immune response. This fact was determined in the germfree mice receiving a diet free of antigen(s) where it was demonstrated that the presence of microflora increased the number of IgA secreting cells in the lamina propria of the intestine, mesenteric node or in the bone marrow (Hoojkaas *et al*., 1984; Bos *et al*., 1987). In similar germfree studies with antigen free diet the levels of the IgM, IgG and IgA in the serum were also diminished (Wostmann and Pleasant, 1991).The level of IgA is mainly dependent on the presence of microflora, whereas for IgG the diet is the more important factor. It would seem that the IgM is not influenced by environmental antigens.

The lipopolysaccharide (LPS) originating in the normal enteric microflora is involved in the maturation of the T cell precursors of the T suppressor cells responsible for oral tolerance at the intestinal level. This was demonstrated in germfree mice that are unable to induce oral tolerance, but this can be reconstituted by the intestinal colonization with the enteric microflora by LPS ingestion (Michalek *et al*., 1983). It is also known that the maturation of lymphocytic function is controlled by the normal intestinal microflora and by the non-colonizing, non-pathogenic exogenous bacteria that pass through the gastrointestinal tract. The maintainence of a constant number of IgA secreting cells in the intestine, in normal conditions, has been attributed to the Gram negative microflora specially *Bacteroides spp*. It was also demonstrated that the muramyl dipeptide (MDP) of the Gram positive cell wall activates immune cells such

as macrophages, and B and T lymphocytes (Lise and Audibert, 1989) associated with the intestinal mucosa.

Development of antibodies

Antibodies start to develop between 2-5 days after the onset of clinical disease in virulent infections and 6-10 days after infection with a virulent strain, although with lower infectious doses a delay of up to 17 days may occur.

Early antibodies can also mean antibodies of a certain class or biological property, for example IgM or Interacting protein (IP) antibodies (primary antibody), which decline rapidly in titer after the primary infection, despite recognizing antigens that also induce persistent antibodies of different classes and biological properties, such as IgG or virus-neutralizing (VN) antibodies. Since the immunoglobulin (Ig) class often determines the biological properties of an antibody, these two Ig are frequently closely linked.

Lymphocytes recognize the antigens by using antibodies and T cell receptors. Antibodies are a class of proteins made by B cells (Finkelman,1995) (B cells or B lymphocytes use membrane-bound antibodies that act as receptors for foreign antigens). When membrane-bound antibody receptors on B cells recognize their antigens, they bind to them and trigger the synthesis of antibodies.

The spleens, is highly organized and is a reservoir for immune cells. It plays an important role in response to pathogens. The spleen contains a circulatory and lymphatic system allowing access to it either through the blood or lymph. Cells in the spleen organize around the blood vessels into two tissues: red pulp and white pulp. Red pulp contains mainly red blood cells, and white pulp is made up predominantly of lymphocytes. The white pulp focuses tightly around the arterioles of the spleen while the red pulp fills the rest of the interstitial space. The location of various lymphocytes in the white pulp further differentiates it. T cells are found near the arterioles and further away are areas of B cells. The spleen is a major area for B cells to congregate

in the body, where they wait to be activated by antigens. During an illness, activated lymphocytes will be released from the spleen to fight the infection.

Immunoglobulin G (IgG) is the most abundant circulating antibody, making up 80% of the total antibodies and 75% of that found in serum. It contains a single antibody protein complex, with two heavy chains and two light chains. IgG is the second type of antibody synthesized in response to an infection and is the only antibody that can pass through the wall of small blood vessels to access antigens present in the extracellular spaces. This immunity will protect a baby for the first 6-12 months of its life, allowing it time for its own immune system to mature. IgG is particularly effective at attacking extra-cellular viruses and protein toxins helps to prevent the systemic spread and facilitates recovery from many infections.

Immunoglobulin A (IgA) is present in serum, mucus, saliva, tears, sweat and milk. Two subclasses with different heavy chains are made, IgA1 and IgA2. IgA1 is synthesized in the bone marrow and makes up most of the serum IgA. IgA2 is synthesized by B cells present under the mucosal surface. The antibodies are synthesized as dimers that are joined by a short J chain polypeptide. As the secreted IgA2 passes through the intestinal epithelium, a second secretory protein attaches. Dimerization and binding of the J and secretory proteins make IgA more resistant to proteases present in the environments that it protects. IgA in breast milk interferes with the colonization of the GI tract by harmful microorganisms in the first few months of life. The mother's IgA in the GI tract of newborns keeps these pathogens at low populations, preventing them from causing serious disease, but still allowing the stimulation of the infant's own immune system. The newborn thus develops its own immunity while being partially protected by the mother. IgA molecules do not activate the classical complement pathway, but may activate the alternative complement pathway.

Immunoglobulin M (IgM) A basic antibody that is present on B cells. It is the primary antibody against A and B antigens on red blood cells. IgM is by far the physically largest antibody in the human circulatory system. For clinical significance: IgM appear early in the course of an infection and usually do not reappear after

further exposure. These two biological properties of IgM make it useful in the diagnosis of infectious diseases. IgM in normal serum is often found to bind to specific antigens, even in the absence of prior immunization. For this reason IgM has sometimes been called a "natural antibody". This phenomenon is probably due to the high avidity of IgM that allow it to bind detectably even to weakly cross-reacting antigens that are naturally occurred. IgM antibodies are mainly responsible for the clumping (agglutination) of red blood cells if the recipient of a blood transfusion receives blood that is not compatible with their blood type.

Immunoglobulin E (IgE) is a class of antibody that has only been found in mammals. It plays an important role in allergy. IgE has also been implicated in immune system responses to most parasitic worms like *Schistosoma mansoni*, *Trichinella spiralis*, and *Fasciola hepatica*, and may be important during immune defense against certain protozoan parasites such as *Plasmodium falciparum*. IgE levels in a normal individual blood level are about 75 ng/ml and it is capable of triggering the most powerful immune reactions.

Lymphocytes make antibodies and regulate the immune system

The immune system has 3 important properties:

1. It is specific.

2. It remembers a previous exposure to an antigen from memory cells.

3. It tolerates the presence of its own macromolecular components.

The spleen is a repository for immune cells. It plays an important role in response to pathogens. The spleen contains a circulatory and lymphatic system allowing access to it either through the blood or lymph. Cells in the spleen organize around the blood vessels into two tissues: red pulp and white pulp. Red pulp contains mainly red blood cells, and white pulp is made up predominantly of lymphocytes. The

white pulp focuses tightly around the arterioles of the spleen while the red pulp fills the rest of the interstitial space. The location of various lymphocytes in the white pulp further differentiates it. T cells are found near the arterioles and further away are areas of B cells. The spleen is a major area for B cells to congregate in the body, where they wait to be activated by antigens. During an illness, activated lymphocytes will be released from the spleen to fight the infection.

Lymphocytes are the other major type of white blood cells, it is important in the immune system, and they work with phagocytic cells to combat infections. Lymphocytes, with the help of antigen-presenting cells, are part of what is called the adaptive immune system. This system has three important features that all depend on lymphocytes.

1. Specificity: B cells/antibodies or T cells react specifically with the antigen that activated them and they will generally not react with any other, differently structured antigens. These reactions are as specific as the binding of substrate to the active site of an enzyme; in fact, antigen-antibody reactions share many features with enzyme-substrate binding.

2. Memory: The immune system remembers an antigen after exposure to it. During the first immunological response to an antigen, lymphocytes capable of responding to that antigen (due to their specific membrane receptors) increase in number and remain present after the antigen is removed. During a second challenge by the same antigen, the immune response of this larger population of lymphocytes produces more antibodies and activates T cells faster, resulting in the swift removal of the entity producing the offending antigen. This type of response is referred to as a memory or secondary response.

3. Tolerance: A healthy mammal does not react to its own macromolecular components, all of which are potentially antigenic. The animal is said to be tolerant of its self antigens. The cell achieves this tolerance by eliminating lymphocytes that react to self.

B lymphocytes - humoral immunity

1. B lymphocytes or B cells are the class of immune cells that synthesize antibodies and are responsible for humoral or antibody-mediated immunity. Antibodies react with antigens and the immune system has a virtually unlimited capacity to create antibodies that will react with millions of potential antigens.

Each B cell contains about 100,000 antibody molecules (IgD or IgM) on its surface. The binding sites of these antibodies are identical so that they all react specifically to the same anti-genic structure, but they are different from the surface antibodies on other B cells. These membrane-bound antibodies serve as receptors for the antigen and contain two additional polypeptides that span the cellular membrane and play a role in transmitting the binding of antigen to antibody to the cytoplasm of the cell. Binding of an antigen by several different antibody receptors on the surface of the cell causes the receptors to cluster in a small area. This clumping of membrane bound antibody molecules then activates a signaling cascade that eventually results in the transcription of genes and thus the production of proteins important for B cell activation.

The activated B cell then goes through a process of rapid division (termed clonal expansion) where many more copies of the cell are made. Some of these cells differentiate into plasma cells that produce large amounts of antibody. The antibody molecules produced specifically react with the antigen that caused the initial active-tion of the B cell. A fraction of the cells from the clonal expansion remain as undif-ferentiated B memory cells that are capable of reacting with a second antigen challenge at a later time. If a future challenge does appear, this secondary response is much more rapid, normally inactivating the pathogen before a detectable infection appears.In mammals, B cells mature in the bone marrow and Mucosa-associated lymphoid tissue (MALT) in the intestines and then travel in the bloodstream eventually settling in immune tissues throughout the body with high concentrations in the lymph nodes and spleen.

2. Antibody production.

The production of antibody involves 3 distinct phases:

2.1 Induction phase:

Antigen reacts with specific T and B cells receptor.

2.2 Expansion and Differentiation phase:

Induced lymphocyte clones proliferation and mature to a functional stage of cells (such as some Antigen receptor cells matured to the antigen effective cells)

2.3 Effector phase:

T cells exert biological effects either: Independently or Through the action of macrophages, complement, other non-specific agents

Humoral immune response is mediated by immunoglobulin (Ig) which is produced by B lymphocytes and protect the host from pathogenic infection or antigen alteration. IgA IgG and IgM are 3main Immunoglobulin which were involved in the immune defense system. IgG and IgA function mainly against pathogens and viruses, inhibit antigens absorption in small intestine and provide long term protection from infection. IgM is the first produced Ig in the immune response, and may leads to the other type of Ig production. Ig production by B lymphocytes is class-specifically regulated by cytokines secretion such as INF-γ, IL-4 and IL-6 which is promote IgA production by differentiated plasma cells.

B lymphocytes respond to the presence of an antigen by differentiating into plasma cells and manufacturing antibodies that react with the antigen that activated them. The activated B cell then goes through a process of rapid division (clonal expansion) where many more copies of the cell are made. Some of these cells differentiate into plasma cells that produce large amounts of antibody. The antibody molecules produced specifically react with the antigen that caused the initial activation of the B cell. A fraction of the cells from the clonal expansion remain as undifferentiated B memory cells that are capable of reacting with a second antigen challenge at a later time. If a future challenge does appear, this secondary response is much more rapid, normally inactivating the pathogen before a detectable infection appears as shown in Figure 1.

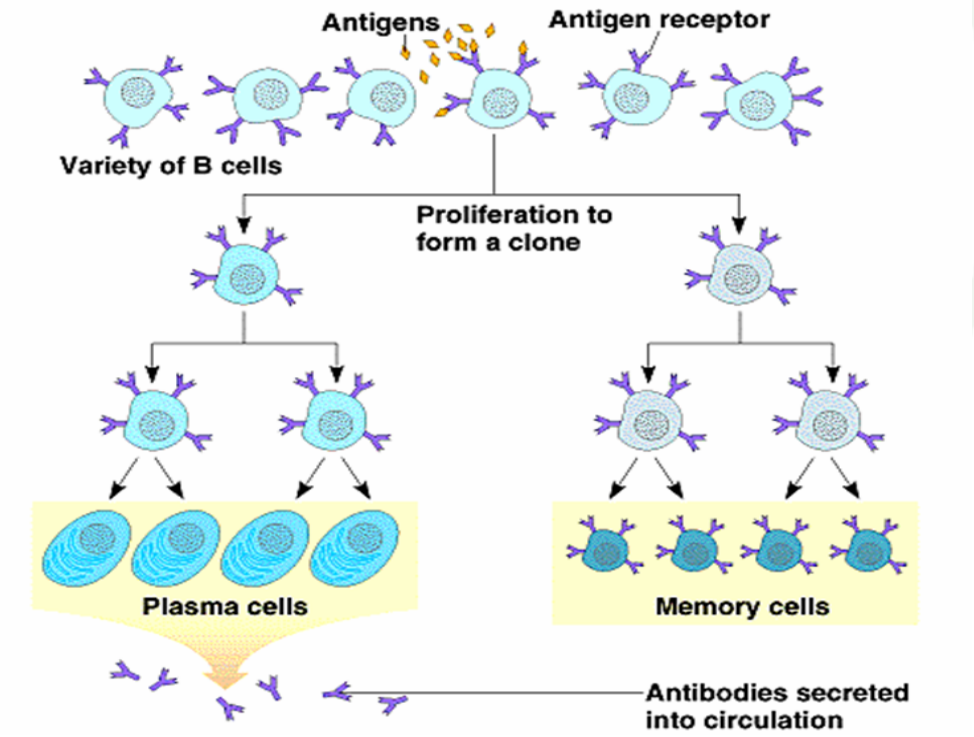

Figure 1 Antigens responding of B lymphocytes (clonal expansion)

Source: The University of Arizona (2000)

T lymphocytes or T cells

a central role in cell-mediated immunity. They can be distinguished from other lymphocyte types, such as B cells and Natural killer cells (NK cells) by the presence of a special receptor on their cell surface called the T cell receptor (TCR).

1. Helper T cells (T_H cells) are the "middlemen" of the adaptive immune system. Once activated, they rapidly divide and secrete small proteins called cytokines that regulate or assist the immune response. Depending on the cytokine signals received, these cells differentiate into T_{H1}, T_{H2}, T_{H17}, or one of other subsets, which secrete different cytokines.

2. Cytotoxic T cells (T_C cells, or CTLs) destroy virally infected cells and tumor cells, and also implicated in transplant rejection. These cells are also known as $CD8^+$ T cells, since they express the CD8 glycoprotein at their surface. Through interaction with helper T cells, these cells can be transformed into regulatory T cells, which prevent autoimmune diseases such as experimental autoimmune encephalomyelitis.

3. Memory T cells are a subset of antigen-specific T cells that persist long term after an infection has resolved. They quickly expand to large numbers of effector T cells upon re-exposure to their relative antigen(s), thus providing the immune system with "memory" against past infections.Memory cells may be either $CD4^+$ or $CD8^+$.

4. Natural Killer T cells (NKT cells) are a special kind of lymphocyte that bridges the adaptive immune system with the innate immune system. Unlike conventional T cells that recognize peptide antigen presented by major histocompatibility complex (MHC) molecules, NKT cells recognize glycolipid antigen presented by a molecule called CD1.Once activated, these cells can perform functions ascribed to both T_h and T_c cells (such as cytokine production and release of cytolytic/ cell killing molecules).

T cell Maturation

1. Maturation of T Cells in the Thymus

requires two steps:

First, the immature, but CD4 or CD8 positive T cells are exposed to cells in the thymus, which have class I and class II histocompatibility antigens on them. T cells which are able to bind to one or the other of these antigens are protected, whereas the others die.

Second, the cells that survive the above selection process are exposed to self antigens that have been taken up and associated with either class I or class II MHC antigen. Those that bind at this stage die (apoptosis).The cells that survive are those that recognize non-self antigens associated with MHC antigens. After a little more maturation, they exit the thymus to perform their role in immune responses.

An immunocompetent but as yet immature B-lymphocyte is stimulated to maturity when an antigen binds to its surface receptors and there is a helper T cell nearby (to release a cytokine). This sensitizes or primes the B cell and it undergoes clonal selection, which means it reproduces asexually by mitosis. Most of the families of the clones become plasma cells. These cells, after an initial lag, produce highly specific antibodies at a rate of as many as 2000 molecules per second for 4-5 days. The other B cells then become long-lived memory cells. (Figure 2)

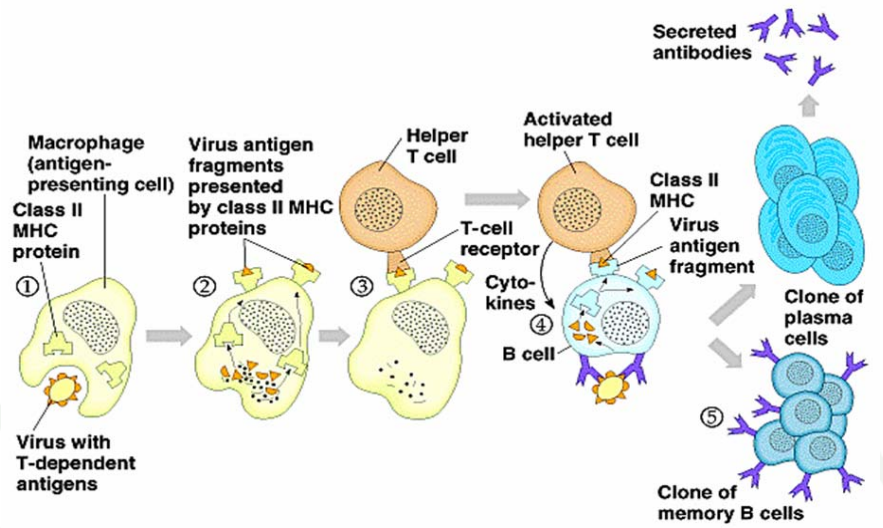

Figure 2 Cell mediated immune response

Source: The University of Arizona (2000)

2. The gastrointestinal defense ecosystem of the host

The host is protected from the attack by potentially harmful microorganisms by the physical and chemical barriers created by the gastrointestinal epithelium. The cells lining the gastrointestinal epithelium and the resident microbiota are two part-ners that properly and/or synergistically function to promote an efficient defensive of the immune system of the host. The gastrointestinal cells that make up the epithelium, provide a physical barrier that protects the host against the unwanted intrusion of microorganisms into the gastrointestinal microbiota, and also protect the host against to the penetration of harmful microorganisms which can hijack the cellular molecules and signaling pathways of the host to become pathogenic.

The gastrontestinal mucosa has a surface coating of mucus that is secreted by the specialized goblet cells. There are two secretory pathways in intestinal mucin secreting polarized cells, the first of which is the regular vesicular constitutive pathway of mucin exocytosis, in which no storage occurs since the small vesicles

transporting the mucins through the constitutive pathway are guided directly to the cell surface by microtubules and undergo immediate exocytosis of their contents. The second pathway for mucin exocytosis involves the packaging and storage of mucins in large vesicles, from which mucin release is regulated by specific stimuli involving signaling molecules. The entrapment of bacteria within the mucus which contains secretory immunoglobulin (s), coupled with peristalsis, results in the rapid expulsion of bacteria from the intestine.

Together with the innate defenses of the host, the constituents of the intestinal immune system of defense reinforce the barrier function of the GI epithelium. Cross-talk mobilizes the cells lining the intestinal epithelium and intestinal-associated immune cells, allowing the host to sense the microbial environment in order to promote a strong defense response when required, by releasing signaling molecules such as cytokines and chemokines, that leads to the recruitment of leukocytes, and initiates the attraction of immune cells.

In innate mucosal immunity, the host defensive mechanisms are activated mainly as a result of the specific recognition of pathogen-associated molecular patterns (PAMPs). In contrast, the endogenous bacterial species of the microbiota all share signature molecules, known as microbe-associated molecular patterns (MAMPs). Epithelial and monocytic cells are specialized cells that sense the environment within the intestinal by means of their pattern-recognition receptors (PRR) (Aderem *et al*.,2000; Didierlaurent *et al*.,2002).

One of the defense mechanisms present in the gastrointestinal tract of the host is the first line of chemical defense involving the production of antimicrobial peptides. This chemical antimicrobial system of defense functions in the mucosa of the gastrointestinal tract, airways, gingival, cornea, reproductive tract and urinary tract. Antimicrobial peptides, first identified in the skin and subsequently in polymorphonuclear neutrophils (PMNs) and macrophages, are produced in the intestine by Paneth cells located in the basal portion of intestinal crypts, underlying the zone of intestinal epithelial cell division. By releasing preformed antimicrobial peptides

known as defensins were first identified by Ouellette *et al*. (1989) in mouse small intestinal cells, and subsequently these antimicrobial peptides have been shown to be produced by human intestinal cells.

Interestingly, recent observations indicate that in response to attack by pathogenic bacteria, the host engage its first line of chemical defence by increasing the production of antimicrobial peptides, such as the α- and β-defensins. In parallel, it is significant that enteric pathogens have developed sophisticated strategies to survive in the gastrointestinal tract by evading the innate mucosal defences. Both *Salmonella* and *Shigella* are able to down regulate host antimicrobials by decreasing the expression of defensins in colonic epithelial cells, colonic human biopsy specimens and in infected mice. (Ayabe *et al*.,2000) .

3. The resident microbiota as a partner of the host GI defense ecosystem

One of the basic physiological functions of the resident microbiota in the intestinal is that it functions as a microbial barrier against microbial pathogens. The mechanisms by which species of the microbiota exert this barrier effect remain largely to be determined. The intestinal microflora can modulate bone marrow and spleen macrophage cytokines production in the different manners.

Intestinal microflora enhancing IL-12 production in the spleen is also potentially important, since this cytokine is implicated in determining the relative levels of Th1 and Th2 responses, and plays an important role in defending the host against intracellular microorganisms. Hudault *et al*.(2001) have shown that resident *E. coli* had a barrier effect when colonizing the intestinal of gnotobiotic C3H/He/Oujco mice orally infected by a lethal strain of *S. typhimurium*, but the mechanism of action remains to be determined. In addition, it has been established that *E. coli* participates in anti-bacterial defense by producing large proteins named colicins that function by forming pores in the cell membrane permeable to nuclease activity against DNA, rRNA and tRNA targets.

Experimental evidence and properly-conducted clinical trials has been published, particularly concerning the effectiveness of selected lactobacilli and bifidobacteria strains in the prevention and treatment of infectious bacterial and viral diarrhea, *Helicobacter pylori* gastroenteritis and the urovaginal infections (Sullivan and Nord,2002).

Some of the probiotics health benefits which have been claimed, include the following: improvement of the normal microflora, prevention of the infectious diseases and food allergies, reduction of serum cholesterol, anticarcinogenic activity, stabilization of the gut mucosal barrier, immune adjuvant properties, alleviation of the intestinal bowel disease symptoms and improvement in the digestion of lactose in intolerant hosts (Perdigon *et al*.,2001).

The ability of probiotics to prevent or reverse several pathological conditions by stimulating the host immune system and all the scientific evidence of immune system activation by probiotics indicate that the ability to generate an immune response should be included in the probiotic definition and may be defined as follows: “live microorganisms, that when included in foods can influence the composition and activity of the gut microbiota, modulate the inflammatory response, improve the nonspecific intestinal barrier, and reinforce or modulate the mucosal and the systemic immune responses.” This definition ascribes to the probiotic microorganisms in the dietary supplement the potential for the prevention of infections, tumor growth, or other systemic pathologies, including effects in mucosal sites distant from the gut, such as the bronchus, mammary glands, the urogenital tract and some probiotics which is commonly used in allergy also showed in Table 3. However, for the best use of these microorganisms, the mechanisms by which they work should be clearly understood. The selection of an appropriate probiotic strain for its inclusion in a probiotic preparation should be made on the basis of its capacity to induce an improved gut immune response without modification of the intestinal homeostasis.

Table 3 Probiotics used in allergy

Organism	Allergic condition
Lactobacillus rhamnosus GG	Asthma
	Rhinitis
	Eczema
	Food allergy
Bifidobacter lactis	Atopic eczema
Lactobacillus paracasei	Allergic rhinitis
Lactobacillus reuteri	Atopic dermatitis

Source: Furrie (2005)

To achieve this task, probiotic strains should have the following properties:

1. High cell viability, resistant to low pH and bile acids in the gastrointestinal condition

2. Ability to persist in the intestine even if the probiotics strain cannot colonize the gut (continuous administration may be necessary)

3. Adhesion to the gut epithelium to cancel the flushing effects of peristalsis.

4. Be able to interact or to send signals to the immune cells associated with the gut.

Immune modulation by LAB probiotics

Beside its role as a barrier to potential pathogens, the intestinal flora is also thought to protect the host by priming the immunological defense mechanisms. Scientific attention is increasingly being focused on the mechanism(s) of the innate immune response of the host to various components of the autochthonous microbiota,

including lactobacilli and bifidobacteria. The interdependency between the epithelium and adjacent lymphoid cells is such that the epithelium is considered to have a central role in the mucosal immune system and is an active participant in both the afferent and efferent limbs of the mucosal immune response. The molecular crosstalk between the epithelium and lymphocytes is just one aspect of a more complex network of intercellular signaling within the intestinal mucosa and upon which the integrity of the mucosa is dependent. Lympho-epithelial communication is bi-directional, and also mediated in large part by shared ligands and receptors. The chemical messengers involved include cytokines, growth factors, local hormones. Disruption of any aspect of the mucosal microenvironment is generally associated with impaired mucosal defense and inflammation. (Gaskins, 1996)

Among the mechanisms suggested by which the selected *Lactobacillus* and *Bifidobacterium* strains may act against microbial pathogens, recent experimental reports have focused on immune stimulation and/or modulation, and evidence is accumulating that these selected strains could influence the immune response in a strain-dependent manner (Servin, 2004). The mechanisms that lactic acid bacteria use to affect the immune system and produce immune stimulating effects are probably, LAB alone or their products are absorbed by M-cells and transported to deeper lying lymphatic follicles where they are checked by immune-competent cells (Brandtzaeg *et al*., 1989). Eventually, LAB and their products are transported for immune analysis to systemic lymphatic tissues-mesenteric lymph nodes or the spleen. LAB were found in Peyer's patches after 6-12 hrs and in mesenteric lymph nodes 48 hours after ingestion.

The gastrointestinal tract is one of the places most exposed to pathogenic microorganisms and non-viable materials including antigens and carcinogens. Lymphatic tissue associated with the intestinal plays a crucial role in the local and systemic immune response and mediates the migration and homing of the activated cells from the intestinal to other sites of the body (Ernst *et al*., 1988). Foreign microorganisms and cell fragments can penetrate the intestinal wall by translocation through the epithelial layer or through Peyer's patches.

Indigenous intestinal bacteria including lactobacilli are able to cross the intestinal mucous layer and they can survive in the spleen or in other organs for many days where they stimulate phago-cytic activity (Deitch *et al*., 1990). The proliferative responses of spleen cells to concavalin A (ConA) and lipopolysaccharide (LPS) were significantly enhanced in mice given different LABs. These cells also produced significantly higher amounts of interferon-γ (IFN-γ) in response to the stimulation with ConA (Gill *et al*., 2000).

The application of some strains of LAB in the host immune modulation

The increased function of phagocytic cells depends on the species or strain of bacteria. The secretion of lysosomal enzymes in macrophages by mice fed fermented milk with *Lactobacillus casei* was more effective compared with the feeding of fermented milk containing *L. acidophilus* and *Streptococcus termophilus* (Perdigon and Alvarez, 1992). The differences in the cell wall composition are probably responsible for the different action of several lactobacilli strains. Moreover, the strains able to survive in the gastrointestinal tract, able to adhere to the intestine mucous membrane, and able to persist at the critical limit are more effective in stimulating phagocytic cells (Schiffrin *et al*., 1997). It is remarkable that fermented products expressed better stimulation of the non-specific immune system probably due to immune-active peptides formed during fermentation from milk proteins (Fiat *et al*., 1993).

A viable strain of *L. plantarum* applied intraperitoneally to a mouse stimulated the delayed type of hypersensitivity and non-viable bacteria had an adjuvant effect. In another study, intraperitoneally applied *L. casei* activated macrophages (phagocytic capacity and enzyme activity) and natural killer cells (Kato *et al.*, 1984). Subcutaneous inoculation of *L. casei* stimulated the production of specific antibodies against Pseudomonas antigens by increasing the circulating IgM antibodies (Saito *et al*., 1983).

Oral application of lactobacilli led to macrophage and lymphocyte stimulation and to the release of the enzymes from murine peritoneal macrophages. From the subsequent studies of Perdigon *et al*. (1990) showed that per orally inoculated *L. casei* activated cells in the intestinal associated lymphatic tissue, which led to the produc-tion of significantly higher anti-salmonella secretory IgA (sIgA) titres in the intestinal fluid. The dose and the length of application of lactobacilli were shown to be very important Perdigon *et al*. (1991). Only mice treated with *L. casei* for 3 consecutive days obtained the thera-peutic effect of the lactobacilli application that correlated with sIgA antibody level. These titres also depended on the number of bacteria applied and *L. casei* was considered as an oral adjuvant in the prevention of intestinal infections.

According to Herich, *et al*. (1999) the 10-day administration of *L. casei* to gnotobiotic piglets experimentally infected with *E. coli* in the same dose was more efficient than the 3-day application in the stimulating non-specific immune functions.

L. acidophilus and *L. casei* strains potentiated IL-6 and IL-12 production by peritoneal cells whereas *L. acidophilus* up regulated IFN-γ and nitric oxide (NO). In contrast, *L. helveticus*, *L. gasseri, L. reuteri,* and *Bifidobacterium* strains attenuated the production of IL-6, IFN-γ and NO by peritoneal cells. TNF-γ was not detectable in peritoneal cultures. None of the bacteria altered ex vivo production of cytokines or NO by Peyer's patch or spleen cell cultures (Tejada *et al*., 1999).

The effect of in vitro exposure to heat-killed cells of *Bifidobacterium* , *L. acidophilus*, *L. bulgaricus*, *L. casei*, *L. gas*seri, *L. helveticus*, and *L. reuteri* strains on cytokine and nitric oxide production has been examined in the RAW 264.7 macro-phage cell line and in murine cultures composed of peritoneal, spleen, and Peyer's patch cells. Both the cell wall and cytoplasmic fractions of lactobacilli were able to stimulate cloned macrophages to produce significant amounts of TNF-γ,IL-6, and nitric oxide. He *et al*.(2002) found that *B. adolescentis* and *B. longum*, known as adult-type *bifidobacteria*, induced significantly more pro-inflammatory cytokine

secretion of IL-12 and TNF-a than did the infant-type *bifidobacteria*, *B. bifidum*, *B. breve*, and *B. infantis*.

It has been shown in monocolonized mice that bacterial translocation was higher for *L. johnsonii* La1 than for *L. paracasei* ST11 and that *L. johnsonii* La1 behaves like a stronger antigen, resulting in greater induction of antibodies (secretory and systemically). *L. paracasei* St11 was overall a poorer inducer of a humoral response, and its antibody isotypes corresponded more to a Th1 T cell helper. *L. acidophilus* Ke-10 was able to restore the proliferation reaction of lymphocytes and their capacity to produce IL-2 in a radiation-induced rat model of immune deficiency. In humans, *L. johnsonii* La1 increased the humoral immune response to an attenuated *Salmonella typhi* Ty21a challenge (Borruel *et al*., 2003)

In healthy volunteers receiving a fermented milk product supplemented with *L. johnsonii* La1 or *B. bifidum* Bb12 strains for 3 weeks, phagocytosis of *E. coli* sp. by leukocytes isolated from the blood, was enhanced in both groups and persisted for 6 weeks after ingesting the strains, even though the fecal lactobacilli and bifidobacteria levels had returned to those prior to consumption. (Ibnou-Zekri *et al*., 2003)

In mice, oral administration of the *L. casei Shirota* or *B. breve* YIT4064 strains activated the humoral immune system. *L. casei Shirota* has been shown to induce the production of several cytokines, such as IFN-γ, IL-1 and TNF-α in mice. Oral administration of *L. casei Shirota* has been found to enhance innate immunity by stimulating the activity of splenic NK cells and oral feeding with killed bacteria was able to stimulate the production of Th1 cytokines, resulting in repressed production of IgE antibodies against ovalbumin in experimental mice. The natural killer (NK) activity of blood mononuclear cells and splenocytes in aged mice fed on a diet containing *L. casei Shirota* was significantly increased. Oral administration of *L. casei Shirota* or *B. breve* YIT4064 strains to infants increased anti-rotavirus IgA production and also significantly reduced the frequency of rotavirus shedding in stool samples. (Yasui *et al*., 1999)

The effect of *Lactobacillus casei rhamnosus* GG, strain involves molecule(s) present in the supernatant of the culture broth. *L. casei rhamnosus* GG diminishes production of TNF-α by the murine macrophage line, RAW 264.7(NO-), and alter the TNF-α/IL-10 balance, in vitro (Pena *et al*., 2003). Interestingly, when media treated with *L. casei rhamnosus* GG are co-incubated with LPS, TNF-α production is significantly inhibited, indicating that soluble molecules were produced and able to inhibit TNF-a production in activated macrophages.

L. casei rhamnosus GG, as a function of the time after administration, enhances T-cell proliferation at the optimal concanavalin A (ConA) concentration and B-cell proliferation at the optimal and supra-optimal concentrations of lipopolysaccharide B-cell proliferation, but decreases marginally the T-cell proliferation at the optimal ConA concentration (Kirjavainen *et al*., 1999). In healthy volunteers receiving *L. casei rhamnosus* GG or placebo for 7 days, an attenuated *Salmonella typhi* Ty21a oral vaccine given to mimic an enteropathogenic infection results in a greater increase in specific IgA among the subjects receiving the vaccine in combination with strain GG. When examining the effect of oral administration of *L. casei rhamnosus* GG on the cellular immune response to intestinal bacteria in a small number of healthy volunteers, Schultz *et al*. (2000) have found that the activation response of CD4+ T-lymphocytes towards isolated and heat-inactivated intestinal bacteria was increased after the probiotic treatment.

Feeding of mice with *L. rhamnosus* HN001, *L. rhamnosus* DR20, *L. acidophilus* HN017, *B. lactis* HN019 or *B. lactis* DR10 strains resulted in a significant increase in the phagocytic activity of peripheral blood leukocytes and peritoneal macrophages compared to that in control mice. *L. acidophilus* treatment in mice enhanced ex vivo basal proliferation and B-cell response at suboptimal and optimal concentrations of LPS, and conversely *L. casei, L. gasseri* and *L. rhamnosus* strains inhibited both basal proliferation and mitogen-stimulated lymphocytes proliferation, particularly at supra-optimal concentrations of ConA and LPS. *L. casei* could prevent enteric infections by stimulating secretory IgA in malnourished animals. Moreover, analysis of the cytokine-producing cells in the lamina propria of intestinal showed that

TNF-α and INF-γ values, determined in macrophages cultured from Peyer's patches, were enhanced. (Gill *et al*., 2000).

In mice fed *with L. acidophilus*, observed the increasing IgG2 response shifting the balance towards Th1, *L. casei, L. delbrueckii* subsp. *bulgaricus* and *L. acidophilus* enhanced the IgG1 response favouring Th2. In addition, oral administration of *L. casei, L. delbrueckii* subsp. *bulgaricus*, *L. acidophilus*, *L. plantarum*, and *L. rhamnosus* is followed by an increase in CD4+ cells, suggesting the possibility that the lactobacilli may interact with Peyer's patches and enhance B- and T-cell migration (Perdigon *et al*., 1999).

Administration of *L. rhamnosus* HN001 to healthy middle-aged and elderly volunteers is followed by a relative increased proportion of PMN cells showing phagocytic activity and by a marked increase in NK cell tumor killing activity (Gill *et al*., 2000).

Bifidobacterium lactis HN019 may enhance aspects of cellular immunity in elderly subjects by increasing the proportions of total, helper, and activated T lymphocytes and natural killer cells. Moreover, the phagocytic capacity of mononuclear and polymorphonuclear phagocytes was also elevated after *B. lactis* HN019 consumption. (Schell *et al*., 2002)

Influent factors to immune modulation activities of LAB (Perdigon and Alvarez, 1992)

1.) A wide difference in the ability of LAB to influence the immune system

2.) The effect of LABs on the immune system depends on the dose

3.) Live cultures are more efficient in some aspects of immune stimulation compared with dead bacteria

4.) LAB applied in fermented products induce higher responses compared with cells applied in non-fermented products

Other factors that could influence the efficiency of LABs by stimulation of immunity are probably the age of the host, the physiological condition of the host, and the quality and quantity of feeding.

Effect of LAB probiotics on non-specific immune response

Non-specific immune response constitutes the first line of defense for the host. It is induced by different stimuli and it is activated very fast. The cell base of non-specific immunity is composed of mononuclear phagocytic cells (monocytes, macrophages), polymorphonuclear leukocytes (mainly neutrophils) and NK cells. Phagocytosis initiates a series of intracellular reactions that continue with the production of reactive oxygen and nitrogen radicals, TNF-α and IL-1 (Tizard, 2000).

Effect of LAB on specific immune response

The specific immune response could be divided into two main categories: humoral immunity and cellular immunity. Antibodies produced in plasma cells (mature B-lymphocytes) mediate humoral immunity. Cellular immunity is mediated by T-lymphocytes, which proliferate after contact with antigens, produce cytokines, and influence the activity of other immunocompetent cells.

The induction of the mucosal immune response is not easy due to the development of oral tolerance, but Perdigon *et al*. (1999) demonstrated that certain lactic acid bacteria are able to induce specific secretory immunity, and others will enhance the intestinal inflammatory immune response. *L. casei* and *L. plantarum* were able to interact with Peyer's patch cells and showed an increase in IgA-, CD4+ cells, and antibodies specific for the stimulating strain. *L. lactis* and *L. delbrueckii* spp. *bulgaricus* induced an increase in IgA+ cells entering the IgA cycle but not CD4+ cells. Nader de Macias *et al*. (1992) described the increased resistance to Shigella

infection mediated by high titres of anti-Shigella antibodies in serum and in intestine secretions in mouse fed fermented milk.

In the experiment of Herias *et al*. (1999), gnotobiotic rats given *L. plantarum* in addition to *E. coli* showed lower counts of *E. coli* in the small intestine and caecum one week after colonisation compared with a group colonised with *E. coli* alone. Rats colonised with *L. plantarum* had signifi-cantly higher total serum IgA levels and marginally higher IgM and IgA antibody levels against *E. coli* than those colonised with *E. coli* alone.

Perdigon *et al*. (1990) found a correlation between the increased immune response and resistance to Salmonella infection in mice fed milk cultures. Similar observations were recorded by Paubert-Braquet *et al*. (1995) in the case of the host resistance to *Salmonella typhimurium*.

The application of *L. casei* and *L. bulgaricus* terminated corticoid-induced immuno sup-pression in mice with *Candida albicans* infection (De Petrino *et al*., 1995). The animals inoculated with lactobacilli showed a significant increase in specific and non-specific immune responses and they reached higher levels of antibodies compared with non-immunosuppressed control animals.

According to Alvarez *et al*. (1998) viable culture of *L. casei* protects the host against *S. typhimurium* infection not only after the first application, but it also maintains the effect after a simple revaccination on day 15 or 30 after the first application. They estimated that the protective effect was obtained when the number of IgA secreting cells in the lamina propria of the intestine and the level of secretory IgA in the intestinal fluid increased. In correlation with this fact, they observed an increased number of polymorph nuclear cells that induce an inflammatory immune response and influence the mucous membrane integrity.

According to Havenith *et al*. (2002) lactobacilli are known as safe bacteria and they have a number of properties that render them highly suited as vehicles for the delivery to the mucosa of compounds that are of pharmaceutical interest. The immunomodulation capacity of lactobacilli together with the possibility of targeting antigens to specific sites of the bacterium offers attractive opportunities for the treatment of infectious diseases, auto-immune diseases, or other immune disorders by modulating the immune response in a directed and predetermined way.

The immune mechanisms induced by probiotic bacteria

In the gut immune response induced by commensal bacteria, the antigen presentation from the luminal flora leads to the generation of large quantities of local immunoglobulin A (IgA) without induction of systemic immunity (Milling *et al*., 2005; Corr *et al*., 2009). The local secretory IgA specific for the pathogen requires the interaction of phagocytic dendritic cells with T and B cells from the Peyer's patches with the antigen-presenting cells in isolated lymphoid follicles or in the mesenteric lymph nodes. The pathway of antigen internalization is crucial for immune cell stimulation and the initiation of mucosal immune responses. Probiotic bacteria may arrive in the intestine along routes which correspond with the different pathways for the internalization of antigens. These bacteria (as whole cells or fragments of antigenic) must interact with the M cells in the Peyer's patches, with gut epithelial cells, and with the associated immune cells. After contact with these cells, the release of cytokines (IL-10 or TGF-β) is induced to up- or down-regulate the immune response. (Ig A secretion) as shown in Figure 3.

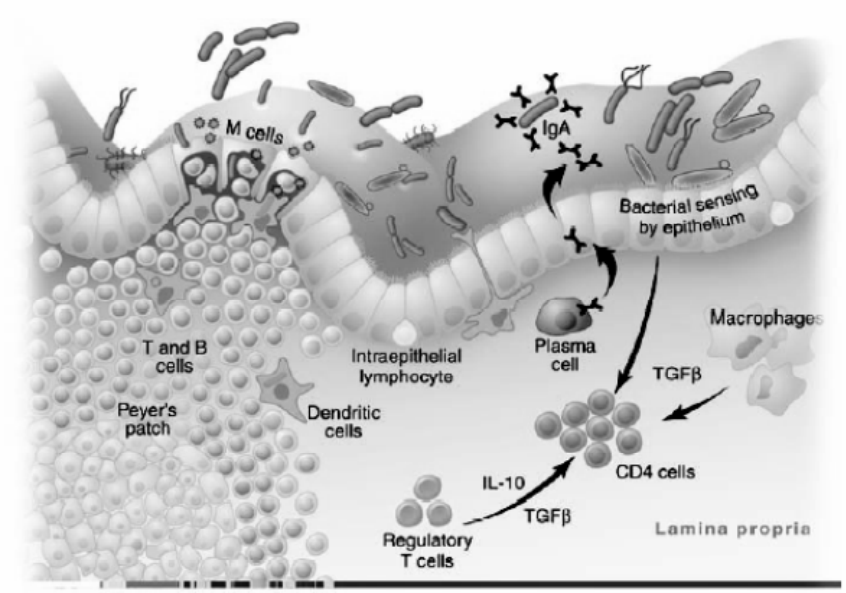

Figure 3 The functioning of the gut mucosal immune system

Source: Maldonado *et al.* (2007)

In order to survive, probiotic bacteria entering by the mouth must be resistant to pH, bile acid, proteolytic enzymes, antimicrobial peptides, intestinal peristalsis, and luminal secretory IgA blocking. This particulate antigen, without a virulence factor, evade all the barriers of the host and up- or down-regulate the gut mucosal immune system by interacting with the epithelial cells and with the immune cells associated with the gut to start the network of immune signals. The increase in the number of IgA-producing cells is the most remarkable property induced by probiotics (Perdigon *et al*., 2002; Corr *et al*., 2009). B cells induced in the Peyer's patches circulate through the mesenteric lymphatic nodes to enter into the blood via the thoracic duct and return to the intestinal mucosa, repopulating distant mucosal sites, such as the bronchus. Some probiotic microorganisms are also able to increase the IgA cycle, and this effect is dose dependent (de Moreno de LeBlanc *et al*., 2005).

T-independent IgA induction was also demonstrated; the cytokines transforming growth factor β (TGF-β), interleukin-4 (IL-4) (Macpherson *et al*., 2004), and IL-2, IL-6, and IL-10 work in a synergistic way from other immune cells different from T cells and can promote the switch from IgM to IgA expression (de Moreno de LeBlanc *et al*., 2004). Some probiotic bacteria can act as adjuvants of the mucosal and systemic immune response. The stimulation with probiotic bacteria induced signals on epithelial and immune cells that evoked different patterns of cytokines in the intestine (Rakoff-Nahoum *et al*.,2006; Maldonado and Perdigon, 2006).

It was previously thought that to have an effect on the immune system, the probiotic strains must remain viable. And this fact is true only for some strains. For *L. delbrueckii* subsp. *bulgaricus*, viability was not necessary for the induction of positive cells producing cytokines, although the number of positive cells was comparatively lower than the number obtained with viable *L. delbrueckii* subsp. *bulgaricus* organisms. The viability was critical for determining the time of residence in the gut with differences between viable and nonviable probiotic bacteria administration; nonviable bacteria were cleared more rapidly. The probiotic bacteria must remain in the gut at least 48 to 72 h to be effective; that is the time required for any particulate antigen to induce gut immunostimulation (de Moreno de LeBlanc *et al*., 2004). This fact is a very important finding, indicating the importance of daily administration in a dose established for each probiotic bacterium to have an adjuvant effect without the induction of oral tolerance.

Probiotic microorganisms are able to induce a gut mucosal immune response (Perdigon *et al*., 2004) which requires the bacteria to interact with the epithelial and immune cells in the gut to induce the network of signals involved in an immune response. Probiotic bacteria may arrive in the intestine along routes which correspond with the different pathways for the internalization of antigens. These bacteria (as whole cells or as the antigenic fragments) must interact with the M cells in the Peyer's patches, with gut epithelial cells, and with the associated immune cells. After contact with these cells, the release of cytokines is induced to up- or down-regulate the immune response.

Mucosal epithelial cells form an efficient barrier which prevents antigens from environmental pathogens from gaining access to the host milieu. Flagellated micro-organisms, including commensals, trigger epithelial homeostatic responses that recruit immune cells of the innate immune system to the epithelium and lamina propria of the intestine to link the innate or/and the adaptive immune response (Rumbo *et al*., 2004).

It has also been shown that commensal bacteria can activate Toll-Like Receptor (TLR) signals (Iwasaki *et al*., 2004). Although the precise location of these receptors in the intestinal epithelial cells (apical or/and basolateral) is controversial (Cario *et al*., 2004), TLR signals are essential, not only for response to pathogens but also to maintain the intestinal barrier function (Rakoff-Nahoum *et al*., 2004).There is an active dialogue between the commensal microorganisms and the host mucosal immune system (Dogi *et al*., 2006). This cross talk elicits different host responses to commensal and pathogenic bacteria. Commensal bacteria may even share molecular patterns recognized by TLRs, which can recognize patterns associated mainly with pathogens.

Vizoso Pinto *et al*. (2009) showed the capability of HT29 intestinal epithelial cells to influence the innate immune response towards *Salmonella enterica* serovar *Typhimurium* and capacity to modulate toll-like receptor (TLR) expression of HT29 cells of the potentially probiotic strain *Lactobacillus plantarum* BFE 1685 isolated from a child's faeces and the probiotic strain *Lactobacillus rhamnosus* GG. They found that TLR2 and TLR9 transcription levels were up-regulated when HT29 cells were incubated with lactobacilli, but not with *S. Typhimurium*, indicated that stimulation of specific signaling pathways occurs in the crosstalk between probiotic bacteria and gut epithelium cells, which can help to explain the adjuvant properties of probiotic lactobacilli.

However, the mucosal immune system of the healthy intestine allows the persistence of this microbiota associated with the intestine and avoids immunological tolerance, maintaining the intestinal homeostasis. Now, there is acceptance of the concept that oral tolerance is not generated by commensal intestinal bacteria; the host would ignore or fail to recognize the presence of indigenous microorganisms (Mac-pherson *et al*., 2004). In the healthy host the penetration of the com-mensal bacteria is usually prevented by the barrier afforded by the intestinal epithelium and the immune cells associated with the mucosa, which are highly adapted to the presence of the normal microbiota (Raibaud, 1992). The signals sent by these microorganisms prevent their penetration and keep them outside the intestinal tissue. If the commensal microorganisms invade the host tissues, the innate immune mechanisms contribute to their rapid clearance, but when pathogens enter the intestine, innate and adaptive mechanisms are coordinately stimulated to respond to the danger signals (Janeway and Medzhitov, 2002).

Probiotic bacteria could be also internalized through M cells in the Peyer's patches or villi or may be sampled by dendritic cells as whole cells or their antigenic fragments (Maldonado and Perdigon, 2005). These may be captured by the other dendritic cells or macrophages associated with the lamina propria to increase the signals to the epithelial cells and/or other immune cells. These bacteria can be trans-ported to the mesenteric lymph nodes, where they interact with T and B cells to induce specific mucosal IgA or suppress T cells (Mowat, 2003).

For the bacterial particles, TLR signal or the CD-206 receptor which is a type I membrane protein which belongs to the group of Pattern recognition receptors involved in the host cell immune response through phagocytosis would be involved, as was demonstrated after *L.casei* CRL431 administration (Abreu, 2010; Maldonado and Perdigon, 2005), The IgA cells in the lamina propria of the small intestine were increased for different LAB tested samples, such as *L. acidophilus*, *L. bulgaricus*, and *S. thermophilus* (Perdigon *et al*., 2005).Specific IgA against the probiotic bacteria and modifications in the number of CD4 population were not found (Vintini *et al*., 2000).

These findings indicated that the antigenic presentation with production of specific antibodies would not be induced.

For the other mucosal immune mechanisms, such as the Th1 cell response, can be modulated by probiotic bacteria, this was demonstrated by other researches in pathological processes such as allergy, inflammatory bowel disease (Cario *et al*., 2004; Rakoff-Nahoum *et al*., 2006), or colon cancer (de Moreno de LeBlanc *et al*.,2004a, 2005b). Previous scientific evidence under physiological conditions suggest that the probiotic bacteria interact with the epithelial cells and preferentially with the immune cells from the innate immune system, reinforcing this barrier (Vinderola *et al*.,2005; Maldonado *et al*., 2007). When they interact with cells from Peyer's patches, they can induce an increase of the IgA cycle. Under physiological condition, probiotic can act as mucosal and systemic adjuvant (Perdigon *et al*., 1999 ; de Moreno de LeBlanc *et al*., 2005a) as the following steps ;

1) the epithelial interaction of the probiotic bacteria,

2) the pathway of internalization of probiotics to the gut,

3) the inducing signals to the immune cells associated with the intestine by an increase in the cytokine production and an increase in the number of IgA-secreting cells;

4) the increase of IgA-secreting cells in other distant mucosal sites, such as the bronchus and mammary glands, as a consequence of gut stimulation by probiotic bacteria.

Maldnado and Perdigon (2006) suggested the proposed model for probiotic interact-tion and gut immune activation that under physiological conditions, probiotic bacteria can act as 2 ways mucosal and systemic adjuvants to modulate the host immune response and the most important signals induced by probiotic bacteria included in daily food would be mediated through the immune cells involved in the

innate immune response. Furthermore, the most important mechanisms involved in the GI immune stimulation are the clonal expansion of B-lymphocyte IgA and the innate immune response. The magnitude of such stimulation did not enhance the inflam-matory immune response but they induced up- or down-regulation of the innate response in order to maintain the intestinal homeostasis.

The local immune response

In past years, several advances have highlighted the primordial role of innate immunity in providing the quick effectors response to infections in vertebrates. Furthermore, natural immunity appears not only to trigger the adaptive immune response but also to direct the type of effector response in clonally selected immune cells that is appropriate to fight efficiently against infections. However, it has become clear that a first line of defense of the organism consists of the local synthesis and release of antimicrobial peptides in tissues, also called barrier epithelia, which are in direct contact with microorganisms. The role of these antimicrobial peptides is illustrated dramatically in the case of cystic fibrosis patients, where β-defensin expressed in the conducting and respiratory airway is inactivated by the high salt concentrations in the airway surface fluid due to a defect in the cystic fibrosis transmembrane conductance regulator. As a result, the airway may become colonized by microbial pathogens, and chronic inflammation ensues.

The local immune response in the gut (Figure 4) induced by the interaction between probiotic bacteria and the epithelial and immune cells associated with the lamina propria of the small intestine. After the interaction with the epithelial cells, probiotic bacteria or their fragments are internalized. The first cells that would interact with them are the antigen-presenting cells (APC), macrophages, and/or dendritic cells associated with the lamina propria of the gut. The interaction with epithelial cells induces IL-6 release. Macrophages and dendritic cells phagocytose the probiotic bacteria or their fragments, and they are induced to produce cytokines such as TNF-α and IFN-γ, which increase epithelial cell stimulation and initiate the cross talk between all the associated immune cells. The ingested bacteria or their particles

could also be eliminated by phagocytosis clearance. IL-6 would favor the clonal expansion of IgA B lymphocytes, increasing the number of IgA-producing cells and the passage of them to plasmatic cells in the lamina propria of the gut. IL-6 together with IL-4 and TGF-β can induce the T-independent switch from IgM to IgA on the surface of B cells and can promote in this way an increase in the number of B cells that are IgA in the lamina propria of the gut.

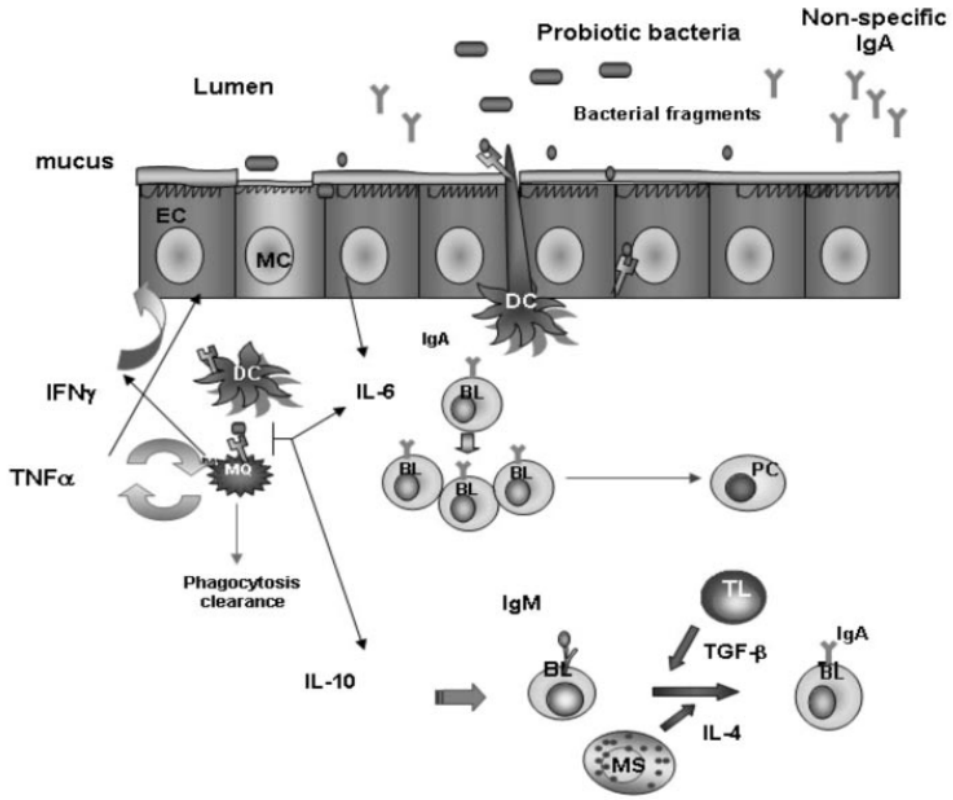

Figure 4 The local immune response by probiotics.
EC: intestinal epithelial cells; MQ: macrophages; TL: T lymphocytes; BL: B lymphocytes; MS: mast cells; PC: plasma cells.

Source: Maldonado *et al*. (2007)

Systemic immune response

Long-term active memory of the host immune system is acquired following infection by activation of B and T cells. Active immunity can also be generated artificially, through vaccination. The principle behind vaccination (also called immunization) is to introduce an antigen from a pathogen in order to stimulate the immune system and develop specific immunity against that particular pathogen without causing disease associated with that organism. This deliberate induction of an immune response is successful because it exploits the natural specificity of the immune system, as well as its induce capacities.

Systemic immune response induced by probiotic bacteria which would be mediated by the network of cytokines induced after probiotic stimulation. From Figure 5, in the Peyer's patches, the probiotic bacteria or their fragments are internalized by M cells or in a paracellular way through follicle-associated epithelial cells of the Peyer's patches. After that, the bacteria or their particles interact with the macrophages and dendritic cells, which are activated to produce cytokines. As consequence of the bacterial stimulation to the immune cells in this inductor site of the immune response, cytokine production is enhanced, as well is the switch from IgM to IgA B cells. Probiotic stimulation can induce the IgA cycle, increasing the number of IgA cells in mucosal sites distant to the intestine. The IgA cells migrate to the mesenteric lym-phoid node and then via the thoracic duct to the circulation, arriving in the bronchus and mammary glands. The cytokines released by probiotic stimulation in Peyer's patches are the biological messengers of the complex network of signals that activate the systemic immune response.

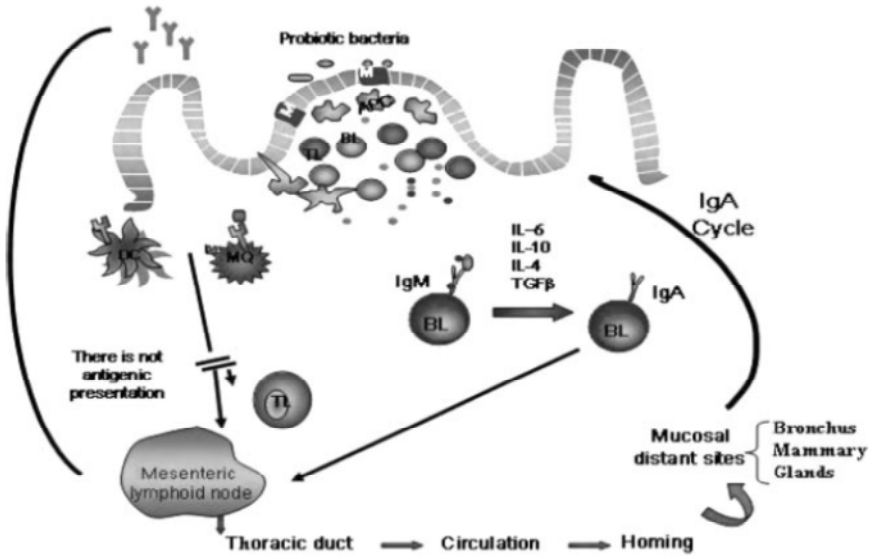

Figure 5 Systemic immune response by probiotics.
DC: dendritic cells; MQ: macrophages cells; APC: antigen-presenting cells; TL: T lymphocytes; BL: B lymphocytes.

Source: Maldonado and Perdigon (2006)

Considerations for probiotics modulation of the immune system (McCracken and Gaskins,1999; Yan and Polk, 2011)

Health benefits emanating from probiotics consumption have in recent years been used as a strong marketing tool, even though supportive information has not been available. The health effects of probiotic have already been identified (Kailasapathy and Chin, 2000), but little attention has been given to their role in manipulating the immune system. Continued progress in probiotics research will require improved understanding of the host intestinal physiology and its relationship with intestinal microbes as followings;

1. Define cellular and molecular modes of host responsiveness.

2. Improve understanding of intestinal physiology, including expression of cell-surface molecules mediating microbial colonization.

3. Study role of individual bacterial species in modifying host physiology and immune functions.

4. Evaluate potential for multiple-strain consortia as probiotics.

5. Determine safety of probiotics for long-term use and for use by immunocompromised individuals.

6. Consider alternatives to live organisms. Specifically, evaluate the modulatory potential of yeast or bacterial cellular components.

It may be necessary to break away from the single probiotic paradigm, which is based largely on studies of immune development in the monoassociated animals. Although the use of monoassociated animals has provided a reasonable understanding of the direct interactions between the host and a specific member of the microflora, this is obviously an oversimplification, as in the conventional animals there may be hundreds of other bacterial species with which to interact. Therefore, a better understanding of the interactions among microbial species may be useful strategies for supplementation of the indigenous microbiota, perhaps by formulation of probiotic cocktails containing multiple strains of bacteria.

Stimulation of antibody production appears to be an important mechanism by which bacteria may interact with the host immune system in numerous ways and act to influence the host immune response and increase resistance to infection (Huang *et al*., 2004; Jian-Kui *et al*., 2009). Cell wall components, which constitute heat-killed cells, may be a factor in the immune modulation (Adams, 2010).Although several studies have suggested that live lactobacilli are most effective at enhancing defense

functions, perhaps cell wall products or lysates of unidentified organisms can generate similar results. Consumption of β-glucans from yeast cell wall extracts has also been shown to increase macrophage activation. The use of abiotic immunomodulators may become very important if, as some have suggested, a stable intestinal ecological system strongly resists establishment of newly introduced organisms.

Recent trends in the marketing of food products have clearly demonstrated a tendency towards claiming specific or general health benefits from the consumption of special food formulations, probiotic bacteria or medical plants. This field of nutraceuticals and health benefits that may ensure from their consumption can now be subjected to rigorous immunological analysis. It may now be timely to determine whether immunological parameters can be used as indicators of health and well-being. Certainly, there is available a range of immunological tools and techniques, as well as animal models, to study the effects of various dietary supplements on the immune response.

Safety of probiotics

As viable probiotic bacteria have to be consumed in large quantities, and over an extended period of time for they to be beneficial effects, the issue of the safety of these organisms becomes a primary concern. The traditional use of probiotic bacteria, particularly lactobacilli in food processing, without significant adverse effects in humans, has long proofed to their safety. Recently, there have been reports of isolated cases of opportunistic infections caused by certain probiotics, such as *Enterococcus* and *Saccharomyces* spp. *Enterococci* are of particular medical relevance because of their increasing importance as a cause of infection originated from the hospital, coupled with evolving antimicrobial resistance. Certain *Enterococcus* strains have a long history of safe to be used as starter cultures in dairy fermentation and are therefore being promoted as probiotics. However, the potential for genetic transfer of virulence factors from medical strains to culture starter strains via a natural conjugation process has now been demonstrated. Recent FAO/WHO guidelines (2006) recommend that probiotic strains should be evaluated for a number of

parameters, including antibiotic susceptibility patterns, toxin production, metabolic and hemolytic activities, infection in immunocompromised animal models, side effects and adverse incidents in humans. (Senok *et al*., 2005)

Risk associated probiotics treatment (McCracken and Gaskins,1999)

Probiotics are often regulated as dietary supplements rather than as pharmaceuticals or biological products. Thus, there is usually no requirement to demonstrate safety, purity, or potency before marketing probiotics. This can lead to significant inconsistencies between the stated and actual contents of probiotic preparations. In Europe, those dietary supplements intended for use by infants and young children do have the specific compositional legal requirements. In the United States, although dietary supplements do not generally require premarket review and approval by the Food and Drug Administration (FDA), those that are marketed specifically for the treatment or prevention of a disease are classified as the biological products and do need review and approval by the Food and Drug Administration. Similarly, in Australia, those probiotics marketed for specific health benefits require premarket review by the Therapeutic Goods Administration and are usually regulated as complementary medicines. In Japan, those probiotic products marketed for a specified health use also require formal premarket review by the Health Ministry. Although most commercially available probiotic strains are widely regarded as safe, there are significant concerns with respect to safety in particular populations.

Choice of probiotic product and regulatory issues

Although there are guidelines regarding the choice of probiotic strains and the assessment of their efficacy and safety, there is still no international regulatory agreement, particularly concerning probiotic food products. Studies or experiments evaluating products on supermarket shelves have found that the contents do not always correspond with the label claims in terms of the type, number and viability of pro-biotic microorganisms. In addition, there is inadequate information available regarding the stability of probiotic in some products such as powdered milk (including

infant formula), especially as the production process is known to cause cell damage and loss of viability of the probiotic cultures. Therefore, appropriate labeling should state the species and strain of probiotic bacteria in the product as well as the viable concen-tration present at the end of the shelf life. (McCracken and Gaskins, 1999)

Lactobacillus johnsonii KUNN19-2 and *Pediococcus pentosaceus* KUNNE6-1.

In the previous study of Kwanmuang (2003), who has isolated and characterized some lactic acid bacteria from commercial Thai fermented pork sausage (Nham) products from around Thailand, and investigated the technological property for using as starter culture in fermented pork sausages again afterward. *Lactobacillus johnsonii* KUNN19-2 and *Pediococcus pentosaceus* KUNNE6-1 were two of those isolated lactic acid bacteria, and found to outstanding show some basically starter culture characteristics in fermented pork sausages, high acid producing together with rapid pH reduction within 24-48 hr of fermentation. Furthermore both strains also displayed some interesting probiotic characteristics of resistant to some antibiotics which usually use in human therapy and animal feed, Chloramphenicol and Tetracycline at 50 µg/mL,Streptomycin at 100 µg/mL of concentration and could produced antibacterial substance to inhibit the growth of *Bacillus cereus, Leuconostoc mesenteroides* and *Staphylococcus aureus*.

The high intrinsic resistance and susceptibility of Lactobacillus strains to a range of antibiotics is important. Strains that show resistance to a specific antibiotic can be given at the time of antibiotic treatment. In the treatment of urogenital tract infections and traveller's diarrhea,better management is obtained when concurrent therapy was made with probiotic lactobacilli and antibiotics to which they are intrinsically resistant,e.g. nitrofurantoin, rifampicin, and ofloxacin. By doing so, intestinal microflora can recover more quickly. An important drawback of antibiotic resistance is that transfer of antibiotic resistance genes is possible. Because antibiotic resistance genes are generally carried on plasmids, they can be transferred to other bacteria by means of conjugation. This may result in highly antibiotic resistant pathogenic

bacteria. So it is important to determine whether antibiotic resistance genes are present on chromosomes or on plasmids. Also in vitro experiments can be conducted to see if transfer is possible between bacterial species. Concisely, some strains are better candidates as probiotic, but none of a strain unfortunately has all the characteristics of a probiotic. However, the candidates of probiotic can be used as a mixture to get all of the characteristics in one product. It can also be concluded from this study that candidates of probiotic strains do exist in several fermented meat products. Nevertheless, in vitro results must be validated. Substantiation of the probiotic use of these candidate strains can only be possible with in vivo studies. Furthermore the stringent, double-blinded, and placebo controlled clinical trials can prove benefits of probiotic strains.

The sixty samples of Nham were collected from around Thailand and used for isolating lactic acid bacteria. The amount of counted lactic acid bacteria colonies in Nham samples were 61-70%. Mostly, the cells were rod and homofermentative lactic acid bacteria. The identification respect on morphological physiological and utilizable of different sugar for acid production. The strains were found as *Lb.johnsonii* 52.17%. The test of antibiotic resistance for homofermentative group,14 isolates were resisted to Chloramphenicol and Tetracycline at 50 µg/mL., Streptomycin at 100 µg/mL of concentration. Seven of isolates could produced antibacterial substance to inhibit the growth of *Bacillus cereus, Leuconostoc mesenteroides* and *Staphylococcus aureus*, then newly named as *Lb. johnsonii* KUNN5-1, *Lb. johnsonii* KUNN8-4, *Lb. johnsonii* KUNN13-1, *Lb. johnsonii* KUNN19-2, *Lb. johnsonii* KUNN15-1, *Pedio-coccus acidilactici* KUNNE 13-1 and *P.pentosaceus* KUNNE6-1. The study of their plasmid found that, one strain has no any plasmid, but the others had some plasmids. *Lacto-bacillus* had more plasmid than *Pediococcus*. These were used as starter culture for Nham production, only four strains could produced high acid concentration in Nham production, there are *Lb. johnsonii* KUNN8-4, *Lb. johnsonii* KUNN19-2, *Lb. johnsonii* KUNN15-1 and *P.pentosaceus* KUNNE6-1.these were grown very good and produced high acid production at 30 ^{o}C of Nham fermentation for 24-30 hrs. Mixed culture of both genus was investigated in the same condition. The result was found that, the pH and lactic acid were 4.7 and 0.819% within 24 hrs, respectively.

For storage time, up to 30 days, every treatments the pH was decreased and the lactic acid was increased. After 30 days, the pH was increased and the lactic acid was decreased. The cell number was decreased after 12 days. At storage time 0-21 days, the firmness of all treatments were increased and higher than control sample. The sensory evaluation of Nham by consumer who eat fresh Nham and found that the mixed culture Nham gave the mean value of texture, color, flavor an acceptance more than control Nham ($P<0.05$).

Lactobacilli are normal inhabitants of the human intestine and vagina. Lactobacilli are gram-positive facultative anaerobes. They are non-spore forming and non-flagellated rod or coccobacilli. The guanine and cytosine content of their DNA is between 32 mol% and 51 mol%. They are either aerotolerant or anaerobic and strictly fermentative. In the homofermentative case, glucose is fermented predominantly to lactic acid. Lactobacilli are also classified as lactic acid bacteria (LAB). To date, 56 species of the genus *Lactobacillus.* have been identified. Lactobacilli used as probiotics include *Lb. acidophilus, Lb. brevis, Lb. bulgaricus, Lb. casei, Lb. cellobiosus, Lb. crispatus, Lb. curvatus, Lb. fermentum, Lb.* GG *(Lb. rhamnosus* or *Lb. casei* subspecies *rhamnosus), Lb. gasseri, Lb. johnsonii, Lb. plantarum* and *Lb. salivarus. Lb. plantarum* . Other probiotic strains of *Lb.* are *Lb. acidophilus* BG2FO4, *Lb. acidophilus* INT-9, *Lb. plantarum* ST31, *Lb. reuteri, Lb. johnsonii* LA1, *Lb. acido-philus* NCFB 1748, *Lb. casei Shirota, Lb. acidophilus* NCFM, *Lb. acidophilus* DDS-1, *Lb. delbrueckii* subspecies *delbrueckii*, *Lb. delbrueckii* subspecies *bulgaricus* type 2038, *Lb. acidophilus* SBT-2062, *Lb. brevis, Lb. salivarius* UCC 118 and *Lb. paracasei* subsp *paracasei* F19.

Lb. johnsonii is predominantly found in the gastrointestinal tract. (Boekhorst *et al*.,2004) *Lb. johnsonii* NCC533, isolated from human faeces, has been extensively studied for its probiotic activities, including immunomodulation (Haller *et al*., 2000a, 2000b) and interaction with the human host (Ibnou-Zekri *et al*.,2003).

Lb. johnsonii NCC533, isolated from human faeces, has been extensively studied for its probiotic activities, including immunomodulation (Haller et al., 2000a, 2000b) and interaction with the human host (Ibnou-Zekri et al.,2003). The viable-cell counts of *Lb. johnsonii* were highly found in the Peyer's patches and mesenteric lymph nodes and also observed that *Lb. johnsonii* induced B-cell stimulation, as characterized by the formation of lymphoid aggregates in Peyer's patches and the presence of IgA^+ B cells in the lamina propria. Colonization with *Lb. johnsonii* promoted substantial production of specific secretory IgA.

Pediococcus pentosaceus;

A gram-positive, facultatively anaerobic, non-motile and non-spore-forming, members of the industrially important lactic acid bacteria. Like other lactic acid bacteria, *P. pentosaceus* are acid tolerant, cannot synthesize porphyrins, and possess a strictly fermentative metabolism with lactic acid as the major metabolic end product (Axelsson, 1998; Garvie, 1986). Phylogenetically Pediococcus and Lactobacillus form a super-cluster that can be divided in to two sub-clusters, all species of Pediococcus fall within the *L. casei* - Pediococcus sub-cluster. Pediococci (cocci; 0.6-1.0 mm in diameter) and lactobacilli (rods) are morphologically distinct. The formation of tetrads via cell division in two perpendicular directions in a single plane is a distinctive characteristic of pediococci. Pediococcus can be described as "the only acidophilic, homofermentative, lactic acid bacteria that divide alternatively in two perpendicular directions to form tetrads". Lactic acid is produced from hexose sugars via the Embden-Meyerhof pathway and from pentoses by the 6-phosphogluconate/ phosphoketolase pathway (Axelsson, 1998). *P. pento-saceus* grow at 40 but not 50^{o}C, between pH 4.5 an 8.0, in 9-10% NaCl, hydrolyzes arginine, can utilize maltose and some strains produce a "pseudo-catalase" (Garvie, 1986; Simpson and Taguchi, 1995).

Pediococcus pentosaceus, a member of the industrially important LAB, can be isolated from a variety of plant materials and bacterial ripened cheeses. This organism has been used as an acid-producing starter culture in sausage fermentations, cucumber and green bean fermentations, soya milk fermentations and silage (Simpson and Taguchi,1995). This strain has been reported to be acid tolerant, cannot synthesize porphyrins and possess a strictly fermentative metabolism with lactic acid as the major metabolic end product (Garvie 1986; Axelsson 1998) In addition, immune enhancement, anticancer and antimicrobial activities has also recently been reported (Choi *et al*.2003).

P. pentosaceus can be isolated from a variety of plant materials and bacterial ripened cheeses. This organism is used as an acid producing starter culture in sausage fermentations, cucumber and green bean fermentations, soya milk fermentations, and silage (Simpson and Taguchi, 1995). *P. pentosaceus* are also a typical component of the adventitious or non-starter microflora of most cheese varieties during ripening (Beresford et al., 2001). In addition,it has been suggested that this organism may have value as an acid-producing starter culture in the dairy fermentations (Caldwell *et al*, 1996 ,1998).

Genetic studies of *P. pentosaceus* have generated a limited quantity of information (1 plasmid sequenced and 8 unique chromosomal sequences) on plasmid and chromosomal encoded genes. However, the vast majority of genes encoding industrially important attributes have yet to be described. Genomic sequence analysis of *P. pentosaceus* genome will help fill key knowledge gaps by providing a comprehensive view of the enzymes and metabolic pathways related to: 1) acid and flavor production in fermented meat and vegetable foods; 2) mechanisms by which by *P. pentosaceus* and other nonstarter LAB grow and direct flavor development in cheese ripening process; and 3) mechanisms by which *P. pentosaceus* and related lactic acid bacteria spoil wine and other alcoholic beverages. In addition, improved knowledge of global gene regulation and integrative metabolism in *P. pentosaceus* will also help to identify rational strategies for metabolic and genetic improvements to industrial strains of lactic acid bacteria.

Pediococcus is one of the main genera used in the fermentation of meats. The species commercially used are *P. acidilactici* and *P. pentosaceus*. The pH of salami products made with starter cultures containing no added manganese lagged behind that of products made with added manganese (5 μg g^{-1}) by 0.2 pH units. A level of 1.2 μg g^{-1} of added manganese was sufficient to achieve optimal fermentation of the meat. A Mettwurst spice blend can provide the fermenting meat with 0.77 μg g^{-1} manganese. Up to 50% of the NaCl in a fermented sausage formulation was substituted with KCl. Pediococci were inhibited by KCl and the inhibition was more pronounced when they were inoculated with a strain of *Lb. plantarum*. (Ketema *et al*.,2009). Upon the inoculation of *P. pentosaceus* which was immobilized in calcium alginate beads and then lyophilized into meat, the immobilized culture was found to ferment more rapidly than a comparable free cell culture (Michael *et al*., 2012) Chlortetracycline was most inhibitory and penicillin was least inhibitory to the fermentation of glucose by *P. pentosaceus* in meat; streptomycin and neomycin were in between. The use of mix starter cultures could be a problem as some strains of pediococci may inhibit the growth of other strains of pediococci, *Lb. plantarum* and *Leuconostoc mesenteroides*.

Pediococci were tested as biopreservatives to control the growth of *Salmonella typhimurium* (in pasteurized liquid whole eggs and cooked, mechanically deboned poultry meat), *Staphylococcus aureus* (cooked, mechanically deboned poultry meat), Listeria (milk), *Pseudomonas* sp. (pasteurized liquid whole eggs and cooked, mechanically deboned poultry meat). Pediococci also increased the shelf life of refrigerated, mechanically deboned poultry meat, ground beef and ground poultry breast.

Fermented chicken cartilage

Fermented chicken cartilage is one of Thai poultry by-products, made from a mixture of Chicken cartilage, sugars, salt, cooked rice, garlic, and spices (black peppers) and lastly, starter culture that may or maybe not add into the mixture. The raw material is packed into plastic bag and placed in the incubator for the fermentation/ripening at the proper temperature and time (normally it takes 60-72 hr at room temperature for natural fermentation and 24-36 hr for those with starter culture added).

Chicken cartilage, the main component of the mixture, cartilage consists of a cellular component distributed in an amorphous extracellular matrix. This matrix is made up of collagen fibers (primarily type II) and proteoglycans, which are complex formed by a protein backbone with leteral branching of sulfated glyco aminoglycans (GAGs), primarily chondroitin sulfate. The gel formed in the cartilage matrix by chondroitin sulfate and water and the structural support formed by the collagen network confer on cartilage its typical hardness, elasticity, surface smoothness and capacity to absorb mechanical stress and strain. There are some interesting medical and scientific information that chicken cartilage has naturally provides glucosamine and chondroitin sulfate that is high quality and is expected to deliver the joint health benefits. And this recently raised the new product category of chicken by-product meal ingredient, food supplement as hydrolyzed chicken cartilage powder and capsule. Furthermore, there are some interesting information in those products which Matrilin-3 gene discovered to prevent onset of Osteoarthritis, chicken cartilage nutritional support for Rheumatoid and injured joints claimed that chicken cartilage is the only available source of Type II Collagen. Gelatin, glucosamine sulfate, and chondroitin sulfate provide only Type I Collagen which does not work as effectively with immune-system-related and joint injury forms of arthritis (Roughle and Lee, 1994)

When a specific joint has been traumatized through sports, accident, surgery, etc. and a specific localized chronic joint problem may result which includes flaring up of rheumatism in the joint. This rheumatism is a body immune system complexity that blocks the bioabsorption of glucosamine sulphate and chondroitin sulphate and the joint repair benefits of Type I Collagen. For reasons not yet defined, Type II Collagen found in Chicken Cartilage is able to bypass this immune system barrier and provide positive improvements in joint pain and functionality. Both Type I and Type II Collagen are compounds known as mucopolysaccharides, which are necessary for the formation of healthy cartilage, collagen and synovial fluid. These mucopolysaccharides also increase joint lubrication through their water-holding capacity. Type II Collagen was thoroughly tested and proven effective in a major double-blind controlled study at Harvard Medical School. (Sai *et al*, 1986; Trentham *et al*., 1993)

Cartilage consists of a cellular component distributed in an amorphous extracellular matrix. This matrix is made up of collagen fibers (primarily type II) and proteoglycans. Proteoglycans are complexes formed by a protein backbone with leteral branching of sulfated GAGs, primarily chondroitin sulfate. The gel formed in the cartilage matrix by chondroitin sulfate and water and the structural support formed by the collagen network confer on cartilage its typical hardness, elasticity, surface smoothness and capacity to absorb mechanical stress and strain.

Chondroitin sulfate has been shown to inhibit cartilage breakdown. Cell culture studies have shown the exogenously supplied chondroitin sulfate competitively inhibits the action of metalloproteases in the cartilage matrix, decreasing the degradation of collagen and proteoglycans. The use of this substance can lead to cartilage repair: pain and stiffness are reduced and can disappear. Furthermore, there is evidence that disease - or age-related decreases in endogenous chondroitin sulfate levels are involved in pathological formation of occlusive thrombi in the microvasculature by helping in the control of blood coagulation. This can be beneficial to synovial membrane and normal production of synovial fluid. When utilized for long periods of time, type II collagen could minimize and even eliminate the disease specific symptoms. Studies have shown that oral administration of $_{14}C$ labeled

collagen leads to an accumulation of radioactivity in cartilage and can help the permanent turnover of cartilage. (Luo *et al*., 2002)

Type II collagen is thought to be able to induce antigen-specific tolerance. This approach, called oral tolerization, takes advantage of a mechanism used by the body to prevent immune reactions to the foods we eat: foreign proteins that enter the body through the digestive system suppress immune responses to those proteins instead of triggering it. Since joint degeneration can be due to an immune response against type II collagen, a daily oral absorption of type II collagen could suppress the auto-immune attack, stop the cartilage breakdown and even facilitate its repair. Collagen in chicken cartilage can help soothes aching joints.

Microbial diversity of fermented sausages

Correct characterization of the intestinal microflora is necessary for the development of probiotics, but this process has been hampered by the biases imposed by traditional culture-based techniques. The use of molecular ecology techniques based on analyses of ribosomal RNA and DNA, and metabolic fingerprinting are beginning to solve these problems. The use of molecular ecology techniques will allow a more accurate assessment of intestinal microbial ecology, particularly during key development windows. Further, improved molecular and culture-based techniques can be used to assess changes in the microbial populations of the intestinal upon administration of probiotic organisms, providing spatial and temporal information concerning colonization

The microbiology of fermented sausages is varied and complex. The type of microflora that develops is often closely related to the ripening method utilized. Normally sausages with a short ripening time have more Lactobacillus spp. from the early stages of fermentation, and some *Micrococcaceae* spp. were found at the end of ripening an acid flavor with little aroma predominates. In contrast, sausages with longer ripening times, *Micrococcaceae* which is have a low rate of acidification and

produce protease and lipase appears as dominant strain in the early stages of fermentation.

A wide variety of microorganisms that have been isolated from sausage fermentation by traditional methods are mainly lactic acid bacteria and *Staphylococcus* and *Kocuria* spp. (Hugas *et al*., 1993). Bacterial starter cultures used in meat fermentation are mostly *Lactobacillus* spp. (Sobrino *et al*., 1991; Garriga *et al*., 1993; Hugas *et al*., 1995) and *Pediococcus* spp.(Bhunia *et al*., 1988; Harris *et al*., 1989) and Rantsiou *et al*. (2005) are also noted that the microbial populations present in the sausages during fermentation was mainly characterized by the stable presence of Lactobacillus spp. (*Lb. curvatus*, *Lb. sakei*, *Lb. paracasei* and *Lb. gar-vieae* and *Bacillus* sp., *Staphylococcus, Ruminococcus* sp. were also detected.

Regarding other bacterial groups, *Bacillus* sp., *Ruminococcus* sp. In addition, *Macrococcus caseolyticus* was identified at the beginning of the transformations and yeast species, *Debaryomyces hansenii*, several *Candida* species, and *Willopsis saturnus* were observed in the DGGE gels.

Bacterial strains found most commonly in Thai fermented meat products are Lactobacilli, Pediococci and Micrococci, but the precise role of these bacteria in the quality of the products are not known (Thiravattanamontri *et al*., 1998). Kunawasen *et al*. (2000) found 12 different genetic groups in commercial Nham and only *Leuconostoc mesenteroides* subsp. *dextranicum* ,*Lb.* spp., *Lb. paracasei* and *P. pentosaceus* were found to be dominant throughout the fermentation with the proportion of population increased and correlated well with the rapidly decreased in pH value.

The conventional methods for the microorganisms characterization are sometime uncertain since require selective enrichment and sub-culturing media. The difficulty of correct identification of these species and the increasing interest in some of their proper-ties, such as their probiotic activities, indicates the need for a simple and reliable molecular method for their definitive differentiation to avoid false declarations of species. In many cases the molecular identification by sequencing of

the 16S rDNA allowed us to only link the strains to a group of species (such as *Lb. Plantarum* group).

One culture-independent method for studying the diversity of microbial community is analysis of PCR (Polymerase Chain Reaction) products, operated with primers homologous to relatively conserved regions in the genome, by using denaturing gradient gel electrophoresis (DGGE) or temperature gradient gel electrophoresis **(**Muyzer *et al*., 1993). The method was able to differentiate closely related bacterial strains by their polymorphic differences. Based on these results, all the bacterial isolates were genetically grouped.

The molecular typing method together with the phenotypic characteristics were used to identify selected strains of lactic acid bacteria from these genetic groups. These approaches allow separation of DNA molecules that differ by single bases and have the potential to provide information about variations in target genes in a bacterial population. By adjusting the primers used for amplification, both major and minor constituents of microbial communities can be characterized.

The PCR-amplified V1 region of the 16S rDNA will be analyzed by DGGE to monitor the evolution of the predominant microorganism populations during the aging or ripening period. The 16S rDNA profiles obtained were compared to determine the active population responsible for the changes that occurred during ripening of the sausages. Bacterial DNA is extracted from the food sample and further processed by polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE method) thus obtaining a sample-specific fingerprint. The fingerprint is an electrophoretic pattern containing bands corresponding to microbial species occurring in the analyzed food sample. Nowadays, the need for safe products with standard and desirable technological properties has resulted in the use of starter cultures for the production of the dry fermented sausages, to control the fermentation and ripening process, inhibiting the growth of other undesirable microorganisms. This has been achieved for other fermented products, such as green olives (Spyropoulou *et al*., 2001).

The most promising bacteria for starter cultures are those which are isolated from the indigenous microflora of traditional products. These microorganisms are well adapted in the meat environment and are capable of dominating the microflora of products. The strains selected as starter or protective cultures must have the most important technological properties and/or bacteriocin production capabilities (Hammes, 1990). That more information about the microflora present and active during chicken cartilage fermentation should help improve the microbiological quality and safety of this food and that the approach which we propose may contribute to new developments in the field of food microbial ecology.

MATERAIALS AND METHODS

Materials

1. Bacterial strains

Two LAB strains isolated from commercial, Thai-style, fermented pork sausage (Nham) product-namely, *Lactobacillus johnsonii* KUNN19-2 and *Pediococcus pentosaceus* KUNNE 6-1-were obtained from the Department of Biotechnology, Faculty of Agro-Industry, Kasetsart University, Bangkok, Thailand.

2. Raw materials

Chicken cartilages and all ingredients (salt, sugar, peppers, cooked rice and garlic)were bought from the local market nearby Kasetsart University, Bangkok, Thailand.

3. Animal model

6-8 week-aged female BALB/c mice (Charles River Laboratories; Missisuaga, ON, Canada)

4. Culture media and reagents

4.1 De Man, Rogosa and Sharpe (MRS) Broth (Merck, Germany)
4.2 De Man, Rogosa and Sharpe (MRS) Agar (Merck, Germany)
4.3 Peptone (Merck, Germany)
4.4 Peptone Water (Oxoid)
4.5 Agar-Agar (Merck, Germany)
4.6 RPMI 1640 medium (GIBCO)
4.7 Fetal bovine serum (Intergen)

4.8 D-glucose (Sigma,UK)

4.9 L-glutamine (Sigma, UK)

4.10 Penicillin-streptomycin (Sigma, UK)

4.11 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium-bromide (Sigma, UK)

4.12 Dimethyl sulfoxide (DMSO)

5. Gastric enzymes and bile

5.1 Pepsin (Porcine pepsin; Sigma Chemical Co.;St. Louis, Mo, USA)

5.2 Pancreatin (Sigma Chemical Co.; St. Louis, Mo, USA)

5.3 Oxgall bile salts (Sigma Chemical Co.; St. Louis, Mo, USA).

6. Chemical reagents

6.1 TE buffer (Tris-EDTA buffer)

6.2 Phenol

6.3 Chloroform

6.4 Isoamyl alcohol

6.5 Isopropanol

6.6 Sodium-acetate

6.7 Ethanol

6.8 Agarose

6.9 Magnesium chloride ($MgCl_2$)

6.10 Taq buffer

6.11 *Taq* polymerase (Eurogentec)

6.12 PCR primers

6.12.1 Primer gc338f

6.12.2 Primer 518r

6.13 Polyacrylamide

6.14 Acrylamide

6.15 Bisacrylamide

6.16 Urea

6.17 Formamide

6.18 SYBR Green

6.19 Sodium Carbonate

6.20 Sodium bicarbonate

6.21 Sodium pyruvate

6.22 100 bp DNA ladder (Fermentas)

7. ELISA reagents

7.1 AffiniPure Goat Anti-Mouse IgG (BD Bioscience, Canada)

7.2 ChromPure Mouse IgG, whole molecule (BD Bioscience, Canada)

7.3 Peroxidase-conjugated AffiniPure Goat Anti-Mouse IgG (BD Bioscience, Canada)

7.4 3,3',5,5'-Tetramethylbenizidine: TMB (Sigma, Canada)

7.5 Sulfuric acid

7.6 Tween-20

7.7 Phosphate-buffered saline (PBS)

8. Equipments and Instruments

8.1 pH meter (Jenco electronic, China)

8.2 Anaerobic jars with BBL gas packs (Becton Dickinson and Co., USA)

8.3 4-digit-Balance (Ohaus model GT 4100)

8.4 Balance (Sartorius model BA-160)

8.5 Hot air oven (Memmert model SLM600)

8.6 Stomacher, Seward model BA8021

8.8 Micropipett (Gilson®)

8.8 Bottle-top dispenser (Brand model Dispensett 3)

8.9 Refregirated centrifugae (Sorvall RC 50 Plus with Rotor F-16/250)

8.10 Incubator (Memmert model BE600)

8.11 Laminar air-flow cabinet (Microflow Biological MDH model 51423/2)

8.12 Autocalve (Tomy model ES-315)

8.13 Vortex mixer, Votex genie-2 model G-560E

8.14 -30 ◦C Freezer (Sanyo model UP-1000)

8.15 Water bath (MEMMERT® model WB22)

8.16 Magnetic stirrer

8.18 Thermal cycler (Thermal cycler 9400, Perkin-Elmer)

8.18 Microplate reader (Biorad model 550)

8.19 Spectrophotometer (Shimadzu model UV-1201)

8.20 Flat-bottomed 48-well tissue culture plate (Fisher Scientific Co.; NY, USA)

8.21 96-well polyvinyl-chloride microplate (Fisher Scientific Co.; NY, USA)

Methods

1. Determination of survival ability passage through the gastrointestinal tract

1.1. Survival ability under gastric conditions

Survival determination under gastric conditions was carried out by the modified method of Huang and Adams (2004). Both *Lb. johnsonii* KUNN19-2 and *P. pentosaceus* KUNNE6-1 were grown in de Man, Rogosa and Sharpe (MRS) broth at 37 ^{o}C for 24 h. To determine acid tolerance, a 1 mL aliquot of the bacterial culture solution was inoculated into 9 mL of sterile saline (0.3% w/v NaCl), adjusted to pH 1.5 with 6 M HCl and incubated for 2 h. The survival ability under gastric conditions was determined by the same manner except that the sterile saline solution containing 0.3 % (w/v) pepsin (Porcine pepsin; Sigma Chemical Co.;St. Louis, Mo, USA) was applied. The mixture solution was incubated at 37 ^{o}C for 2 h. The viable cells of both 0 and 2 h cultivation time were determined by a standard plate count using MRS agar at 37 ^{o}C for 48 h under anaerobic conditions in anaerobic jars with BBL gas packs (Becton Dickinson and Co.; Sparks, MD, USA). Cell survival was calculated as the percentage of colonies grown on MRS agar at detection time compared to the initial bacterial concentration (0 h).

1.2. Survival under intestinal conditions

Cell survival under intestinal conditions was performed by the modified method of Fernandez *et al*. (2003). Culture was grown in MRS broth at 37 ^{o}C for 24 h. An amount of 1 mL of bacterial culture solution was inoculated into 9 mL of pancreatic solution containing 0.3% w/v sterile saline adjusted to pH 8.0, 0.1% (w/v) pancreatin (Sigma Chemical Co.; St. Louis, Mo, USA) and 0.3% Oxgall bile salts (Sigma Chemical Co.; St. Louis, Mo, USA). Cell survival was determined as mentioned above. To determine the effect of two continuous stress conditions of stomach and small intestinal transit that might interact in a synergistic action, a 1mL aliquot of the culture solution after pre-exposure under gastric conditions for 2 h was re-suspended into 9 mL of pancreatic solution as described above. Samples were further incubated at 37 ^{o}C for 1, 2 and 4 h. The residual viable cells were determined by a standard plate count using MRS agar at 37 ^{o}C for 48 h under the anaerobic conditions. Bacterial survival was then calculated as the percentage of colonies grown on MRS agar at detection time compared to the initial bacterial concentration.

2. Determination of immunomodulation

2.1. Preparation of bacterial heat-killed cells

Lactobacillus johnsonii KUNN19-2, *Pediococcus pentosaceus* KUNNE6-1 were grown in MRS broth at 37 ^{o}C for 24 hours, washed in sterilized Phosphate-buffered saline (PBS) and re-suspend in PBS again to obtain the final concentrations of 1×10^{6}, 1×10^{7} and 1×10^{8} CFU.mL^{-1}. After that bacterial suspensions were subjected to 100 ^{o}C heat treatment for 30 min (Haller *et al*., 2002). Cell suspension were then confirmed the absence of both strains viable cells by a standard plate count on MRS agar.

2.2. Mice spleen cells preparation

Female BALB/c mice (Charles River Laboratories; Missisuaga, ON, Canada) aged 6–8 wk were maintained under pathogen-free conditions at the Central Animal Facility at the University of Guelph, Ontario, Canada until used following the standard guidelines of the Canadian Council on Animal Care. BALB/c mice spleen cells were aseptically removed and suspended in working media (RPMI1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), 4.5 $mg.mL^{-1}$ D-glucose,1.5 $mg.mL^{-1}$ sodium bicarbonate, 1mM sodium pyruvate, 2 mM L-glutamine, and penicillin-streptomycin (50 $Units.mL^{-1}$)). Spleen cell suspensions of 1.25×10^6 cells per well were seeded into the flat-bottomed 48-well tissue culture plate (Fisher Scientific Co.; Corning, NY, USA) containing the heat-killed cell of *Lb. johnsonii* KUNN19-2 or *P. pentosaceus* KUNNE6-1 at the concentrations of 1×10^6, 1×10^7 and 1×10^8 $CFU.mL^{-1}$ with the addition of either concanavalin A (Con A) 0.25 $\mu g.mL^{-1}$ or lipopolysaccharide (LPS) 2.5 $\mu g.mL^{-1}$. The mixtures were incubated at 37 ^oC under 5% CO_2 for 5 d. Spleen cells supernatant was collected on day 5 to determine the Immunoglobulin G (IgG) concentration by an indirect enzyme-linked immunosorbent assay (ELISA).

2.3. Cell proliferation

Cell proliferation of BALB/c mice spleen cells suspension which was co-incubated with either Con A or LPS and heat-killed cells of *Lb. johnsonii* KUNN19-2 or *P. pentosaceus* KUNNE6-1 were measured on day 5 of the incubation period using MTT assay (Mosmann, 1983). To determine the effect of heat-killed cells of both LAB on T and B cells (T and B lymphocytes), the proliferation responses of challenged spleen cells to the T-cell mitogen and B-cell mitogen induced by ConA and LPS, respectively, were measured by *in vitro* MTT assay. Their viable cells were determined by the absorbance at 570 nm of blue color formazan product produced by yellow tetrazolium salt MTT reaction. Then, lymphocyte proliferation was determined as the percentage of viable cells from the stimulated mice spleen cells compared to the control (non-stimulated spleen cells).

2.4. Measurement of Antibody Concentration

An indirect ELISA was used to measure antibody concentrations present in supernatants. Total IgA level was measured using an Indirect Double Sandwich ELISA. Coat the 96-well polyvinylchloride plates with AffiniPure Goat Anti-Mouse IgG (BD Bioscience, Canada) at a concentration of 1 µg /ml (0.1 ml per well) in 0.06 M-sodium bicarbonate buffer (pH 9.6) at 4°C overnight. Plates were washed in PBS with 0.05% Tween-20 (PBS-T), then blocked with a solution of PBS-T + 1% BSA at 37°C for 1 hr. Standard (ChromPure Mouse IgG, whole molecule, BD Bioscience, Canada) and samples to be tested were added (0.1 ml per well) and then incubated at 37°C for 2 h. After plates were washed with PBS-T, 0.1 ml of Peroxidase-conjugated AffiniPure Goat Anti-Mouse IgG (BD Bioscience, Canada) detection antibody was added to each well, at concentrations specified by the supplier, incubated at 37°C for 1 hr. Washed plates with PBS-T, then 0.1 ml of substrate (3,3',5,5'-Tetramethylbenizidine: TMB, Sigma, Canada) was added into each well and 0.5 M H_2SO_4 was used as stop solution. Read plates at a wavelength of 450 nm. IgG concentrations were determined by comparison to standards curve.

3. Technological properties as starter cultures in chicken cartilage fermentation of *Lactobacillus johnsonii* KUNN19-2 and *Pediococcus pentosaceus* KUNNE6-1.

3.1 Fermented chicken cartilage probiotic sausage preparation

The required quantities of chicken cartilage and salt were manually mixed for 2-5 mins. Then ground garlic, white pepper and sugar were added and further mixed for 1 min. Cooked rice was then mixed additionally for 2 min. Finally, cell suspension of *Lb. johnsonii* KUNN19-2*, P. pentosaceus* KUNNE6-1 at the concentration of 10^6 CFU/g were added into the mixture and mixed for 1 min. The mixture was then stuffed into plastic bag and tied with thread. Each piece of product weighted 20-25 g. After that, the samples were kept at 35°C in an incubator where fermentation took place before being randomly selected for analysis at 0, 12, 24, 36, 48, 60 and 72 h of fermentation process for the following analysis.

3.2 pH measurements.

pH value of fermented chicken cartilage were measured (AOAC, 2000) by pH meter (Jenco electronic, China). Three independent measurements were obtained for each sample. Means and standard deviations were calculated.

3.3 Microbiological analysis.

Fermented chicken cartilage samples were subjected to a microbiological analysis to monitor the dynamic change in the population responsible for ripening of fermented sausage and their hygienic quality. 25 g of each sample was transferred into a sterile stomacher bag, 225 ml of saline-peptone water (8 g of NaCl per liter, 1 g of bacteriological peptone per liter) was added, and the preparation was mixed for 1.5 min in a stomacher machine. Additional decimal dilutions were prepared, and the following analyses were carried out on duplicate MRS agar plates, incubated at 37°C for 24-48 h under anaerobic conditions in anaerobic jars with BBL gas packs (Becton Dickinson and Co.; Sparks, MD, USA). After counting, means and standard deviations were calculated.

3.4 Microbial diversity analysis

3.4.1 DNA extraction. (Modified from Ampe *et al*.,1999)

3.4.1.1 DNA extraction from pure cultures

Pure cultures were prepared on MRS agar (Becton, Dickinson and Co., Sparks, Md., U.S.A.) from stock cultures frozen in MRS broth supplemented with 10% glycerol. Cultures were sub-cultured twice before used for experimental purposes. Agar plates and broth cultures were incubated for 24 h at 30 ◦C under aerobic conditions in anaerobic jars with BBL gas packs (Becton Dickinson and Co.; Sparks, MD, USA).

One-milliliter sample of overnight cultures were centrifuged at 8,000 rpm, 4 ^{o}C for 10 min. The cell pellets were re-suspended in 1,000 µl TE buffer (Tris-EDTA buffer), then transfer into 1.5 ml screw-cap tubes containing 0.3 g of glass beads. The three minutes treatment at the maximum speed, with 10-s ice incubated intervals between treatments, were performed with a bead beader (Mini Bead Beader). The tubes were then centrifuged at 11,000 rpm at 4°C for 20 min, the aqueous phases were collected. Total DNA was then twice extracted with phenol-chloroform-isoamyl alcohol (25:24:1), followed by final extraction with chloroform-isoamyl alcohol (24:1).Finally, the DNA was precipitated with isopropanol and 3M Sodium-acetate, washed with 70% ethanol, and vacuum dried. The resulting pellets were resuspended in 20 µl of autoclaved deionized water. The quality of the extracts were checked on 1.5% agarose gel.

3.4.1.2 DNA extraction from fermented chicken cartilage

Ten grams fermented chicken cartilage samples were homogenized in a stomacher bag with 90 ml of normal saline (0.85% NaCl) for 2 min. After each preparation had settled for 1 min, a 1 ml sub-samples was placed in 1.5 ml screw-cap tubes containing 0.3 g of glass beads. The three minutes treatment at the maximum speed, with 10-s ice incubated intervals between treatments, were performed with a bead beader (Mini Bead Beader). The tubes were then centrifuged at 11,000 rpm at 4°C for 20 min, the aqueous phases were collected. Total DNA was then twice extracted with phenol-chloroform-isoamyl alcohol (25:24:1), followed by final extraction with chloroform-isoamyl alcohol (24:1). Finally, the DNA was precipitated with isopropanol and 3M Sodium-acetate, washed with 70% ethanol, and vacuum dried. The resulting pellets were re-suspended in 20 µl of autoclaved deionized water. The quality of the extracts were checked on 1.5% agarose gel.

3.4.2 Polymerase chain reaction (PCR).

Different regions of the 16S rDNA were amplified with the primers gc338f and 518r spanning the V3 region of the 16S ribosomal DNA (rDNA) (Table 4) (Ampe *et al*.,1999).

Table 4 PCR primers used in this study

Primer	Sequence(5´-3´)
gc338f	CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGG ACT CCT ACG GGA GGC AGC AG
518r	ATT ACC GCG GCT GCT GG

Amplification was performed with a thermal cycler (Thermal cycler 9400, Perkin-Elmer). Each mixture (final volume, 25 µl) contained 2 µl of template DNA, each primer at a concentration of 20 ρM, 25 mM $MgCl_2$, 2.5 µl of 10x taq buffer, and 1.25 U of *Taq* polymerase (Eurogentec). PCR conditions consisted of initial denaturation at 95°C for 5 min, 34 cycles of denaturation at 95°C for 30s, annealing at 60°C for 45 s and elongation at 72°C for 45 s, and final extension at 72°C for 5 min. Aliquots (5 µl) of the amplification products were analyzed first by electrophoresis in 1.5 % agarose gels.

3.4.3 Denaturing gradient gel electrophoresis (DGGE) analysis.

DGGE analysis of the PCR products obtained from single cultures and directly from fermented chicken cartilage. Electrophoresis was performed in a 0.8 mm polyacrylamide gel (8% [w/v] acrylamide-bisacrylamide [37.5:1]) by using two different ranges of denaturants to optimize separation of the products from the population involved in fermentation. Denaturant gradients, from 35 to 60% (100% denaturant was 7 M urea plus 40% [w/v] formamide) increasing in the direction of electrophoresis, was used. The gels were subjected to a constant voltage of 120 V for 5 h at 60°C with Bio-Rad DCode apparatus, and after electrophoresis they were

stained for 20 min in 10 ml of 1× TAE containing 10 µl SYBR Green and photographed under UV illumination.

4. Statistical analysis.

All the experiments were carried out in triplicate. All data were presented as mean ± SE. Statistical analysis was performed using SPSS software (SPSS Inc., United States). Data were analyzed by one way analysis of variance (ANOVA), and the differences between experimental groups were evaluated using Duncan's multiple range test,and the values were considered statistically significant when $P < 0.05$.

RESULTS AND DISCUSSION

1. Probotic properties of *Lactobacillus johnsonii* KUNN19-2 and *Pediococcus pentosaceus* KUNNE6-1

1.1 Survival ability under gastric and intestinal conditions

The effects of acidic gastric conditions on the viability of *Lb. johnsonii* KUNN19-2 and *P. pentosaceus* KUNNE6-1 are presented in Table 5. Both strains showed tolerance to acid conditions over a period of 2 h. The survival percentage of *Lb. johnsonii* KUNN19-2 (70.97%) was significantly higher than *P. pentosaceus* KUNNE6-1 (60.40%). In the stomach, beside the low pH of 1–3, bacteria are also stressed by proteolytic enzyme secreted in the stomach (Olejnik *et al*., 2005). Therefore, the effect of pepsin on the viability of both bacteria was investigated. It was found that the presence of 0.3% pepsin in acidic conditions decreased the survival percentage of *Lb. johnsonii* KUNN19-2 and *P. pentosaceus* KUNNE6-1 to 58.80 and 40.51, respectively. It was clearly shown that cell survival of *Lb. johnsonii* KUNN19-2 under acidic broth plus 0.3% pepsin was significantly higher than *P. pentosaceus* KUNNE6-1.

Table 5 Survival ability of *Lactobacillus johnsonii* KUNN19-2 and *Pediococcus pentosaceus* KUNNE6-1 in simulated gastric juice.

Treatment	Survival percentage	
	Lb. johnsonii KUNN19-2	*P. pentosaceus* KUNNE6-1
0.3M HCl, pH 1.5	70.97 ± 0.44^{aA}	60.40 ± 0.41^{aB}
0.3M HCl, pH 1.5, 0.3% pepsin	58.80 ± 0.21^{bA}	40.51 ± 0.74^{bB}

Data are shown as mean ± standard deviation.

$^{a-b}$ and $^{A-B}$ = Mean values within a column (lowercase letters) and row (uppercase letters), respectively, not identified with a common superscript are significantly different ($P < 0.05$).

Survive passage through the small intestine is another barrier for probiotic bacteria. The adverse conditions of the small intestine include the presence of bile salts and pancreatin. Resistance to bile salts is considered as an important property in strains considered as probiotic. In this study simulation of intestinal conditions was then performed in an aqueous solution containing both pancreatin and bile. The tolerance of both targeted LAB under the simulated conditions was tested after 1, 2 and 4 h as shown in Table 6. The survival cells of both strains decreased significantly as the contact time increased. After 4 h, the survival percentage of *Lb. johnsonii* KUNN 19-2 and *P. pentosaceus* KUNNE 6-1 decreased to 57.14 and 54.47%, respectively. Since the physical and chemical surroundings change from the stomach through the intestinal region, cells passing through the GI tract may encounter some stresses that affect cell viability. Thus, it is important to evaluate both conditions as a continuous process. This study successfully combined the effects of both gastric and intestinal fluids to cell tolerance under simulated GI tract conditions. Survival cells of both *Lb. johnsonii* KUNN 19-2 and *P. pentosaceus* KUNNE 6-1 showed a similar decreasing pattern with contact time with a drastic decrease in the first hour of

incubation, and then remaining at 30.84% and 19.40%, respectively, after 4 h incubation.

In contrast, the viable cells of the strains KUNN19-2 and KUNNE6-1 remaining from simulated intestinal conditions alone at 4 h were higher at 57% and 54 %, respectively. It was clarified that the cells exposed to gastric conditions became weakened with low survival.

Table 6 Survival ability of *Lactobacillus johnsonii* KUNN19-2 and *Pediococcus pentosaceus* KUNNE6-1 in simulated gastric and intestinal fluid up to 4 h of incubation.

Time (h)	Survival percentage			
	Lb. johnsonii KUNN19-2		*P. pentosaceus* KUNNE6-1	
	Intestinal condition	Gastric and intestinal condition	Intestinal condition	Gastric and intestinal condition
1	67.02 ± 1.12^{aA}	49.01 ± 1.62^{aB}	65.01 ± 1.76^{aA}	32.01 ± 4.38^{aC}
2	62.39 ± 3.16^{bA}	38.83 ± 1.03^{bB}	60.23 ± 3.14^{bA}	23.67 ± 2.36^{bC}
3	57.14 ± 1.73^{cA}	30.84 ± 1.35^{cC}	54.47 ± 1.59^{cB}	19.40 ± 0.96^{cD}

Data are shown as mean ± standard deviation.
$^{a-d}$ and $^{A-D}$ = Mean values within a column (lowercase letters) and row (uppercase letters), respectively, not identified with a common superscript are significantly different ($P < 0.05$).

The most critical characteristics of probiotic strains are acid and bile salt resistance; without these they could not reach the human intestine, where they are expected to exert their health promoting effects. The capacity of the strains to adhere to the intestinal mucosa is crucial if they are to promote changes in intestinal ecology (Erkkilä and Petäjä, 2000). As mentioned earlier, one of the main roles of meat LAB starter cultures is the rapid production of organics acids; this inhibits the growth of

unwanted flora and enhances product safety and shelf-life. The antimicrobial effect of organic acids lies in the reduction of pH, and in the action of undissociated acid molecules (Podolak *et al*., 1996). It has been proposed that low external pH causes acidification of the cytoplasm. The lipophilic nature of the undissociated acid allows it to diffuse across the cell membrane (Kashket, 1987) collapsing the electrochemical proton gradient. Alternatively, cell membrane permeability may be affected,disrupting substrate transport systems (Snijders *et al*., 1985).

The survival of bacteria in the gastric juice depends on their ability to tolerate low pH. The transit time can be from less than 1 h to 3–4 h depending on the individual and the diet. Several studies has proposed that strains intended for probiotic purposes should be screened for tolerance to pH 2.5 in an HCl-acidified culture medium for 4 h and also reported about LAB isolated from sausages such as *L. sakei*, *L. plantarum*, *L. pentosus*, *P. acidilactici* and *P. pentosaceus*, can tolerate such acidic conditions (Erkkilä and Petäjä, 2000; Klingberg *et al*., 2006; Pennacchia *et al*., 2004).

Bacteria that survive the acidic conditions of the stomach must then face the detergent-like function of the bile salts released into the duodenum after the ingestion of fatty meals. Microorganisms can reduce the emulsifying effect of the bile salts by hydrolyzing them with bile salt hydrolase enzymes (BSHs), thus decreasing their solubility (Erkkilä and Petäjä, 2000). BSH activity has been described in some intestinal lactobacilli such as *L. acidophilus*, *L. casei,* and *L. plantarum* (Gilliland and Speck, 1977, Haller *at al*., 2001; Begley *et al*., 2006). Furthermore, some LAB strains from sausages, such as *L. sakei*, *L. plantarum*, *L. pentosus*, and *P. acidilactici*, have been shown to resist bile salts (0.3% (w/v) oxgall) (Erkkilä and Petäjä , 2000; Klingberg *et al*., 2006; Pennacchia *et al*., 2004).

In order to perform functionally and efficiently as a probiotic, bacteria must survive during passage through the GI tract and still be alive at their functional site. In the upper part of the GI tract, bacteria will be subjected to several stress factors. In the stomach, they are stressed by very low pH within the range 1.5–3.0 and by the proteolytic enzyme, pepsin. Normally bacteria can be introduced to the stomach and

reside there under acidic gastric conditions for 1–4 h, depending on the diet, which is long enough to kill the majority of bacteria (Olejnik *et al*., 2005). In the current study, with simulated gastric juice at pH 1.5 with 0.3% pepsin, *Lb. johnsonii* KUNN19-2 exhibited a significantly higher survival percentage than *P. pentosaceus* KUNNE6-1. This was in agreement with previous studies by Haller *et al*. (2001) and Vizoso *et al*. (2006) who concluded that *Lb. johnsonii* spp. had good survival rates under acidic conditions. The possible acid resistance mechanism was explained as resulting from the bacteria perhaps trying to maintain pH homeostasis by discharging H+ from cells by H^+-ATPase (Matsumoto *et al*., 2004). Furthermore, an increase of *atp*BEF genes of the *atp* operon, whose genes encode the various subunits of the F_1F_0-ATPase, may function in the ATP dependent extrusion of protons and maintenance of cytoplasmic pH which is involved in stress responses relating to the enhanced acid tolerance properties of LAB reported by Klaenhammer and Kullen (1999). Survival during passage through the small intestine in the presence of bile salts and pancreatin is another barrier for bacteria to overcome in order to have a positive effect on their host. The pH of the human small intestine is around 8.0 with the concentration of bile acids between 5,000 and 20,000 μM (Hofmann, 1991) and the general transit time of food is between 1 and 4 h (Huang and Adam, 2004). Several previous studies (Goldin and Gorbach 1992; Casey *et al*., 2004) proposed that 0.3% bile salt (equivalent to approximately 7,353 μM) was considered as a critical bile concentration, which is high enough to screen for the resistant strains. In this study *Lb. johnsonii* KUNN19-2 exhibited higher bile salt toleration compared to *P. pentosaceus* KUNNE6-1 which might have been due to bile salt hydrolase (BSH) enzyme activity (Erkkilä and Petäjä, 2000; Haller *et al*., 2001; Begley *et al*., 2006).

In addition, another protective mechanism in the host GI tract might possibly occur from the protein layer on the outside of the bacteria cell wall, known as s-layer (Sleytr and Beveridge, 1999).These proteins are hypothesized and claimed to to be involved in cell protection, adhesion and trapping of some molecules including ions (Avall-Jaaskelainen and Palva, 2005). Frece *et al.* (2005) reported the functional role of the s-layer of *Lb. acidophilus* M92 which is tolerant to the pancreatin and pepsin existing in the small intestine and in gastric juice**,** respectively. The viability of

Lb. acidophilus M92 cells without an S-layer was reduced in simulated gastric juice at low pH (2–3) and lost all viability within 1 h in simulated pancreatic juice with bile salts (3 $g.L^{-1}$). In the current study, *Lb. johnsonii* KUNN19-2 and *P. pentosaceus* KUNNE6-1 showed a substantial decrease in viability under gastric and bile conditions and retained their viability after 1.5 h under gastric and after 4 h under intestinal conditions. This may imply a potential protective role of the S-layer of both tested strains.

1.2. Immune modulation of *Lactobacillus johnsonii* KUNN19-2 and *Pediococcus pentosaceus* KUNNE6-1

1.2.1 Proliferation responses of mice splenocytes

The proliferative responses of the BALB/c mice spleen cells challenged with LPS and heat-killed cells of *Lb. johnsonii* KUNN19-2 or heat killed cells of *P. pento-saceus* KUNNE6-1 at the concentrations of 1×10^6, $1 \times 10^7$and 1×10^8 $CFU.mL^{-1}$ were measured for 5 d. The results showed that cell proliferation of the challenging treatment of *Lb. johnsonii* KUNN19-2 and *P. pentosaceus* KUNNE6-1 was signi-ficantly higher than with one of the LPS alone at a dose of 1×10^8 or 1×10^6 $CFU.mL^{-1}$ as shown in Figure 6.

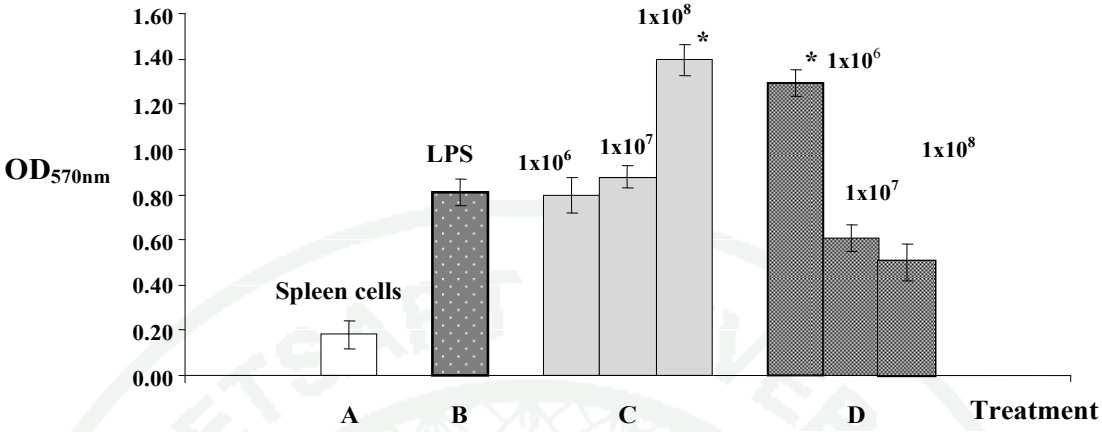

Figure 6 Proliferation response of BALB/c mice spleen cells alone and BALB/c mice spleen cells with the induction of LPS and heated-killed cells. (A) BALB/c mice spleen cells (□); (B) BALB/c mice spleen cells induced by LPS (▩); (C) BALB/c mice spleen cells induced by LPS and heat-killed cells of *Lb. johnsonii* KUNN19-2 (▨); and (D) BALB/c mice spleen cells induced by LPS and eat-killed cells of *P. pentosaceus* KUNNE6-1 (▩) (* = Mean proliferation response significantly higher than other treatments ($P < 0.05$); Error bars indicate standard deviation range.)

However LPS-induced proliferative responses of spleen cells challenged with heat-killed cells of *Lb. johnsonii* KUNN19-2 were significantly increased, whereas the LPS-induced proliferative responses of spleen cells challenged with heat-killed cells of *P. pentosaceus* KUNNE6-1 were significantly decreased when a challenging dose of 1×10^6, 1×10^7 or 1×10^8 CFU.mL^{-1} increased.

These results indicated that heat-killed cells of both strains exhibited a dose-dependent effect on B-cell multiplication. The proliferative lymphocyte responses to ConA increased significantly only in spleen cells challenged with heat-killed cells of *Lb. johnsonii* KUNN19-2 at the concentration of 1×10^8 CFU.mL^{-1} (Figure 7). Therefore, in this study, heat-killed cells of *Lb. johnsonii* KUNN19-2 showed potential as an inducer of both T- and B-lymphocyte proliferation while those of *P. pentosaceus* KUNNE6-1 showed potential for only B-lymphocyte proliferation.

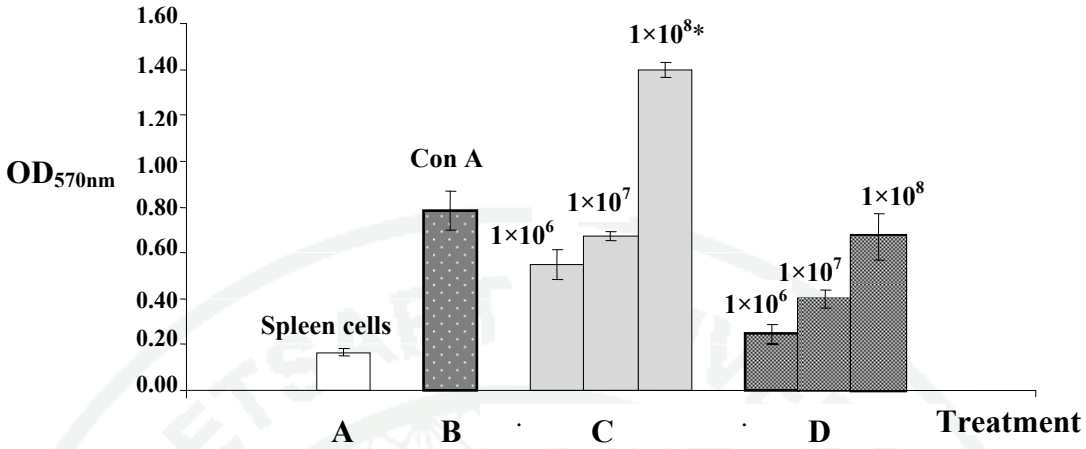

Figure 7 Proliferation response of BALB/c mice spleen cells alone and BALB/c mice spleen cells with the induction of ConA and heated-killed cells. (A) BALB/c mice spleen cells (□); (B) BALB/c mice spleen cells induced by ConA (▩); (C) BALB/c mice spleen cells induced by Con A and heat-killed cells of *Lb. johnsonii* KUNN19-2 (□); and (D) BALB/c mice spleen cells induced by Con A and heat-killed cells of *P. pentosaceus* KUNNE6-1 (▩) (* = Mean proliferation response significantly higher than other treatments ($P < 0.05$); Error bars indicate standard deviation range.)

1.2.2 Effect of heated-killed cells of *Lactobacillus johnsonii* KUNN19-2 and *Pediococcus pentosaceus* KUNNE6-1 on antibody production of BALB/c mice spleenocytes response to lipopolysaccharide (LPS) and concanavalin A (ConA)

To test the effect of heat-killed cells of either *Lb. johnsonii* KUNN19-2 or *P. pentosaceus* KUNNE6-1 on the antibody production responses to LPS and ConA, mice splenocytes were stimulated with heat-treated cells of tested strains over a cell concentration range of 1×10^6, 1×10^7 and 1×10^8 CFU.mL^{-1}. The results showed that both LAB could induce the production of IgG responses to the LPS depending on the challenged cell concentration and strain dependence as shown in Table 7. For *P. pentosaceus* KUNNE6-1, the highest IgG production of 3.83µg.mL-1 was observed at 1×10^6 CFU.mL^{-1} of challenge concentration; the induced IgG concentration showed a decreasing trend with an increase in the challenged cell

concentration. In contrast, *Lb. johnsonii* KUNN19-2 at 1×10^8 CFU.mL^{-1} could induce the highest IgG production of 3.66 μg.mL^{-1}.

Table 7 Effect of heat-killed cells of *Lactobacillus johnsonii* KUNN19-2 and *Pediococcus pentosaceus* KUNNE6-1 to Immunoglobulin G (IgG) production of BALB/c mice spleen cells responses to lipopolysaccharide (LPS)

Treatment	IgG concentration (μg.mL^{-1}) (mean ± SD) *
Spleen cells	0.31 ± 0.04 ^{e}
LPS	2.32 ± 0.02^{c}
Lb. johnsonii KUNN19-2 (1×10^6 CFU.mL^{-1})	2.50 ± 0.02^{c}
Lb. johnsonii KUNN19-2 (1×10^7 CFU.mL^{-1})	3.04 ± 0.03^{b}
Lb.johnsonii KUNN19-2 (1×10^8 CFU.mL^{-1})	3.66 ± 0.03^{a}
P. pentosaceus KUNNE6-1 (1×10^6 CFU.mL^{-1})	3.83 ± 0.06^{a}
P. pentosaceus KUNNE6-1 (1×10^7 CFU.mL^{-1})	2.94± 0.04^{d}
P. pentosaceus KUNNE6-1 (1×10^8 CFU.mL^{-1})	2.89 ± 0.02^{d}

* $^{a-e}$ = Mean values within a column that are not identified with a common lowercase superscript are significantly different ($P < 0.05$).

It was shown that the increment of IgG production depended on the challenged cell concentrations applied. The IgG production of mice splenocytes was stimulated by both heat-treated strains responses to Con A. It was found that only heat-killed cells of *Lb. johnsonii* KUNN19-2 at 1×10^8 CFU.mL^{-1} could significantly induce IgG production of 4.24 μg.mL^{-1} as shown in Table 8 while the heat-killed cells of *P. pentosaceus* KUNNE6-1 could not.

Table 8 Effect of heat-killed cells of *Lactobacillus johnsonii* KUNN19-2 and *Pediococcus pentosaceus* KUNNE6-1 to immunoglobulin G (IgG) production of BALB/c mice spleen cells responses to concanavalin A (Con A).

Treatment	IgG concentration ($\mu g.mL^{-1}$) (mean ± SD)*
Spleen cells	0.76 ± 0.01^{g}
Con A	2.75 ± 0.08^{b}
Lb. johnsonii KUNN19-2 (1×10^{6} CFU.mL^{-1})	2.34 ± 0.06^{d}
Lb. johnsonii KUNN19-2 (1×10^{7} CFU.mL^{-1})	2.72 ± 0.02^{c}
Lb. johnsonii KUNN19-2 (1×10^{8} CFU.mL^{-1})	4.24 ± 0.03^{a}
P. pentosaceus KUNNE6-1(1×10^{6} CFU.mL^{-1})	2.20 ± 0.04^{f}
P. pentosaceus KUNNE6-1(1×10^{7} CFU.mL^{-1})	2.07 ± 0.03^{e}
P. pentosaceus KUNNE6-1(1×10^{8} CFU.mL^{-1})	2.67 ± 0.10^{c}

* $^{a-g}$ = Mean values within a column that are not identified with a common lowercase superscript are significantly different ($P < 0.05$).

Stimulation of antibody production appears to be an important mechanism by which bacteria may interact with the host immune system in numerous ways and act to influence the host immune response and increase the resistance to infection (Huang *et al*., 2004; Jian-Kui *et al*., 2009). Few studies have examined the direct mitogenic effects of the probiotic or other lactic acid bacteria, and focused on cellular division as a sign of mitogenic activity, rather than induction of antibody production (Shimizu *et al*., 1988; Kado-Oka *et al*., 1991, Tejada *et al*., 1999).

In this study, *Lb. johnsonii* KUNN19-2 being the most effective inducer of antibody production, also the most potent inducer of splenocytes proliferation (T and B lymphocytes activation). While direct mitogenic stimulation resulting in antibody production by B-cells (B lymphocytes) would explain many of the elevated antibody levels observed in challenge studies, the immunostimulatory effect of LAB may be

dependent on their interaction with other cell types. Some bacteria may act on T-lymphocytes, stimulating the production of cytokines essential for sending signal to B- lymphocytes to induce antibody production (Easo *et al*., 2002). Cytokine induction by several strains of LAB, including *L. acidophilus* (strains E507, La1, La2 and NCK 56) and *L.delbreuckii* subsp. *bulgaricus* has been reported (Guencheva *et al*., 1992; Solis Pereyra and Lemonnier, 1993; Popova *et al*., 1993; Miettinen *et al*., 1996, 1998; Marin *et al*., 1997, 1998, He *et al.*,2002, Borruel *et al*., 2003), but the ability of the LAB in general to directly activate T-cells appears to be less potent, and may require co-stimulatory signals (Marin *et al*., 1998). Whether mediated directly via B-cell activation or indirectly via effects on cytokine production (T-cell activation), stimulation of antibody production by the LAB appears to be an important mechanism through which these probiotic bacteria may act to influence the immune response and increase resistance to infection.

In the current study, heat-treated *Lb. johnsonii* KUNN19-2 and *P. pentosaceus* KUNNE6-1 in an *ex vivo* system were tested to determine whether non-viable LAB could modulate antibody production and it was found that both strains were able to induce the lymphocyte proliferation and modulate IgG production. The results indicated that a heat-resistant structural component of both strains could effectively induce the polyclonal B-cells, and were responsible for the majority of antibody production, and this may underline reports of the potential beneficial health effects of consumption of the heat-killed cells of these selected strains. Cell wall components, which constitute heat-killed cells, may be a factor in the immune modulation (Adams, 2010). Therefore, the identification and comparison of the cell wall composition of these strains might be one of the interesting subjects for future study. *Lb. johnsonii* KUNN19-2 and *P. pentosaceus* KUNNE6-1 have already been proposed as good starters for Nham production in Thailand. After fermentation, the starter used may or may not survive. However, their cell components especially from *Lb. johnsonii* KUNN19-2 have shown an excellent immune response. Such a substantial benefit suggests a new opportunity for future Nham product to be specifically formulated for health, especially immunity enhancement. However, both nutrition levels and dosage should be the focus of more detailed study in the future.

2. Technological properties of *Lactobacillus johnsonii* KUNN19-2 and *Pediococcus pentosaceus* KUNNE6-1

In general, *Lactobacillus sakei*, *Lb. curvatus*, *Lb. plantarum*, *Lb. pentosus* , *Lb. casei*, *Pediococcus pentosaceus* and *P. acidilactici* which were isolated from fermented meat products and also naturally found in meat raw materials, are the species most used as commercial meat LAB starter cultures (Hammes and Hertel, 1998). As LAB originating from fermented meats are particularly well adapted to the ecological niche of meat fermentation and thus should be considered for selection as starter cultures (Hugas and Monfort, 1997). In this study, *Lactobacillus johnsonii* KUNN19-2 and *Pediococcus pentosaceus* KUNNE6-1 which were isolated from commercial Thai-style fermented pork sausage were tested for the technological properties as a starter culture in chicken cartilage fermentation.

2.1 Quality changes during chicken cartilage fermentation using *Lactobacillus johnsonii* KUNN19-2 and *Pediococcus pentosaceus* KUNNE6-1 as starter culture.

2.1.1 pH changes

Fermented chicken cartilage is one of typical fermented products. This kind of product is characterized by low acidity with the final pH of approximately 4.6 (Jaichumjai, *et al*., 2011). Changes of pH during fermentation of chicken cartilage was investigated for both selected strains (*Lactobacillus johnsonii* KUNN19-2 and *Pediococcus pentosaceus* KUNNE6-1). It was found that for the natural fermentation samples (control), pH value was gradually changed from the first hour of fermentation and 84 h were needed to finally reach pH 4.58. Compared to controlled samples, chicken cartilage inoculated with *Lb. johnsonii* KUNN19-2 performed the fastest acidification (Figure 8), within 24 hr, the product was acidified to a pH level of 4.59, whereas a slower acidification was observed for *P. pentosaceus* KUNNE6-1, reaching pH 4.58 after 48 h of fermentation.

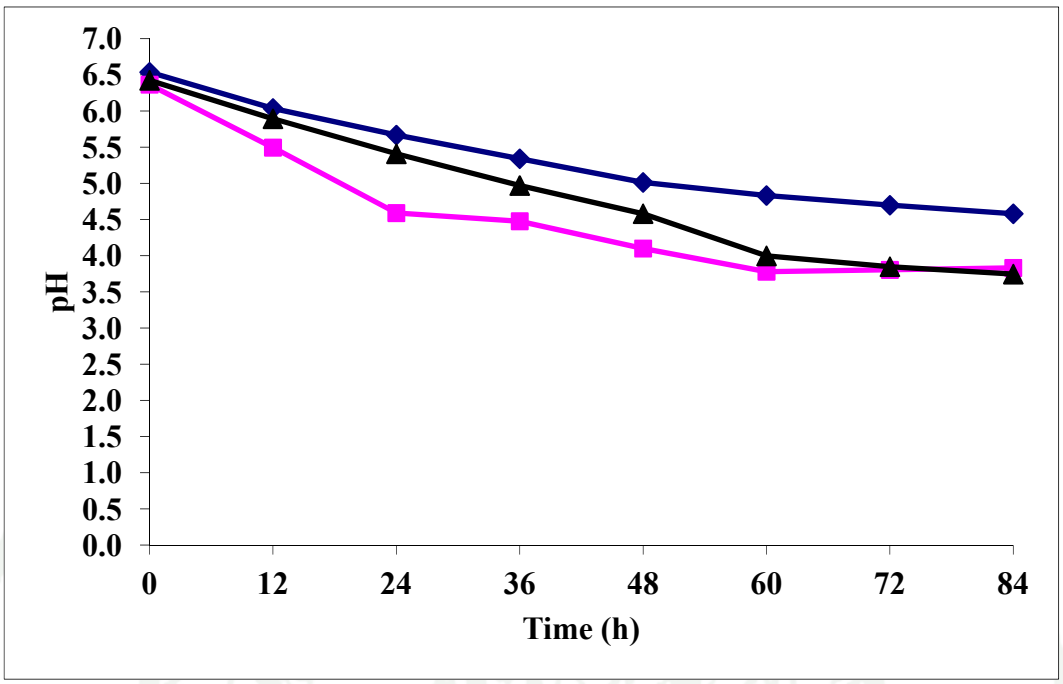

Figure 8 pH changes during fermentation of Fermented chicken cartilage inoculated with *Lb. johnsonii* KUNN19-2 (—■—) and *P. pentosaceus* KUNNE6-1 (—▲—) compared to natural fermented (control) samples (—◆—).

2.1.2 Lactic acid bacterial counts (total plate count on MRS agar).

Lactic acid bacterial counts (LAB counts) during chicken cartilage fermentation using *Lactobacillus johnsonii* KUNN19-2 and *Pediococcus pentosaceus* KUNNE6-1 as the starter culture were performed by using total plate count on MRS agar where the results are presented in Figure 9.

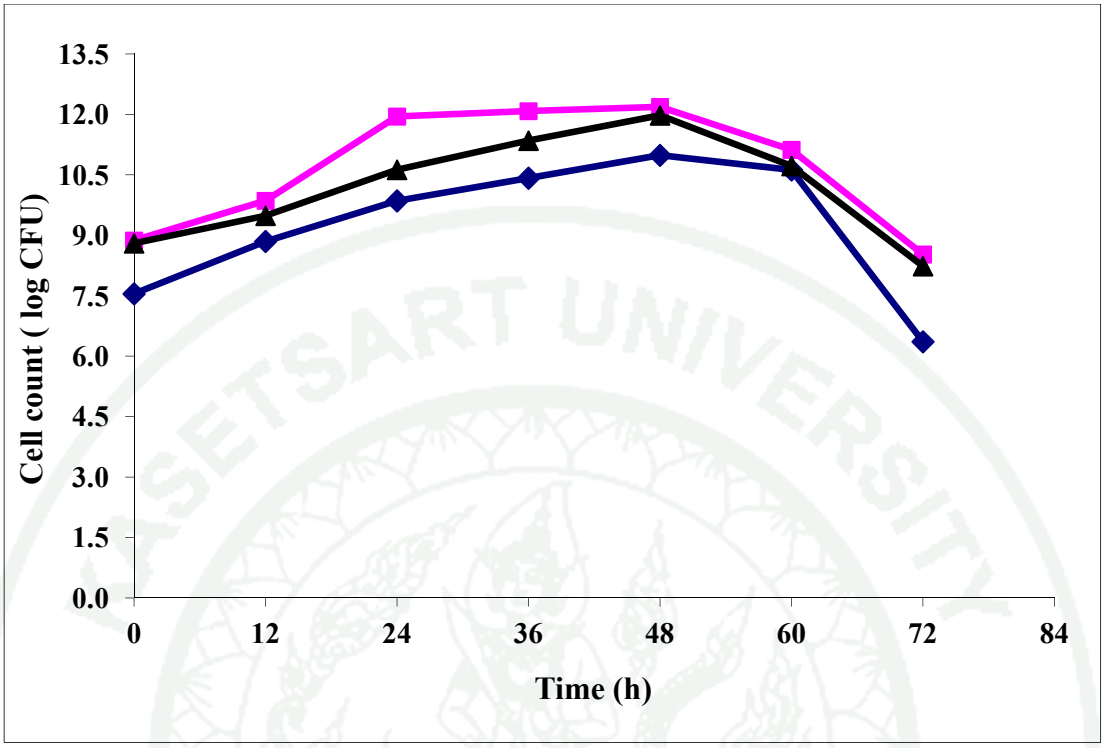

Figure 9 LAB counts during fermentation of fermented chicken cartilage inoculated with *Lb. johnsonii* KUNN19-2 (—■—) and *P. pentosaceus* KUNNE6-1 (—▲—) compared to natural fermented (control) samples (—◆—).

The desire pH of fermented chicken cartilage was around 4.6, which is typical for this kind of meat products, and this was the result of the classical trend of microbial growth in products, where LAB are increasing in numbers at the very beginning of the fermentations, producing acids. The natural fermentation (control sample) shows gradually increase of the LAB populations, starting with 7.549 log $CFUg^{-1}$ and reaching values of 10.986 log $CFUg^{-1}$ at 48 h of fermentation, while chicken cartilage fermented with both strains had LAB counts higher than control sample at the beginning and rapidly increased during fermentation. LAB counts in samples inoculated with *Lb. johnsonii* KUNN19-2 increased faster and had 11.948 log $CFUg^{-1}$ when reached the desire pH (4.59) within 24 h, whereas sample inoculated with *P. pentosaceus* KUNNE6-1 had 11.980 log $CFUg^{-1}$, pH 4.58 at 48 h of fermentation. From this study, it was found that LAB count of all samples were

drastically decreased after 60 h, but control sample remained only 6.359 log $CFUg^{-1}$ whereas both samples inoculated with *Lb. johnsonii* KUNN19-2 and *P. pentosaceus* KUNNE6-1 still remained high LAB counts (8.525 and 8.230 log $CFUg^{-1}$ respectively) at the end of fermentation process (72 h).

In meat fermentations, beside creates the biochemical conditions to attain the new sensory properties of the ripe products through modification of the raw materials, the other main functions of LAB are to obtain a rapid pH drop of the batter, which in turn offer product safety by inactivating unwanted organisms especially pathogens and food poisoning and further obtain product stability and shelf life by inhibiting undesirable changes caused by spoilage microorganisms. (Lücke, 2000).

The ability of the starter culture to compete with the natural microbiota of the raw material and to undertake the desirable metabolic activities is considered by its growth rate and survival in the fermentation conditions such as an anaerobic atmosphere, temperature, rather high salt concentrations and low pH. The salt concentration which commonly used in the sausage batter is about 2% (A_w = 0.94-0.98) and can eventually reach up to 15% (A_w = 0.85-0.86) in the final product (Montel, 1999). The manufacturing temperature ranges from 4 to 10^oC when preparing the batter and from 30 to 35 ^oC during the fermentation period. The initial pH of the meat batter, which is generally around 6.5 decreases during fermentation and reaches to the pH values between 4.5-5.0. Thus, over the entire meat fermentation process the growth rate at different temperatures, the tolerance of salt concentrations and of pH in the range 4.5-6.5 are limiting factors affecting the persistence and competitiveness of the starter culture.

Lactic acid bacteria are traditionally used to produce or to preserve various food products such as fermented milks, meats, and vegetables. Their ability to initiate rapid acidification of the raw material is essential to improve the flavor, texture, and safety of these products. One of the most important characteristics of *Lactobacillus* in fermented meat products is the production of lactic acid. The acidification has positive effects on safety and on the sensory characteristics of the product. The pH

decrease in fermented sausages provides the coagulation of myofibrillar proteins, resulting in the increase of firmness and cohesiveness of the final fermented product, and contributes to the flavor and red color. Furthermore inhibition of spoilage and pathogenic microorganisms is also provided by the fast decrease of pH and lactic acid production in appropriate quantities. The fast decrease in pH values during fermentation of sausages can also contribute to the prevention of the accumulation of biogenic amines, which are harmful to health (Ammor and Mayo, 2007).

Lactic acid is a weak acid ($pK_a = 3.86$), with a pH dependent, dissociated lactate level that is prevalent at low pH. The concentration of the lactate form was well correlated with cellular mortality and membrane damage. A high level of undissociated lactate led to a high level of dead cells and then to a poor physiological state of the population (Rault *et al*., 2009). The undissociated form of this organic acid is a strong growth inhibitor for the organism, penetrate to the cytoplasmic membrane, resulting in reduced intracellular pH and disruption of the trans-membrane proton motive force (Amrane and Prigent, 1999).

The lactic acid inhibition mechanism may be described by combining total lactic acid concentration and pH, thus taking into account the membrane integrity damage induced by the ionic form at high pH values. From a mechanistic point of view, this phenomenon can be explained by considering pH homeostasis in the cells. Lactic acid affects pH homeostasis through a mechanism that depends upon both proton concentration, normally referred to the weak acid inhibition mechanism that inhibits cellular reactions, and lactate concentration, which might affect membrane integrity and cellular viability (Rault *et al*., 2009).The acidification of the cytoplasm induced by the non-dissociated form of the weak organic acid leads to the collapse of the proton motive force. This phenomenon inhibits nutrient transport and enzymatic reactions and leads to DNA alteration and biomass inactivation (Even *et al*.,2002; Gonçalves *et al*.,1998).Maintaining the extracellular pH at a high value helps the cells stabilize their intracellular pH at a sufficiently high value, thus decreasing the inhibiting effect of lactic acid.

In general bacteria display a characteristic four-phase pattern of growth. The initial lag phase is a period of slow growth during which the bacteria are trying to adapt to the conditions in the medium or food matrix. This is followed by a logarithmic or log Phase, during which growth is exponential, with cells doubling every replication cycle. After that stationary phase occurs when the nutrients become limiting or the concentration of metabolic waste in the medium becomes high, and the rate of bacterial cell replication is reasonably equal to the rate of cell death. Finally death phase occurs when cells die faster than they are replaced. In the first period corresponded to the exponential phase. The intracellular pH and the pH gradient remained stable at neutral. When entering the stationary phase, once more acid was produced, the cells displayed a very low specific acidification activity and decreased cell viability, indicating that they were in a unpleasant physiological state. This may because of the metabolic activity of the cells was mainly devoted to their growth during the log phase instead of their physiological state (Rault *et al*., 2008).

It was found in this study that LAB counts of all fermented chicken cartilage samples showed the reasonably related results with pH changes as the higher LAB counts, the greater and faster pH decreasing (Figure 8). Both selected strains especially *Lb. johnsonii* KUNN19-2 showed the good technological properties to produce high acid and remain the high cell numbers throughout the fermentation process and this also as well implied to acid tolerance properties of this organism.

2.3 Bacterial ecology of Fermented Chicken Cartilage inoculated with *Lactobacillus johnsonii* KUNN19-2 and *Pediococcus pentosaceus* KUNNE6-1

In order to overcome the uncertainty and time consumed by the phenotypic methods which were relied on physiological or biochemical for LAB identification , molecular methods such as PCR-denaturing gel electrophoresis (Cocolin *et al*., 2004) species-specific PCR (Aymerich et al., 2006), real-time PCR (Furet *et al*., 2004) have been developed and employed for LAB species identification. The most common isolated LAB from sausages are *Lactobacillus*, *Pediococcus*, *Leuconostoc*, *Weissella* and *Enterococcus* (Ammor *et al*., 2005;

Aymerich *et al*., 2006; Garriga *et al*., 2005; Santos *et al*., 2005). The dominant species are usually members of *Lactobacillus* (Ammor *et al*., 2005; Martn *et al*., 2005).

In order to demonstrate the role of the organisms (particularly the LAB) in the production of fermented chicken cartilage, it is essential to quantify the predominating groups of organisms and to investigate the dynamics of the overall community. In addition, the desire quality of the product and product shelf life strongly rely on the way the fermentation was performed. Identifying the key organisms is a necessary step in the development of a mixed or multi-strains starter culture that would standardize the manufacturing of this product.

The present work involves the use of LAB not only to provide organoleptic properties of fermented chicken cartilage but also for bioprotection and health benefit from probiotic effects of both tested strains; *Lb. johnsonii* KUNN19-2 and *P. pentosaceus* KUNNE6-1 as well

From the results of survival abilities in the gastrointestinal tract and immune stimulation, *Lactobacillus johnsonii* KUNN19-2 has already shown that it has outstanding potential to be used as probiotic. Therefore, the next step is study the application of this strain in chicken cartilage fermentation.

Total DNA was extracted three times independently from each fermented chicken cartilage sample and pure culture of *Lb. johnsonii* KUNN19-2 and *Pediococcus pentosaceus* KUNNE6-1 and used in amplification reactions.

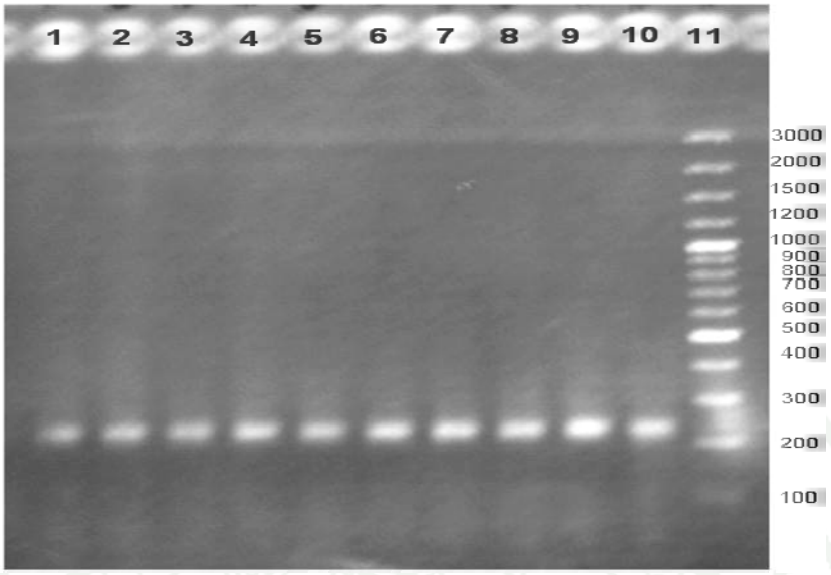

Figure 10 PCR product bands from extracted DNA of pure culture of *Lb. johnsonii* KUNN19-2 (Lane1-6), *P. pentosaceus* KUNNE6-1 (Lane 7-10) and Marker DNA100 bp ladder (Lane 11) in 1.5% agarose gel.

The PCR product bands from extracted DNA of pure culture of *Lb. johnsonii* KUNN19-2 (Lane1-6), *P. pentosaceus* KUNNE6-1 (Lane 7-10) and Marker DNA100 bp ladder (Lane 11) in 1.5% agarose gel are shown in Figure 10. The PCR products obtained for all tested samples in this study was approximately 250 base pairs long**.**

The melting behavior of the PCR products from extracted DNA of pure strains and fermented chicken cartilage samples were examined then used as the DNA template in DGGE analysis. The great majority of the tested samples produced a single DGGE band with a melting position identical to that of one of the bands identified in the fermented chicken cartilage DNA fingerprint.

The fingerprints obtained with the duplicates natural fermented chicken cartilage (control samples) contained multiple light bands (Figure 11). The lowest band (band 9) disappeared during 24-36 hr of fermentation (Lane 6 -10) and another higher intensity band appeared at 48 h (band 8). This fingerprint showed is well related with the total plate count on MRS agar of control samples (Figure 9) that cell

counts reached the highest number during 48-60 h of fermentation time and decreased after that and this may because of the lower pH (higher acidity) of sample according to lactic acid produced from bacteria.

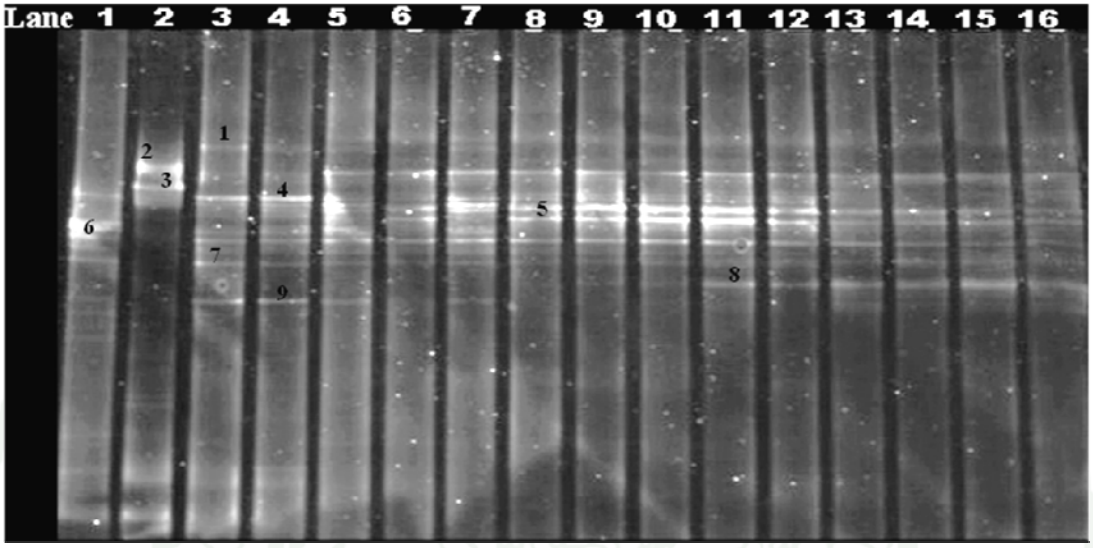

Figure 11 Bacterial DGGE of DNA extracted from natural fermented chicken cartilage (control samples) in every 12 h of fermentation time.

Lane 1. Fresh chicken cartilage
Lane 2. Pure culture: *Lb. johnsonii* KUNN19-2
Lane 3-4. Control, 0 h.
Lane 5-6. Control, 12 h.
Lane 7-8. Control, 24 h.
Lane 9-10. Control, 36 h
Lane 11-12. Control, 48 h
Lane 13-14. Control, 60 h
Lane 15-16. Control, 72 h

The PCR-DGGE technique is widely employed in microbial diversity study because it is capable of providing a fingerprint of the bacterial community in the sample after a direct DNA extraction. Briefly, food sample is subjected to DNA extraction obtaining a mixture containing DNA from the bacterial species occurring in the sample. The DNA mixture is then used as template in PCR amplifications of particular variable DNA regions of taxonomic interest by obtaining an amplified product that is a mixture of amplicons from the species present in the initial sample. All the amplicons have the same size but different sequences, and can be thus

separated by DGGE. The final result is a fingerprint that is specific of the analyzed sample and contains a series of bands relative to the microbial species present in the food sample. Identification of those species can be finally achieved by purifying and sequencing the bands in the DGGE profile.

PCR-DGGE patterns of fermented chicken cartilages from this study did not perform sequencing step, but were further analyzed by cluster analysis. The resulting fingerprints were analyzed using Syngene GeneDirectory Application - Version 2.01.02 . The software was used to calculate the genetic similarity with Dice coefficients, cluster DNA profiles using the Unweighed Paired Group Arithmetic Average linkages (UPGMA) method and create dendrograms for the isolates.

The UPGMA method assumes a constant evolutionary rate occurs between the different lineages and identifies patterns for similarity, allowing the tree to be constructed in a step-wise manner (Opperdoes, 1997). A position tolerance of 0.5% was chosen for the generation of all obtained dendrograms from this experiment.

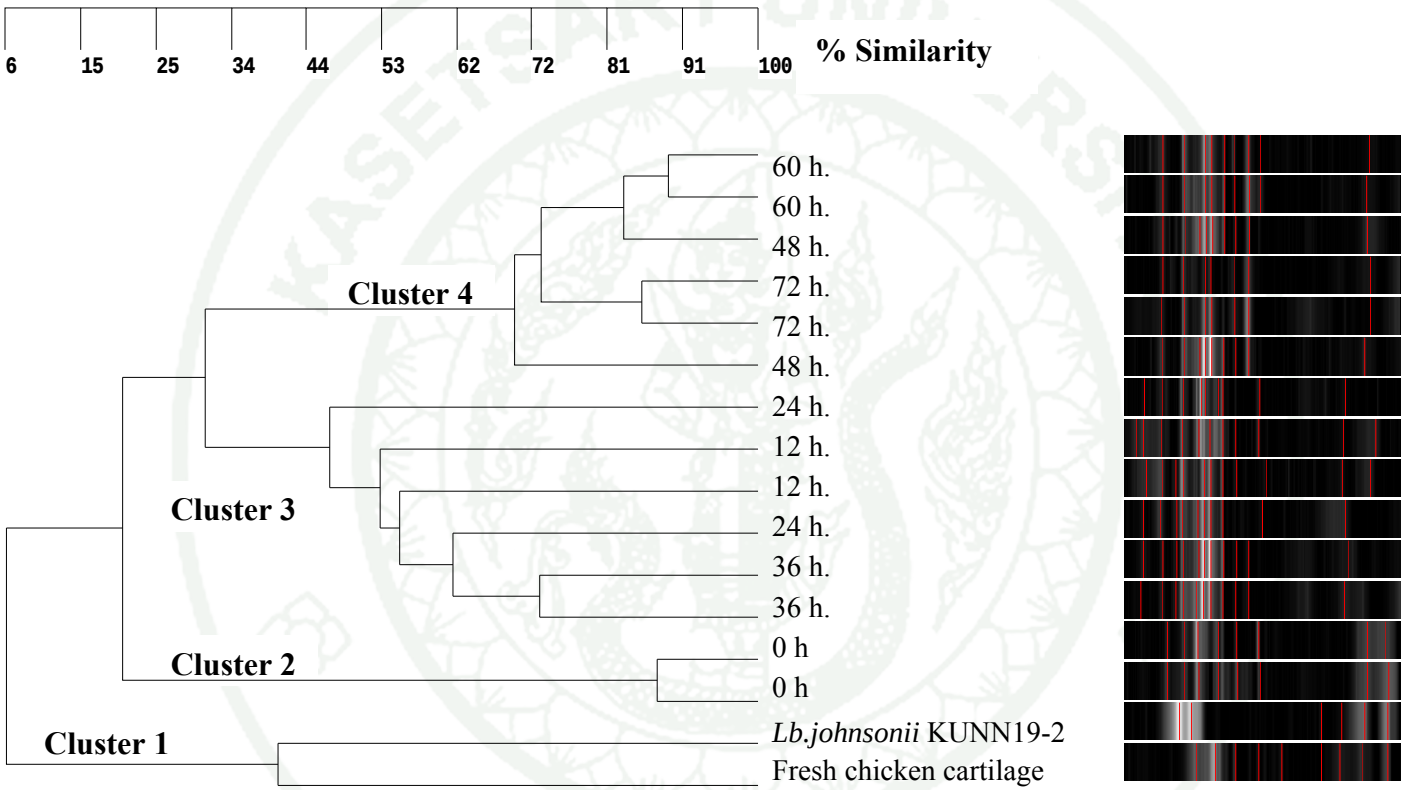

Figure 12 Phylogenetic dendrograms of the bacterial population isolated from natural fermented chicken cartilage (control samples) in every 12 h interval during the fermentation process, based on the UPGMA linkage of Dice coefficients (expressed as % similarity values for convenience in the X axis)

Cluster analysis is an exploratory technique designed to identify discrete patterns withi multivariate data (Kaufman and Rousseeuw, 1990; van Tongeren, 1995).The method works by determining similarity or dissimilarity among samples and grouping them accordingly. The obtained dendrograms of natural fermented chicken cartilage (control samples) from the cluster analysis are shown in Figure 12. No differences in the profiles were observed for the duplicate fermentations or within each sampling point.

With regards to the natural fermentations (control samples), the profiles clustered in 4 groups. Clusters 1(38% similarity) was related to fresh chicken cartilage samples and pure culture; *Lb. johnsonii* KUNN19-2 , cluster 2 (86% similarity) was natural fermented chicken cartilage (control) samples at the beginning of fermentation process (0 h).The clustering analysis demonstrated that the shift of microbial diversity during natural fermentation of chicken cartilage took place between 12 and 72 h: from 12 to 36 h, all patterns belonged to cluster 3 (46% similarity) with high intensity bands (band 5 in Figure 11), while the lowest band (band 9 in Figure 11) disappeared. After 48 h (from 48 to 72 h), the patterns were grouped into cluster 4 (69.5% similarity), with another higher intensity band appeared at 48 h (band 8 in Figure 11). The exhibition of microbial shift corresponded to the increase in cell counts during 12-48 h and the highest counts at 48-60 h during natural fermentation of chicken cartilage as shown in Figure 9.

From Figure 13, showed the DGGE fingerprinting of chicken cartilage inoculated with *Lb. johnsonii* KUNN19-2 at every 12 h of fermentation process. It was also found that no differences in the profiles of duplicate fermentations or within each sampling point. From the obtained DGGE fingerprint, it apparently showed that the microbial diversity of the inoculated chicken cartilage sample with *Lb. johnsonii* KUNN19-2 was lower compared to the natural fermented (control) sample.

It was found that one or two high intensity band (band 3 and 7) appeared at the beginning with another high intensity band (band 4) which has started to appear from the 24th h until the end, and this indicated that 1-2 dominate strains during the first 48 h of fermentation process and later that the time of 60 h of process, there was one more band of another strain (band 8) appeared to be the dominant. From this fingerprint, the very similar DGGE profiles, at least with respect to the most intense bands, of the different time interval samples strongly suggested that the same species were dominated in all samples through the fermentation process, although they were presented in different relative proportions.

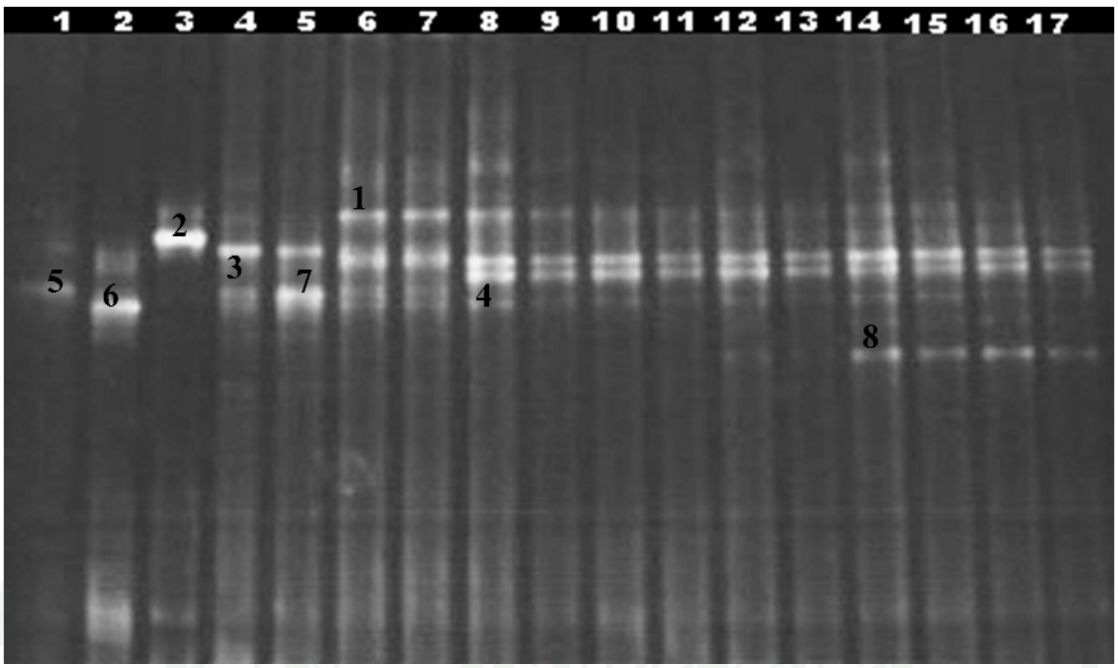

Figure 13 Bacterial DGGE of DNA extracted in every 12 h of fermentation time from Fermented chicken cartilage inoculated with *Lactobacillus johnsonii* KUNN19-2

Lane 1. Fresh chicken cartilage

Lane 2. Pure culture: *P. pentosaceus* KUNNE6-1

Lane 3. *Pure culture*: *Lb. johnsonii* KUNN19-2

Lane 4-5. *Lb. johnsonii* KUNN19-2 inoculated samples, 0 h.

Lane 6-7. *Lb. johnsonii* KUNN19-2 inoculated samples,12 h.

Lane 8-9. *Lb. johnsonii* KUNN19-2 inoculated samples, 24 h

Lane 10-11. *Lb. johnsonii* KUNN19-2 inoculated samples, 36 h

Lane 12-13. *Lb. johnsonii* KUNN19-2 inoculated samples, 48 h

Lane 14-15. *Lb. johnsonii* KUNN19-2 inoculated samples, 60 h

Lane 16-17. *Lb. johnsonii* KUNN19-2 inoculated samples, 72 h

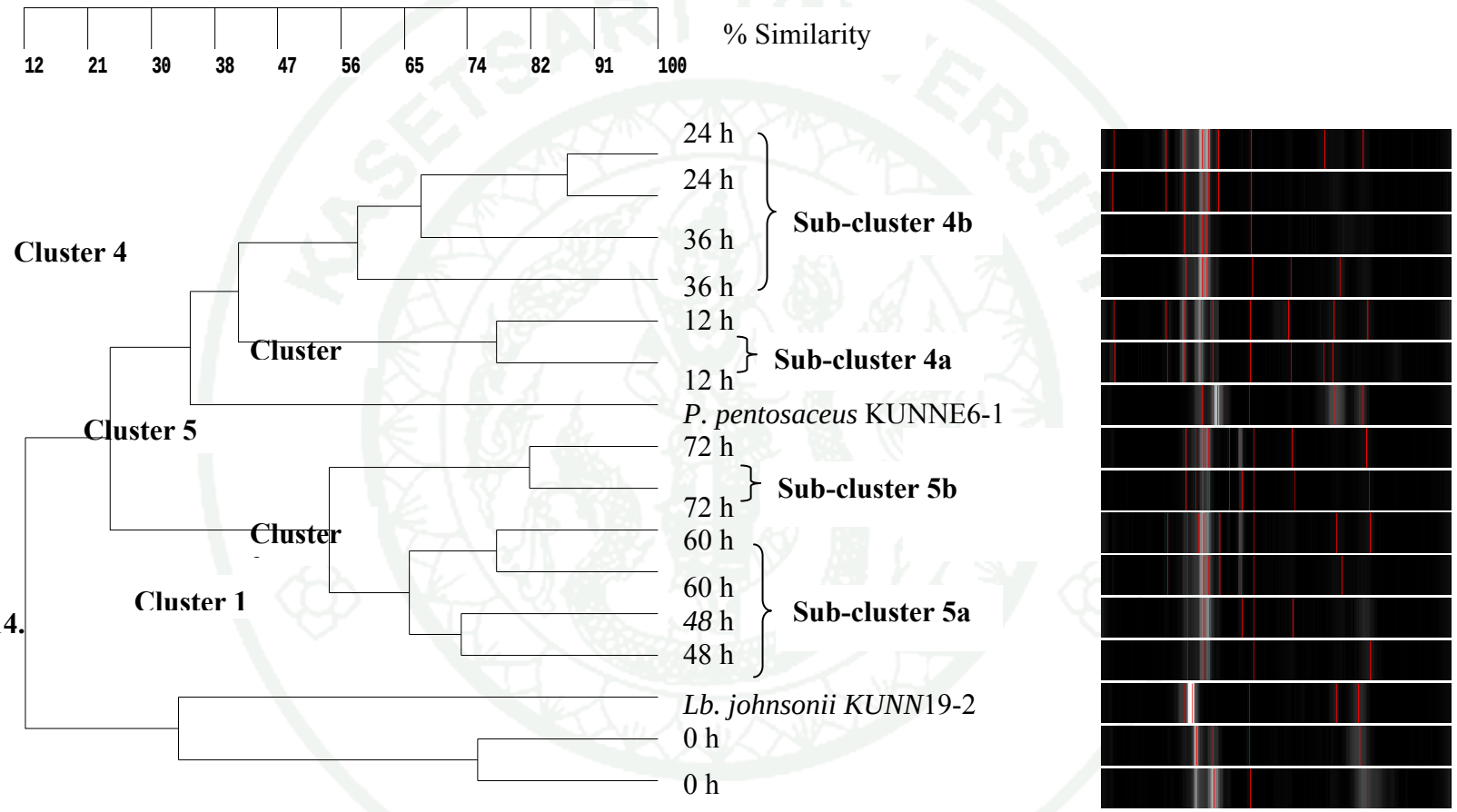

Figure 14.

Figure 14 Phylogenetic dendrograms of the bacterial population isolated from fermented chicken cartilages inoculated with *Lb. johnsonii* KUNN19-2 in every 12 h interval during the fermentation process, based on the UPGMA linkage of Dice coefficients (expressed as % similarity values for convenience in the X axis).

The similarity between the DGGE patterns of the bacterial community in fermented chicken cartilage inoculated with *Lactobacillus johnsonii* KUNN19-2 was then further evaluated. The dendrogram was characterized by the presence of five well-defined clusters, and no differences in the profiles were as well observed for the duplicate fermentations or within each sampling time interval of this sample as shown in Figure 14.

Cluster 1 was with 74% similarity, related to fermented chicken cartilage inoculated with *Lb. johnsonii* KUNN19-2 at the beginning fermentation time (0h) while pure culture of *Lb. johnsonii* KUNN19-2 and *P. pentosaceus* KUNNE6-1 with an identical DGGE band pattern was separately grouped in cluster 2 and cluster 3 respectively. The change in microbial diversity of *Lb. johnsonii* KUNN19-2 inoculated sample was divided by cluster analysis into 2 main groups, according to the presence of DGGE bands during fermentation process.

From Figure 14, dendrograms at the first 12 to 36 h fermentation were grouped by 41% similarity into cluster 4. In this cluster, found that 1-2 high intensity bands (band 3 and 7 in Figure 13) appeared at the beginning with another high intensity band (band 4 in Figure 13) which has started to appear from the 24^{th} h until the end. However at the 12^{th} h, the disappearance of band 7 with one more new high intensity band (band 1 in Figure 13) was noticed, it then were grouped in the sub-cluster 4a with 78% similarity. From this result, it indicated that 1-2 dominate strains are responsible for the drastic pH reduction and eventually reached the desire pH during the first 24 h of chicken cartilage fermentation process. In this stage, the change has apparently occurred and started to be seen from the 24^{th} h of fermentation by the presence of double DGGE band (band 4 in Figure 13). Therefore, sample DGGE fingerprints of fermented chicken cartilage from 24 to 36 h that reached pH 4.6 with the remaining highest bacterial cell count were grouped by 66.5 % similarity into sub-cluster 4b as seen in Figure 14.

The last cluster (cluster 5, 52.5% similarity) of *Lb. johnsonii* KUNN19-2 inoculated chicken cartilage was grouped for sample DGGE fingerprints from 48 to 72 h of fermentation process which showed one more high intensity band of another strain (band 8 in Figure 13), and appeared to be the dominant in the fermentation process. This cluster analysis has obviously demonstrated that diversity of this sample started to change again at 48 h of fermentation process, as another group of bacteria was also dominate. During 48-60 h of fermentation process, the pH value of sample started to drop from 4.80 to 3.78 while the total cell count on MRS agar remain as high as 11.188 - 12.18 log $CFUg^{-1}$, so the results of DGGE fingerprints were grouped together in sub-cluster 5a (65 % similarity) and finally at the 72^{th} h which noticed that one DGGE band of sample is absent (band 1 in Figure 13), and it was found that sample pH value has shifted from 3.78 to 3.83, whereas the cell count has drop into around 8.525 $CFUg^{-1}$, this dendrogram is then grouped into sub-cluster 5b with 82 % similarity.

Before DGGE analysis, the DNA fragment of interest should be amplified using the following PCR cycling conditions (i) Heating at 94–96 °C, 1–9 minutes. (ii) Denaturation at 94–98 °C, 20–30 second to disrupt the hydrogen bonds between complementary bases, yielding single-stranded DNA molecules. (iii) Annealing at 50–65 °C, 20–40 second to allow annealing of the primers to the single-stranded DNA template meanwhile polymerase binds to the primer-template hybrid and begins DNA formation. (iv) Extension step: the DNA polymerase (Taq polymerase) synthesizes a new DNA strand complementary to the DNA template strand. At each extension step which is performed at 72 ^{o}C, the amount of DNA target is doubled, leading to exponential amplification of the specific DNA fragment. These steps are repeated from 28-35 times. Since the reaction is essentially exponential and since each cycle is about 5 minutes, a large quantity of DNA can be produced for analysis in as little as several hours.

However particular combinations of target gene fragments and primers may give rise to unusual profiles when analyzed using DGGE. One example is double bands (artifactual double bands) that occurred, which by definition should yield single bands and this have been reported by other researchers (Muyzer *et al*.,1993; van Hannen *et al*.,1998; Janse *et al*., 2003). Artifactual bands hamper interpretation and analysis of DGGE gels since they lead to an overestimation of sequence diversity. Moreover, the increased overlap of bands will make identification and retrieval of bands for sequencing more troublesome. Janse *et al.* (2004) whose work encountered DGGE profiles in which each prominent band was accompanied at close distance by another band, explained the formation of double bands. It could be that during each PCR cycle some secondary product is formed due to prematurely halted elongation, the enzyme TAQ polymerase may be obstructed by secondary structures. Finally they suggested that extended incubation at a high temperature could disrupt such structures and at the same time allow the enzyme to complete the elongation, then could eliminate the artifactual double bands in DGGE.

In this experiment, it was found that a greater microbial diversities were recovered from naturally fermented chicken cartilage, and this matrix was also carried out in exclusive uncontrollable process conditions thereby yielding products with variation in quality and organoleptic properties. This biodiversity and the associated complexity in the inoculated sample DGGE profiles, is sharply reduced during the fermentation time compared to natural fermented (control) sample, and just a few species appear to predominate and are commonly detected throughout the rest of the process. This may assumed that the added starter culture help to preferably promote the growth of dominant group while suppress some microflora in the food matrix by producing antibacterial substances.

When a final product is taken into consideration, then the microorganisms that have managed to persist and most likely were responsible for the transformation process should be detected. However, in this experiment, microorganisms that expected to play a role at earlier stages and survive until the end cannot be seen.

In this study, *Lb. johnsonii* KUNN19-2 was at first added for about 10^6 CFU per gram chicken cartilage and was expected to study its performance as starter culture but unfortunately cannot be seen even at the first fermentation period (0 h). The reasons of this were assumed and explained as follows;

i) The error from inoculating amount added into the batter at the beginning step of chicken cartilage fermentation, an uneven mixing step during sample preparation or uneven sampling method in DNA extraction process. Insufficient cell counts might then caused the missing DGGE band. Cocolin *et al*. (2004) reported that *Lactococcus lactis* subsp. *lactis*, *L. casei*, and *E. casseliflavus*, isolated at day 0, did not give a specific band at either the DNA or RNA level, meaning that the relative population were below the detection limit of 10^4 $CFUg^{-1}$

ii) Due to food processing and food constituents, the DNA in sample matrixes can be present in low amounts and also degraded. Cankar *et al*. (2006) stated that from the perspective of a detection laboratory such fragmentation in defining matrixes and identifying substances that affect PCR in each product is impossible.

The more complex is the food matrix, the more difficult it is to achieve good extraction and to get rid of all the impurities that can negatively affect the amplification step. The case of food matrixes is particularly difficult; in fact, the presence of natural constituents such as lipids, proteins, carbohydrates, and salts, may render the DNA extraction very hard and some of these molecules can persist until the end of the extraction and are found in the extract. When the extracted DNA is used as template for the PCR, the matrix residues might act as inhibitors and caused nucleic acid lost and the missing bands in some cases. (Wilson,1997).Therefore, the DNA extraction procedure needs to be optimized in order to obtain the highest yield in extraction, and achieve the right degree of purification to have a DNA pure enough to be used as template for PCR reactions.

iii) The PCR itself may be a source of bias in molecular studies of fermented chicken cartilage samples. It was found that preferential amplification might be caused by re-annealing of the template DNA, which compromises the hybridization of the primers (Suzuki and Giovannoni, 1996). Because of preferential amplification, a mixture of bacterial DNA from a food complex community may be only partially amplified by PCR, thereby leading to a product where some of the original members of the community are missing, and this may implied to this experiment concerning the missing bands of *Lb. johnsonii* KUNN19-2 even it was added at the begining. Preferential amplification may represent a problem for the PCR-DGGE analysis of microbial communities from food. In fact, the number of species detected may not be real because of a lack of amplification by PCR of a specific DNA template. Therefore, the choice of the primer couple and the fragment to target is fundamental, and validation of the extraction procedure for different sample matrixes is necessary and is done by assessing the ability of the extraction method to provide suitable DNA from the specific matrix. (Cocolin *et al*., 2004)

Nowadays, the need for safe products with standard technological properties has resulted in increasing the use of starter cultures in fermented food, to control the process and inhibiting other undesirable microorganisms. The most promising bacteria for starter cultures are those which are isolated from the indigenous microflora of traditional products. These microorganisms are well adapted in the meat environment and are capable of dominating the microflora of products. The strains selected as starter cultures must have the most important technological properties (Hammes,1990). That information about the microflora present and active during chicken cartilage fermentation will benefit the production of fermented meat products. It also contributes to new developments in the field of microbial ecology.

Lactic acid bacteria (LAB) have long been used as starter cultures in the production of fermented sausages and other meat-derived commodities. They are known to alter the flavor, texture and appearance of foods, to retard spoilage and to reduce contamination (Hammes and Tichaczek, 1994). Metabolic byproducts of LAB have been shown to inhibit the growth of several important pathogens and to increase

product shelf life (Daeschel, 1989; Abee *et al*., 1995). These cultures are generally designed to meet food safety, shelf-life, technological effectiveness and economic feasibility criteria. Besides all these traditional properties, novel starter cultures should take into account the development and spreading of bacterial resistance to antibiotics and could protect consumers from harmful bacteria either by a rapid acidification or by the production of antimicrobials (bacteriocins) as natural food preservation (Stiles and Hastings, 1991; McMullen and Stiles, 1996; Hugas and Monfort, 1997; Hugas, 1998; De Martinis *et al*.,2002).Specially-selected cultures may also provide probiotic benefits, and, if properly modified, they may even be endorsed with nutraceutical traits (Jimenez-Colmenero *et al*., 2001). LAB-fermented food products may also be healthy because of probiotic effects; several LAB genera, such as *Lactobacillus*, *Leuconostoc*, *Lactococcus*, have been proved to possess probiotic activity. Probiotic species such as *Lactobacillus acidophilus* have been used safely for more than 70 years (Hammes and Tichaczek, 1994; Salminen *et al*.,1998).

Combining the ability to lowering pH and maintaining the high number cell counts during chicken cartilage fermentation. According to its resistance to some antibiotics and inhibition of some food poisoning bacteria (Kwanmuang, 2003) and immunity enhancement thus, *Lb. johnsonii* KUNN19-2 is the interesting starter culture for using in chicken cartilage fermentation.

CONCLUSION AND RECOMMENDATIONS

The ability to survive passage through the gastrointestinal tract and the immune modulation capability of two LAB strains-*Lb. johnsonii* KUNN19-2 and *Pediococcus. pentosaceus* KUNNE6-1-and their potential to be used as probiotics for human consumption were investigated. Both selected strains showed the capability to survive through the host GI tract, by tolerating acid and bile under simulated stomach and small intestine conditions, respectively.

Furthermore, both strains could also act in an immune regulatory manner *ex vivo* via the enhancement of the immune response by activating T and B lymphocytes to produce immunoglobulin G. This could be preliminary information for the characterization of both strains to be defined as probiotics with beneficial health effects on the immunological response. These constitute the basis for this strain to be used as health-promoting bacteria and warrant further clinical and in vivo investigations.

A greater microbial diversities were recovered from naturally fermented chicken cartilage, while the DGGE fingerprinting profile of *Lb. johnsonii* KUNN19-2 inoculated chicken cartilage were defined in three identical groups by cluster analysis technique. This referred to the shift in microbial diversity during fermentation process. Furthermore, this also indicated that 1-2 strains were dominated through the fermentation process, although they were presented in different relative proportions.

Lactobacillus johnsonii KUNN19-2 in this study showed some interesting technological properties as the starter culture in chicken cartilage product due to its ability to lowering the pH to the required level of fermented meat within 24 hr and maintaining a high number cell counts (total plate count on MRS agar) until the end of fermentation process (8.525 log $CFU.g^{-1}$). Although this experiment failed to monitor the performance of *Lb. johnsonii* KUNN19-2 in chicken cartilage fermentation but has suggested for the validation of the extraction procedure for

different sample matrixes to provide suitable DNA from the specific matrix for the future study.

LITERATURE CITED

Abreu M.T. 2010. Toll-like receptor signalling in the intestinal epithelium: how bacterial recognition shapes intestinal function. **Nat. Rev. Immunol.** 10: 131-44.

Adams, C.A. 2010. The probiotic paradox: Live and dead cells are biological response modifiers. **Nutr. Res. Rev.** 1-10.

Aderem, A. and R.J. Ulevitch. 2000. Toll-like receptors in the induction of the innate immune response. **Nature**. 406: 782-787.

Agren, L.C., L. Ekman, B. Lowenadler, J.G. Nedrud, and N.Y. Lycke. 1999.Adjuvanticity of the cholera toxin A1-based gene fusion protein, CTA1-DD, is critically dependent on the ADP-ribosyltransferase and Ig-binding activity. **J. Immunol**. 162: 2432-2440.

Ahn, Y.T., G.B. Kim, Y.S. Lim, Y.J. Baek and Y.U. Kim. 2003. Deconjugation of bile salts by *Lactobacillus acidophilus* isolates. **Int. Dairy J**. 13: 303-311.

Akira, S., K. Takeda and T. Kaisho. 2001. Toll-like receptors: critical proteins linking innate and acquired immunity. **Nat. Immunol**. 2: 675-680.

Alakomi, H.L., E. Skytta, M. Saarela, T. Mattila-Sandholm, K. Latva-Kala and I. Helander. 2004. Lactic acid permeabilizes Gram-negative bacteria by disturbing the outer membrane. **Appl. Environ. Microbiol**. 66: 2001-2005.

Alander, M., R. Satokari, R. Korpela, M. Saxelin, T. Vilpponen-Salmela,T. Mattila-Sandholm and A von. Wright. 1999. Persistence of colonization of human colonic mucosa by a probiotic strain, *Lactobacillus rhamnosus* GG, after oral consumption. **Appl. Env. Microbiol**. 65: 351-354.

Altermann, E., W.M. Russell, A. Azcarate-Peril, R. Barrangou, B. L. Buck, O. McAuliffe, N. Souther, A. Dobson, T. Duong, M. Callanan, S. Lick, A. Hamrick, R. Cano and T. R. Klaenhammer. 2005. Complete genome sequence of the probiotic lactic acid bacterium *Lactobacillus acidophilus* NCFM. **PNAS**. 102 :3906-3912.

Alvarez S., N. Gobbato, E. Bru, A.P. De Ruiz Holgado and G. Perdigón. 1998. Specific immunity induction at the mucosal level by viable *Lactobacillus casei*: A perspective for oral vaccine development. **Food Agric. Immunol**. 10: 79-87.

Ammor, M.S. and B. Mayo. 2007. Selection criteria for lactic acid bacteria to be used as functional starter cultures in dry sausage production: an update. **Meat Sci**. 76: 138-146.

_____., E. Dufour, M. Zagorec, S. Chaillou and I. Chevallier. 2005. Characterization and selection of *Lactobacillus sakei* strains isolated from traditional dry sausage for their potential use as starter cultures. **Food Microbiol**. 22: 529-538.

Ampe, F., N.B. Omar, C. Moizan, C. Wacher and J.P. Guyot. 1999. Polyphasic study of the spatial distribution of microorganisms in Mexican pozol, a fermented maize dough, demonstrates the need for cultivation-independent methods to investigate traditional fermentations. **Appl. and Environ. Microbiol**. 65: 5464-5473.

Amrane, A. and Y. Prigent. 1999. Differentiation of pH and free lactic acid effects on the various growth and production phases of *Lactobacillus helveticus*. **J. Chem. Technol. Biot**. 74:33-40.

Angel C.R., R.A. Dalloul, N.M. Tamim, T.A. Shellem and J.A. Doerr. 1999. Performance and nutrient use in broilers fed a lactobacillus –based probiotic, p. 98. *In* **Poutry Sci. Association 8 th Annual Meeting Abstracts**. August 8-11. Springdale, Arkansas.

Ananou, S., M. Maqueda, M. Martínez-Bueno1 and E. Valdivia. 2007. Biopreservation, an ecological approach to improve the safety and shelf-life of foods. **Communicating Current Research and Educational Topics and Trends in Applied Microbiology**. 475-486.

AOAC. 2000. Official methods of analysis. **AOAC International**. Maryland, USA.

Arihara, K., H. Ota, M. Itoh, Y. Kondo, T. Sameshima, H. Yamanaka, M. Akimoto, S. Kanai and T. Miki. 1998. *Lactobacillus acidophilus* group lactic acid bacteria applied to meat fermentation. **J. Food Sci**. 63: 544-547.

Aslim, P., Z. N. Yuksekdag, E. Sarikaya, and Y. Beyatli. 2005. Determination of the bacteriocin-like substances produced by some lactic acid bacteria isolated from Turkish dairy products. **LWT – Food Sci. Technol**. 38: 691-694.

Audisio, M.C., G. oliver and M.C. Apella. 1999. Antagonistic effect of *Enterococcus faecium* J96 against human and poultry pathogenic salmonella spp. **J. Food Prot**. 62(7): 751-755.

Åvall-Jääskeläinen, S. and A. Palva. 2005. Lactobacillus surface layers and their applications. **FEMS Microbiol. Rev**. 29: 511-529.

_____., K. Kyla-Nikkila, M. Kahala, T. Miikkulainen-Lahti and A. Palva. 2002. Surface display of foreign epitopes on the *Lactobacillus brevis* S-layer. **Appl. Environ. Microbiol.** 68 (12): 5943-5951.

Åvall-Jääskeläinen, S., A. Lindholm and A. Palva. 2003. Surface display of the receptor-binding region of the Lactobacillus brevis S-layer protein in Lactococcus lactis provides nonadhesive lactococci with the ability to adhere to intestinal epithelial cells. **Appl.Environ. Microbiol**. 69: 2230-2236.

Axelsson, L.T., T.C. Chung, W.G. Dobrogosz and S.E. Lindgren. 1989. Production of a broad spectrum antimicrobial substance by *Lactobacillus reuteri*. **Microb. Ecol. Health Dis**. 2: 131-136.

_____. 1998. Lactic acid bacteria: classification and physiology, pp. 1-72. *In* S. Salminen and A.von wright, eds. **Lactic Acid Bacteria: Microbiology and Functional Aspects**. Marcel Dekker,Inc.New York.

Ayabe, T., D.P. Satchell, C.P. Wilson, W.C. Parks, M.E. Selsted and A.J. Ouellette. 2000. Secretion of microbicidal alphadefensins by intestinal Paneth cells in response to bacteria. **Nat. Immunol**. 1: 113-118.

Ayebo, A.D., K.M. Shahani and R. Dam. 1981. Antitumor component of yoghurt fractionation. **J. Dairy Sci**. 64: 2318-2324.

Aymerich T., B. Martin, M. Garriga, M.C. Vidal-Carou, S. Bover-Cid and M. Fugas. 2006. Safety properties and molecular strain typing of lacid acid bacteria from slightly fermented sausages. **J. Appl. Microb**. 100: 40-49.

Bacus, J. 1984. Update meat fermentation. **Food Tech**. 38: 59:63.

Balasubramanya, N.N., B.R. Lokesh, H.P. Ramesh and T.P. Krishnakantha. 1995. Effect of lactic microbes on superoxide anion generating ability of peritoneal macro-phages and tissue histopathology of murines. **Indian J. Dairy Biosci**. 6: 28-33.

Banks, J.G., R.G. Broad and N.H.C. Sparks. 1986. Natural antimicrobial system and their potential in food preservation of future. **Biotech. appl. Biochem**. 8: 103-147.

Battcock, M. and S. Azam-Ali. 1998. **Bacterial fermentation.** Available Souece: http://www.fao.org/docrep/x0560e/x0560e10.htm, November 27, 2007.

Bearso, S., B. Bearason and J.W. Foster. 1997. Acid stress responses in Enterobacteria. **FEMS**. **Microl.Lett**. 147: 173-180.

Bendelac, A. 1995. CD1: presenting unusual antigens to unusual T lymphocytes. **Science** 269: 185-192.

Begley, M., C.G.M. Gahan, and C. Hill. 2005. The interaction between bacteria and bile. **FEMS Microbiol. Rev**. 29: 625-651.

Begley, M., C. Hill and C.G.M. Gahan. 2006. Bile Salt Hydrolase Activity in Probiotics. **Appl. Environ. Microbiol**. 1729-1738.

Beresford, T.P., N.A. Fitzsimons, N.L. Brennan and T.M. Cogan. 2001. Recent advances in cheese microbiology. **Int. Dairy J**. 11: 259-274.

Berg, R.D. 1983. Host immune response to antigens of the indigenous intestinal flora. pp. 101-126. **In** D. J. Hentges (ed.) **Human intestinal microflora in health and disease.** Academic Press, New York.

_____. 1998. Probiotic, probiotics or 'conbiotics'. **Trends Microbiol**. 6: 89-92.

Bernet-Camard, M.F., V. Lievin, D. Brassart, J.R. Neeser, A.L. Servin and S. Hudault. 1997. The human *Lactobacillus acidophilus* strain LA1 secretes nonbacteriocin antibacterial substances active in vitro and in vivo. **Appl. Environ. Microbiol**. 63: 2747-2753.

Bhakdi, S., T. Klonisch, P. Nuber and W. Fischer. 1991. Stimulation of monokine production by lipoteichoic acids. **Infec. Immun**. 59: 4614-4620.

Bhunia, A.K., M.C. Johnson and B. Ray, 1988. Purification, characterization and antimicrobial spectrum of a bacteriocin produced by *Pediococcus acidilactici*. **J. Appl. Bacteriol**. 65: 261-268.

Bibiloni, R., R.N. Fedorak, J.W. Tannock, K.L. Madsen, P. Gionchetti, M. Campieri, C. De Simone and R. Balfour Sarpor. 2005. DSL #3 probioticsmisture induces remission in patients with active ulcerative colitis. **Am. J. Gastroenterol**. 100: 1539-1546.

Bloksma N., E. De Heer, M. Van Dijk and M. Willers. 1979. Adjuvanticity of Lactobacilli. I. Differential effects of viable and killed bacteria. **Clin. Exp. Immunol**. 37: 367-375.

Boekhorst, J., R.J. Siezen, and M.C. Zwahlen. 2004. The complete genomes of *Lactobacillus plantarum* and *Lactobacillus johnsonii* reveal extensive differences in chromosome organization and gene content. **Microbiol**. 150: 3601-3611.

Bohme, H.M., F.D. Mellet, L.M.T. Dicks and D.S. Basson. 1996. Production of salami from ostrich meat with strains of *Lactobacillus sake*, *Lactobacillus curvatus*, and *Micrococcus* sp. **Meat Sci**. 44: 173-180.

Bongaerts, G.P. and R.S. Severijnen. 2005. Preventive and curative effects of probiotics in atopic patients. **Med. Hypotheses**. 64: 1089-92.

Boonthanom, S., P. Wiriyacharee and N. Chinprahast. 1994. Effect of sodium nitrate and sodium nitrite on Nham quality produced with glucono-delta-lactone and mixed starter cultures. **J. of Agri**. 10(3): 296-312.

Boot, H.J. and P.H. Pouwels. 1996. Expression, secretion and antigenic variation of bacterial S-layer proteins. **Mol. Microbiol.** 21 (6): 1117-1123.

Boot, H.J., C.P.A.M., Kolen, J.M. van Noort and P.H. Pouwels. 1993. S-Layer Protein of *Lactobacillus acidophilus* ATCC 4356: purification, expression in *Escherichia coli*, and nucleotide sequence of the corresponding genet. **J. of Bacteriol**. 175 (19): 6089-6096.

Boot, H.J., Kolen, C.P.A.M. and P.H. Pouwels. 1995. Identification, cloning, and nucleotide sequence of a silent S-layer protein gene of *Lactobacillus acidophilus* ATCC 4356 which as extensive similarity with the S-layer protein gene of this species. **J. of Bacteriol**. 177 (24): 7222-7230.

_____., _____ and _____. 1996a. Interchange of the active and silent S-layer protein genes of *Lactobacillus acidophilus* by inversion of the chromosomal *slp* segment. **Mol. Microbiol**. 21 (4): 799-809.

_____, _____, B. Pot, K. Kersters and P. H. Pouwels. 1996b. The presence of two S-layer protein-encoding genes is conserved among species related to *Lactobacillus acidophilus*. **Mol. Microbiol** 142 (9): 2375-2384.

Borruel, N., F. Casellas, M. Antolin, M. Llopis, M. Carol, E. Espiin, J. Naval, F. Guarner, F. and J.R. Malagelada. 2003. Effects of nonpathogenic bacteria on cytokine secretion by human intestinal mucosa. **Am. J. Gastroenterol**. 98: 865-870.

Bos N., C. Neeuwsen, H. Hooijkaas, R. Benner, B. Wostmann and J. Pleasants. 1987. Early development of Ig-secreting cells in young of germ-free BALB/c mice fed a chemically defined ultrafiltered diet. **Cell Immunol**. 105: 235-245.

Brandtzaeg, P. 1989. Overview of the mucosal immune system. **Curr. Top. Microbiol. Immunol**. 146: 13-23.

Brashears, M.M. and W.A. Durre. 1999. Antagonistic action of *L. lactis* toward *Salmonella spp*. And *Escherichia coli* 0157:H7 during growth and refrigerated storage. **J. Food Prot**. 62 (11): 1336-1340.

Bry, L., P.G. Falk, T. Midtvedt and J. I. Gordon J. I. 1996. A model of host-microbial interactions in an open mammalian ecosystem. **Science**. 273 (5280): 1380-1383.

Buck, B.L., E. Altermann, T. Svingerud and T.R. Klaenhammer. 2005. Functional analysis of putative adhesion factors in *Lactobacillus acidophilus* NCFM. **Appl. Environ. Microbiol.** 71: 8344-8351.

Buddhiman, T., P.T. Jyoti, S. Ulrich, M.A.P.F. Charles, G. Michael and H.H. Wilhelm. 2008. Phenotypic and genotypic identification of lactic acid bacteria isolated from ethnic fermented bamboo tender shoots of North East India. **Int. J. of Food Microbiol**. 121 (1): 35-40.

Buntin, N., S. Chanthachum and H. Tipparat. 2008. Screening of lactic acid bacteria from gastrointestinal tracts of marine fish for their potential use as probiotics. **Songklanakarin J. Sci. Technol**. 30 (Suppl.1): 141-148.

Caldwell, S., R.W. Hutkins, DJ McMahon, CJ Oberg, and J.R. Broadbent. 1998. Lactose and galactose uptake by genetically engineered *Pediococcus* species. **Appl. Microbiol. Biotechnol**. 49: 315-320.

Caldwell, S., D.J. McMahon, C.J. Oberg, and J.R. Broadbent. 1996. Development and characterization of lactose-positive *Pediococcus* species for milk fermentation. **Appl. Environ. Microbiol**. 62: 936-941.

Callegari, M.L., B. Riboli, J.W. Sanders, P.S. Cocconcelli, J. Kok, G. Venema and L. Morelli. 1998. The S-layer gene of Lactobacillus helveticus CNRZ 892: cloning, sequence and heterologous expression. **Microbiol**. 144(3):719-726.

Cankar, K., D. Štebih, T. Dreo1, J. Žel1 and K. Gruden. 2006. Critical points of DNA quantification by real-time PCR-effects of DNA extraction method and sample matrix on quantification of genetically modified organisms. **BMC Biotechnol**. 6 (37): 1-15.

Cario, E., G. Gerken and D.K. Podolsky. 2004. Toll-like receptor-2 enhances ZO-1 associated intestinal epithelial barrier integrity via protein kinase C. **Gastroenterol** 127: 224-238.

Casey, P.G., G.D. Casey, G.E. Gardiner, M. Tangney, C. Stanton, R.P. Ross, C. Hill and G.F. Fitzgerald. 2004. Isolation and characterization of anti-*Salmonella* lactic acid bacteria from the porcine gastrointestinal tract. **Lett. Appl. Microbiol**. 39: 431-438.

Castellano, P. and G. Vignolo. 2006. Inhibition of *Listeria innocua* and *Brochothrix thermosphacta* in vacuum-packaged meat by addition of bacteriocinogenic *Lactobacillus curvatus* CRL705 and its bacteriocins. **Lett. Appl. Microbiol**. 43: 194-199.

Castellano, P., R. Raya and G. Vignolo. 2003. Mode of action of lactocin 705, a two-component bacteriocin from *Lactobacillus casei* CRL705. **Int. J. Food Microbiol**. 85: 35-43.

Chalermchaikit, T. 2001. Prevalence and drug resistance of *Salmonella* contaminated in raw chicken meat. **J. Sci. Res. Chula. Univ.** 20 (6): 14-17.

Charalampopoulos, D., S.S. Pandiella, and C. Webb**.** 2003. Evaluation of the effect of malt, wheat and barley extracts on the viability of potentially probiotic lactic acid bacteria under acidic conditions. **Int. J. Food Microbiol**. 82: 133-141.

Charteris, W.P., P.M. Kelly, L. Morelli and J.K. Collins. 1998. Development and application of an in vitro methodology to determine the transit tolerance of potentially probiotic Lactobacillus and Bifidobacterium species in the upper human gastrointestinal tract. **J. Appl. Microbiol**. 84: 759-768.

Charteris, W.P., P.M. Kelly, L. Morelli and J.K. Collins. 2000. Effect of conjugated bile salts on antibiotic susceptibility of bile salt-tolerant *Lactobacillus and Bifidobacterium* isolates. **J. Food. Prot.** 63: 1369-1376.

Chateau, N., A.M. Deschamps and A. Hadjsassi. 1994. Heterogeneity of bile salts resistance in Lactobacillus isolates of a probiotic consortium.Lett. **Appl. Microbail**. 18: 42-44.

Choi, C.W., M.H. Park, S.H. Hwang, S.G. Woo, M.K. Song, J.J. Im, S.G. Hong and J.H. Kim. 2003. **Novel microorganism *Pediococcus pentosaceus* EROM 101, having immune enhancement, anticancer and antimicrobial activities. Free patents online**. Available Source: http://www.freepatentsonline.com/20050131288.html, November 27, 2007.

Chou, L. S. and B. Weimer. 1999. Isolation and characterization of acid and bile tolerant isolated from strains of *Lactobacillus acidophilus*. **J. Dairy Sci**. 82: 23-31.

Christensen, H.R., H. Frokiaer and J.J. Pestka. 2002. Lactobacilli differentially modulate expression of cytokines and maturation surface markers in murine dendritic cells. **J. Immunol**. 168: 171-178.

Chiu, H.H., C.C. Tsai, H.Y. Hsih and H.Y. Tsen. 2008. Screening from pickled vegetables the potential probiotic strains of lactic acid bacteria able to inhibit the Salmonella invasion in mice. **J. Appl. Microbiol.** 104: 605-612.

Cocolin, L., K. Rantsiou, L. Iacumin, R. Urso, C. Cantoni and G. Comi. 2004. Study of the ecology of fresh sausages and characterization of populations of lactic acid bacteria by molecular methods. **Appl. Environ. Microbiol**. 70: 1883-1894.

Cocolin, L., M. Marisa, C. Carlo and C. Giuseppe. 2001. Denaturing gradient gel electrophoresis analysis of the 16S rRNA gene V1 region to monitor dynamic changes in the bacterial population during fermentation of Italian sausages. **Appl. Environ. Microbiol.** 67: 5113-5121.

Cocolin, L., P. Dolci, K. Rantsiou, R. Urso, C. Cantoni and C. Giuseppe. 2009. Lactic acid bacteria ecology of three traditional fermented sausages produced in the North of Italy as determined by molecular methods. **Meat Sci**. 82: 125-132.

Coconnier, M.H., M.F. Bernet, S. Kerneis, G. Chauviere, J. Fourniat, and A.L. Servin. 1993. Inhibition of adhesion of enteroinvasive pathogens to human intestinal Caco-2 cells by *Lactobacillus acidophilus* strain LB decreases bacterial invasion. **FEMS Microbiol. Lett**. 110: 299-305.

Coconnier, M.H., T.R. Klaenhammer, S. Kerneis, M.F. Bernet and A.L. Servin. 1992. Protein-mediated adhesion of *Lactobacillus acidophilus* BG2FO4 on human enterocyte and mucus-secreting cell lines in culture. **Appl. Environ. Microbiol**. 58: 2034-2039.

Coconnier, M.H., V. Levien, E. Hemery and A.L. Servin. 1998. Antagonistic activity against Helicobacter infection in vitro and in vivo by the human *Lactobacillus acidophilus* strain LB. **Appl. Environ. Microbiol**. 64: 4573-4580.

Corcoran, B.M., R.P. Ross, G.F. Fitzgerald and C. Stanton. 2005. Survival of probiotic lactobacilli in acidic environments is enhanced in the presence of metabolizable sugars. **Appl. Environ. Microbiol.** 71: 3060-3067.

Conway, P.L., Gorbach, S. and B. Goldin. 1987. Survival of lactic acid bacteria in the human stomach and adhesion to intestinal cells. **J. Dairy Sci**.70: 1-12.

Conway, P. L. 1996. Selection criteria for probiotic microorganisms. **Asia Pacific J. Clin. Nutr.** 5: 10-14.

Cooffman, R. L., D. Lebman, and B. Shrader. 1989. Transforming growth factor specifically enhances IgA production by lipopolysaccharide-stimulated murine B lymphocytes. **J. Exp. Med**. 170: 1039-1044.

Cook, M.T., T. George, D. Charalampoulos and V.V. Khutoryanskiy. 2012. Microencapsulation of probiotics for gastrointestinal delivery. **J. Control. Release.** 162: 56-67.

Corsetti, A., P. Lavermicocca, M. Morea, F. Baruzzi, N. Tosti and M. Gobbetti. 2001**.** Phenotypic and molecular identification and clustering of lactic acid bacteria and yeasts from wheat (species *Triticum durum* and *Triticum aestivum*) sourdoughs of Southern Italy**. Int. J. of Food Microbiol**. 64 (1-2): 95-104.

Corr, S.C., C. Hill and C.G. Gahan. 2009. Understanding the mechanisms by which probiotics inhibit gastrointestinal pathogens. **Adv. Food. Nutr**. 56: 1-15.

Cotter, P.D. and C. Hill**.** 2003. Surviving the acid test: responses of gram positive bacteria to low pH. **Microbiol. Mol. Biol. Rev**. 67: 429-453.

Chung, H.S.Y., B. Kim., S.L. Chun and G.E.Ji. 1999. Screening and selection of acid and bile resistant bifidobacteria. **Int. J. Food. Microbiol.** 47: 25-32.

Daeschel, M. A. 1989. Antimicrobial substances from lactic acid bacteria for use as food preservatives. **Food Technol**. 43: 164-167.

Dameyer, D., J. Hoozee and H. Mesdom. 1974. Specificity of lipolysis during dry sausage ripening. **J. Food Sci**. 39: 293-296.

Dawshen, S. 2007. **Immune system**. Available source: http://www.kidshealth.org/teen/your_body/body_basics/immune.html, November 27, 2007.

De Ambrosini, M., V. I. S. N. Gonzalez and G. Oliver. 1999. Study of adhesion of *L. casei* CRL431 to ileal intestinal cell to mice. **J. Food Prot**. 62: 1430-1434.

De Boever, P., R. Wouters, L. Verschaeve, P. Berckmans, G. Schoeters and W. Verstraete. 2000. Protective effect of the bile salt hydrolase-active *Lactobacillus reuteri* against bile salt cytotoxicity. **J. of Appl. Microbiol.** 53: 709-711.

De Boever, P. and W. Verstraete. 1999. Bile salt deconjugation by *Lactobacillus plantarum* 80 and its implication for bacterial toxicity. **J. of Appl. Microbiol.** 87: 345-352.

de Leeuw, E., X. Li X and W. Lu. 2006. Binding characteristics of the *Lactobacillus brevis* ATCC 8287 surface layer to extracellular matrix proteins. **FEMS Microbiol. Lett.** 260: 210-215.

De Martinis, E.C.P, V.F. Alves and B.D.G. Franco. 2002. Fundamentals and perspectives of the use of bacteriocins by lactic acid bacteria in meat products. **Food Rev. Int**. 18: 191-208.

de Moreno de LeBlanc, A. and G. Perdigon. 2004a. Yoghurt feeding inhibits promotion and progression of experimental colorectal cancer. **Med. Sci. Monit**. 10: 96-104.

_____, J. Valdez, and G. Perdigon. 2004b. Regulatory effect of yoghurt on intestinal inflammatory immune response. **Eur. J. Inflamm**. 2: 21-61.

de Moreno de LeBlanc, A., C. Matar, N. LeBlanc, and G. Perdigon. 2005a. Effects of milk fermented by *Lactobacillus helveticus* R389 on a murine breast cancer model. **Breast Cancer Res**. 7: 477-486.

_____, C. Maldonado Galdeano, S. Chaves and G. Perdigon. 2005b. Oral administration of *L. casei* CRL 431 increases immunity in bronchus and mammary glands. **Eur. J. Inflamm**. 3: 23-28.

_____, C. Matar, E. Farnworth and G. Perdigon. 2006. Study of cytokines involved in the prevention of a murine experimental breast cancer by kefir. **Cytokine.** 34: 1-8.

De Petrino, S.F., M.E.B.B De Jorrrat, O. Meson and G. Perdigon. 1995. Protective ability of certain lactic acid bacteria against an infection with *Candida albi-cans* in a mouse immuno-suppression model by corticoid. **Food Agric. Immunol.** 7:365-373.

Deitch E., E. Specian, E. Steffen and R. Berg. 1990. Translocation of Lactobacillus murinus from the gastrointestinal tract. **Curr. Microbiol**. 20: 177-184.

Deraz, S.F., E.N. Karlsson, M. Hedstrom, M.M. Andersson and B. Mattiasson. 2005. Purification and characterization of acidocin D20079, a bacteriocin produced by *Lactobacillus acidophilus* DSM 20079. **J. Biotechnol**. 117: 343-354.

Derek A.A., V. D. B. Joost, M. K. M. Inge, T. P. Jack and J. A. V. M.Antonius. 2009. Anaerobic homolactate fermentation with *Saccharomyces cerevisiae* results in depletion of ATP and impaired metabolic activity. **FEMS Yeast Res.** 9(3): 349–357.

De Simone, C., R. Vesely, B. Bianchi-Salvidori and E. Jirillow. 1993. The role of probiotics in modulation of the immune system in man and in animals. **Int. J. Immunotherapy** IX: 23-28.

de Vrese, M., A. Stegelmann., B. Ritcher, S. Fenselau, C. Laue and J. Schrezenmeir. 2001. Probiotics: compensation for lactase insufficiency. **Am. J. Clin. Nutr**. 73: S421-S429.

De Vuyst, L. 1994. Nisin production variability between natural *Lactococcus lactis* subsp. lactis strains. **Biotechnol. Lett**. 16: 287-292.

Di Giacinto, C., M. Marinaro, M. Sanchez, W. Strober, and M. Boirivant. 2005. Probiotics ameliorate recurrent Th1-mediated murine colitis by inducing IL-10 and IL-10-dependent TGF-beta-bearing regulatory cells. **J. Immunol**. 174: 3237-3246.

Didierlaurent, A., J.C. Sirard, J.P. Kraehenbuhl and M.R. Neutra. 2002. How the gut senses its content. **Cell Microbiol**. 4: 61-72.

Dogi, C. A. and G. Perdigo´n. 2006. Importance of the host specificity in the selection of probiotic bacteria. **J. Dairy Res**. 73: 357-366.

Drosinos, E.H., M. Mataragas, N. Xiraphi, G. Moschonas, F. Gaitis and J. Metaxopoulos. 2005. Characterization of the microbial flora from a traditional Greek fermented sausage. **Meat Sci**. 69: 307-317.

Dunne, C., L.O' Mahony, L. Murphy, G. Thornton, D. Morrissey, S. O'Halloran, M. Feeney, S. Flynn, G. Fitzgerald, C. Daly, B. Kiely, G. C. O'Sullivan, F. Shanahan and J. K. Collins. 2001. In vitro selection criteria for probiotic bacteria of human origin: correlation with in vivo findings. **Am J Clin Nutr**. 73(2): 386S-392S.

Easo, J.G., J.D. Measham, J. Munroe and J.M. Green-Johnson. 2002. Immuno-stimulatory actions of Lactobacilli: Mitogenic induction by *Lactobacillus delbrueckii* subsp.*bulgaricus* and *Lactobacillus acidophilus*. **Food and Agri. Immunol**. 14: 73-83.

Erkkila S. and E. Petaja. 2000. Screening of commercial meat starter cultures at low pH in the presence of bile salts for potential probiotic use. **J. Meat Sci**. 55: 297-300.

Erkkila, S., M. L. Suihko, S. Eerola, E. Petaja and T. Mattila-Sandholm. 2001. Dry sausages fermented by *Lactobacillus rhamnosus* strains. **Int. J. Food Microbiol**. 64: 205-210.

Ernst P. B., Scicchitano R., Underdown B.J. and J. Bienenstock. 1988. **Immunology of the Gastro-intestinal Tract and Liver**, pp. 125-144. *In*: M.F. Heyworth, A.L. Jones, eds. Raven Press., New York.

Even, S., N.D. Lindley, P. Loubiere and M. Cocaign-Bousquet. 2002. Dynamic response of catabolic pathways to autoacidification in *Lactococcus lactis*: transcript profiling and stability in relation to metabolic and energetic constraints. **Mol. Microbiol**. 45: 1143-1152.

FAO/WHO. 2006. **Guidelines for the evaluation of probiotics in food.Joint FAO/WHO Working Group Report on Drafting Guidelines for the Evaluation of Probiotics in Food**. Available Source: ftp://ftp.fao.org/es/esn/food/wgreport2.pdf., March 25, 2006.

_____. 2001. Evaluation of health and nutritional properties of powder milk and live lactic acid bacteria. Cordoba, Argentina. **Food and Agriculture Organization of the United Nations and World Health Organization Expert Consultation Report**. 1-34.

Fermandez, M., L. De la Hoz, O. Diaz, M.I. Cambero and J. Ordonez. 1995. Effect of the addition of pancreatic lipase on the ripening of dry sausages : part I. Microbial,physico-chemical and lipolytic changes. **Meat Sci**. 40: 159-170.

Fernandez, M. F., S. Boris and C. Barbes. 2003. Probiotic properties of human lactobacilli strains to be used in the gastrointestinal tract. **J Appl. Microbiol**. 94: 449-455.

Fernandez, C.F., R.C. Chandan and K.M. Shahani. 1992. Fermented dairy products and health, pp. 297-331. *In* B.J.B. Wood, ed. **The Lactic acid bacteria in health and disease**. Elsevier Appl. Sci. Pub., London.

Ferrie E. 2005. Probiotics and allergy. **Proceedings of the Nutrition Society**. 64: 465-469.

Fiat A.M., D. Migliore-Samour, P. Jolles, L. Drouet, C. Bal Dit Sollier and J. Caen. 1993. Biologically active peptides from milk proteins with emphasis on two examples concerning antithrombotic and immunomodulating activities. **J. Dairy Sci**. 76: 301-310.

Finegold, M.V., L. Sutter and G. E. Mathisen. 1983. Normal indigenous intestinal flora, pp. 3-31. *In* D. J. Hentges, ed. **Human intestinal microflora in health and disease**. Academic Press, New York, NY.

Finkelman, F.D. 1995. Relationships among antigen presentation, cytokines, immune deviation, and autoimmune disease. **J. Exp. Med**. 182: 279-282.

Foegeding, P., A. Thomas, D. Pilkington and T. Klaenhammer. 1992. Enhanced control of *Listeria monocytogenes* by in situ-produced pediocin during dry fermented sausage production**. Appl. Environ. Microbiol**. 58: 884-890.

Fortier, L.C., R. Tourdot-Mare´chal, C. Divie`s, B.H. Lee, and J. Guzzo. 2003. Induction of Oenococcus oeni H^+-ATPase activity and mRNA transcription under acidic conditions. **FEMS Microbiol. Lett**. 222: 165-169.

Frece J., B. Kos, I.K. Svetec, Z. Zgaga, V. Mrsa and J. Suskovic. 2005. Importance of S-layer proteins in probiotic activity of *Lactobacillus acidophilus* M92. **J. of Appl. Microbiol.** 98: 285-292.

Fukushima, M. and M. Nakano. 1996. Effects of a mixture of organisms *Lactobacillus acidophilus* or Streptococcus faecalis on cholesterol metabolism in rats fed on fat-and-cholesterol-enriched diet. **Br. J. Nutr**. 76: 857-867.

Fukushima, Y., Y. Kawata, H. Hara, A. Terada and T. Mitsuoka. 1998. Effect of a probiotic formula on intestinal immunoglobulin A production in healthy children. **Int. J. Food Microbiol.** 42: 39-44.

Fuller, R. 1989. Probiotics in man and animals. **J. Appl. Bacteriol**. 66: 365-369.

_____. 1991. Probiotics in human medicine. **Gut.** 32: 439-442.

_____. 1997. The importance of lactobacilli in maintaining normal microbial balance in the crop. **British Poultry Sci**. 18: 85-94.

_____, M.E. Coates and G.F. Harrison. 1997. The influence of specific bacteria and filterable agent on the growth of gnobiotic chicks. **J. Appl. Bacteriol**. 46: 335-342.

Furet, J.P., P. Quenee and P. Tailliez. 2004. Molecular quantification of lactic acid bacteria in fermented milk products using real-time quantitative PCR. **Int. J. Food Microbiol**. 97: 197-207.

Furrie, E., S. Macfarlane, A. Kennedy, J.H. Cummings, S.V. Walsh, D.A.O'Neil and G.T. Macfarlane. 2005. Synbiotic therapy (*Bifidobacterium longum*/Sinergy) initiates resolution of inflammation in patients with active ulcerative colitis: a randomized controlled pilot trial. **Gut**. 54: 242-249.

Galdeano,C.M., A. de Moreno de LeBlabc and G. Vinderola. 2007. Proposed model: Mechanisms of immunomodulation induced by probitic bacteria. **Clin.Vaccine Immunol**. 14 (5): 485-492.

Ganzle, M., C. Hertel, J. van der Vossen, and W. Hammes. 1999a. Effect of bacteriocin-producing lactobacilli on the survival of Escherichia coli and Listeria in a dynamic model of the stomach and the small intestine. **Int. J. Food Microbiol**. 48: 21-35.

_____, S. Weber and W. Hammes. 1999b. Effect of ecological factors on the inhibitory spectrum and activity of bacteriocins. **Int. J. Food Microbiol**. 46: 207-217.

Garriga, M., B. Marcos, B. Martín, M.T. Veciana-Nogués, S. Bover-Cid, S. Hugas and T. Aymerich,T. 2005. Starter cultures and high pressure processing to improve the hygiene and safety of slightly fermented sausages. **J. Food. Prot.** 68: 2341-2348.

_____, M. Hugas, T. Aymerich and J. M. Monfort. 1993. Bacteriocinogenic activity of lactobacilli from fermented sausages. **J. Appl. Bacteriol**. 75: 142-148.

Garrote, G.L., L. Delfederico, R. Bibiloni and A.G. Abraham. 2004. Lactobacilli isolated from kefir grains: evidence of the presence of S-layer proteins. **J. Dairy Res**. 71 (2): 222-230.

Garvie, E.I. 1986. Genus *Pediococcus*. pp. 1075-1079. *In* P. H. A. Sneath, N.S. Mair, M. E. Sharpe and J. G. Holt, eds. **Bergey's Manual of Systematic Bacteriology**. vol 2, 9th ed. Williams and Wilkins, Baltimore.

Gaskins H.R. 1996. Immunological aspects of host/microbiota interactions at the intestinal epithet-lium, pp. 537-587. *In* R.I. Mackie, B.A.White and R.E. Isaacson,eds. **Gastrointestinal microbiology**. Chapman and Hall, New York.

Gibson, G.R. and R. Fuller. 2000. Aspects of in vitro and in vivo research approaches directed toward identifying probiotics and probiotics for human use. **J. Nutr**. 130: 391S-395S.

Gill H. S. 1998. Stimulation of the immune system by lactic cultures. **Int. Dairy J**. 8: 535-544.

Gill, H. S., K. J. Rutherfurd, J. Prasad, and P. K. Gopal. 2000. Enhancement of natural and acquired immunity by Lactobacillus rhamnosus (HN001), *Lactobacillus acidophilus* (HN017) and *Bifidobacterium lactis* (HN019). **Brit. J. Nutr**. 83: 167–176.

Gill, H.S. and K.J. Rutherfurd. 2001. Viability and dose–response studies on the effects of the immune enhancing lactic acid bacterium *Lactobacillus rhamnosus* in mice. **Brit. J. Nutr**. 86: 285-289.

Gilland, S.E. and D.K. walker. 1990. Factors to consider when selecting a culture of Lactobacillus as a dietary adjunct to produce a hypocholesterolemic effect in human. **J. of dairy Sci**. 73: 905-911.

Gilliland S.E. and M.L. Speck. 1977. Deconjugation of bile acids by intestinal lactobacilli. **Appl. Environ. Microbiol**. 33: 15-18.

Gilliland S.E., T.E. Staley and L.J. Bush. 1984. Importance in bile tolerance of *Lactobacillus acidophilus* used as a dietary adjunct. **J. of Dairy Sci**. 67: 3045-3051.

Goldin B.R. and S.L. Gorbach.1992. Probiotics for Humans, pp. 355–376. *In* R. Fuller (eds.). **Probiotics: The Scientific Basis**. Chapman & Hall. London. UK.

Goncalves, L. M.D., A. Ramos, J.S. Almeida, A.M.R.B. Xavier and M.J.T. Carrondo. 1997. Elucidation of the mechanism of lactic acid growth inhibition and production in batch cultures of *Lactobacillus rhamnosus*. **Appl. Microbiol. Biotechnol**. 48:346-350.

Goutefongea, R. 1992. Salting and curing, pp. 115-137. *In* J.P.Girard, ed**. Technology of Meat and Meat products**. Ellis Horwood Limited,London.

Granto D., F. Perotti, I. Masserey, M. Rouvet, M. Golliard, A. Servin and D. Brassart. 1999. Cell surface-associated lipoteichoic acid acts as an adhesion factor for attachment of *Lactobacillus johnsonii* La1 to human enterocyte-like Caco-2 cells. **Appl. Environ. Microbiol**. 65: 1071-1077.

Greene, J. D. and T.R. Klaenhammer. 1994. Factors involved in adherence of lactobacilli to human Caco-2 cells. **Appl. Environ. Microbiol**. 60: 4487-4494.

Gueimonde M., C. Garrigues, D. van Sinderen, C.G. de los ReyesGavilan and A. Margolles A. 2009. Bile-inducible efflux transporter from *Bifidobacterium longum* NCC2705, conferring bile resistance. **Appl Environ Microbiol.** 75: 3153-3160.

Guencheva, G., P. Popova, G. Davidkova, V. Mincheva, S. Mihailova, A. Bogdanov, E. Pacelli and A. Auteri. 1992. Determination of cytokine release after in vivo and in vitro administration of Deodan (a preparation from *L. bulgaricus* "LB51") by the rabbit pyrogen test. **Int. J. Immunopharmac**. 14: 1429-1436.

Hagen, K.E, L.L. Guan, G.W. Tannock, D.R. Korver, G.E. Allison. 2005. Detection, characterization, and in vitro and in vivo expression of genes encoding S-proteins in *Lactobacillus gallinarum* strains isolated from chicken crops. **Appl. Environ Microbiol**. 71: 6633-6643.

Haller, D., C. Bode C, W.P. Hammes, A. Pfeifer, E.J. Schiffrin and S. Blum. 2000a. Non-pathogenic bacteria elicit a differential cytokine response by intestinal epithelial cell/leucocyte co-cultures. **Gut**. 47: 79-87.

Haller, D., S. Blum, C. Bode, W. P. Hammes and E. J. Shiffrin. 2000b. Activation of human peripheral blood mononuclear cells by nonpathogenic bacteria in vitro: evidence of NK cells as primary targets. **Infect. Immun**. 68: 752-759.

Haller D., H. Colbus, M.G. Ganzle, P. Scherenbacher, C. Bode and W.P. Hammes. 2001. Metabolic and functional properties of lactic acid bacteria in the gastrointestinal ecosystem: A comparative *in vitro* study between bacteria of intestinal and fermented food origin. **System. Appl. Microbiol**. 24: 218-226.

Haller, D., P. Serrant, D. Granato, E.J. Schiffrin and S. Blum. 2002. Activation of human NK cells by Staphylococci and Lactobacilli requires cell contact-dependent co-stimulation by autologous monocytes. **Clin. Diag. Lab. Immunol**. 9(3): 649-657.

Hamilton-Miller, J.M. and S. Shah. 1998. Vancomycin susceptibility as an aid to the identification of lactobacilli. **Lett. Appl. Microbiol**. 26: 153-154.

Hamilton-Miller J.M.T., S. Shah S and J.T. Winkler. 1999. Public health issues arising from microbiological and labeling quality of foods and supplements containing probiotic microorganisms. **Public Hlth. Nutr**. 2: 223-229.

Hammes, W.P. and C. Hertel. 1996. Selection and improvement of lactic acid bacteria used in meat and sausage fermentation. **Lait** 76: 159-168.

_____ and _____. 1998. New developments in meat starter cultures. **Meat Sci.** 49: S125-S138.

_____ and P. S. Tichaczek. 1994. The potential of lactic acid bacteria for the production of safe and wholesome food. **Z Lebensm Unters Forsch**. 198: 193-201.

Harris, L.J., M.A. Daeschel, M.E. Stiles and T.R. Klaenhammer. 1989. Antimicrobial activity of lactic acid bacteria against *Listeria monocytogenes*. **J. Food Protec**. 52: 384-387.

Havenaar, R., B. Ten Brink, J. Huis in't Veld. 1992. Selection of strains for probiotic use., pp. 209-224. *In*: R. Fuller,ed. **Probiotics:The Scientific Basis**. Chapman & Hall, London, England.

Havenith C.E.G., J.F.M.L. Seegers and Pouwels P.H. 2002. Gut-associated lactobacilli for oral immunisation. **Food Res. Int**. 35: 151-163.

He, F., Morita, H., Ouwehand, A.C., Hosoda, M., Hiramatsu,M., Kurisaki, J., Isolauri, E., Benno, Y. and S. Salminen. 2002. Stimulation of the secretion of pro-inflamma-tory cytokines by *Bifidobacterium* strains. **Microbiol. Immunol**. 46: 781-785.

Heasman, M. and J. Mellentin. 1998. Battling for Europe's gut. **Int. Ingred**. 4: 40-41.

Henrich, R. and Levkut, M. 2002. Lactic acid bacteria,probiotics and immune system. **Vet.Med.-Czech.** 47: 169-180.

Herias, M.V., C. Hessle, E. Telemo E., T. Midtvedt, L.A. Hanson and A.E. Wold. 1999. Immuno-modulatory effects of *Lactobacillus plantarum* colonising the intestine of gnotobiotic rats. **J. of Bacteriol**. 116: 283-290.

Herıas, M.V., J.F.J.G. Koninkx, J.G. Vos, J.H.J. Huis in't Veld and J.E.van Dijk. 2005. Probiotic effects of Lactobacillus casei on DSS-induced ulcerative colitis in mice. **Int. J. Food Microbiol.** 103: 143-155.

Herich R., Bomba A., Nemcova R. and Gancarcikova S. 1999. The influence of short-term and con-tinuous administration of Lactobacillus casei on basic haematological and immuno-logical parameters in gnotobiotic piglets. **Food Agric. Immunol**. 11: 287-295.

Hermelijn, H.S., A. Engering, D. van der Kleij, E.C. de Jong, K. Schip perd, T.M.M. van Capela, B.A.J.Zaat, M. Yazdanbakhsh, E.A. Wierenga, Y.van Kooyk and M.L. Kapsenberg. 2005. Selective probiotic bacteria induce IL-10-producing regulatory T cells in vitro by modulating dendritic cell function through dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin. **J. Allergy Clin. Immunol**. 115: 1260-1267.

Heumann D., Barras C., Severin A., Glauser M.P., Tomasz A. 1994. Gram-positive cell walls stimulate synthesis of tumor necrosis factor alpha and interleukin-6 by human monocytes. **Infect. Immun**. 62: 2715-2721.

Hinkens, J., Faith, N., Lorang, T., Bailey, P., Buege, D., Kaspar,C., Luchansky, J. 1996. Validation of pepperoni processes for control of Escherichia coli O157:H7. **J. Food Prot**. 59: 1260-1266.

Hirayama, K. and J. Rafter. 1999. The role of lactic acid bacteria in colon cancer prevention: mechanistic considerations. **Antonie Leeuwenhoek**. 76: 391-394.

Hofmann A. 1991. Enterohepatic circulation of bile acids, pp.567-580. *In* S.G. Schultz, J.G. Forte and B.B. Rauner, eds. **Handbook of Physiology: The gastrointestinal system. American Physiological Society**. Bethesda, MD**.**

Holzapfel, W., Habere, P. Snel. J. Schillinger, V. and Huis in't Veld, J. 1998. Overview of gut flora and probiotics. **Int. J. of Food Microbiol**. 41: 85-101.

Hong, S. I., Y. J. Kim, and Y. R. Pyun**.** 1999. Acid tolerance of *Lactobacillus plantarum* from kimchi. **Technol.** 32: 142-148.

Hooijkaas H., Benner R., Pleasants J. and B. Wostmann. 1984. Isotypes and specificities of immunoglobulins produced by germ-free chemically defined ultra-filtred antigen free diet. **Eur. J. Immunol**. 14: 127.

Huang M.K., Y.J. Choi, R. Houde, J.W. Lee, B. Lee and X. Zhao. 2004. Effects of Lactobacilli and an Acidophilic fungus on the production performance and immune responses in broiler chickens. **Poultry Sci**. 83: 788-795.

Huang Y. and M.C. Adams. 2004. In vitro assessment of the upper Gastrointestinal tolerance of potential probiotic dairy propionibacteria. **Int. J. Food Microbiol**. 91: 253-260.

Hudault, S., Guignot, J. and Servin, A.L. 2001. *Escherichia coli* strains colonizing the gastrointestinal tract protect germfree mice against Salmonella typhimurium infection. **Gut.** 49: 47-55.

Hugas, M. 1998. Bacteriocinogenic lactic acid bacteria for the biopreservation of meat and meat products. **Meat Sci**. 49: S139-S150.

_____ and J.M. Monfort. 1997. Bacterial starter cultures for meat fermentation**.** **Food Chem**. 59(4): 547-554.

_____, M. Garriga, M.T. Aymerich and J.M. Monfort. 1995. Inhibition of Listeria in dry fermented sausages by the bacteriocinogenic Lactobacillus sake CTC494. **J. Appl. Bacteriol.** 79: 322-330.

_____, M. Garriga, T. Aymerich, and J.M. Monfort. 1993. Biochemical characterization of lactobacilli from dry fermented sausages. **Int. J. Food Microbiol**. 18**:** 107-113.

Husband, A.J., and J.L. Cowans. 1978. The origin and antigen dependent distribution of IgA-containing cells in the intestine. **J. Exp. Med**. 148: 1146-1160.

Hynönen, U., B. Westerlund-Wikström, A. Palva and T.K.Korhonen TK. 2002. Identification by flagellum display of an epithelial cell- and fibronectin-binding function in the SlpA surface protein of *Lactobacillus brevis*. **J. Bacteriol**. 184: 3360-3367.

Ibanez C., L. Quintanilla, C.Cid, I. Astiasaran and J. Bello. 1996. Dry Fermented Sausages Elaborated with *Lactobacillus plantarum-Staphylococcus carnosus* Part I: Effect of Partial Replacement of NaCl with KCl on the Stability and the Nitrosation Process. **Meat Sci.** 44 (4): 227-234.

Ibnou-Zekri, N., S.Blum, E.J. Schiffrin and T. Von Der Weid. 2003. Divergent patterns of colo-nization and immune response elicited from two intestinal Lactobacillus strains that display similar properties in vitro. **Infect. Immun**. 71: 428-436.

Incze, K. 1998. Dry fermented sausages. **Meat Sci.** 49: S169-S177.

Ishikawa, H., I. Akedo, T. Otani, T. Suzuki, T. Nakamura, I. Takeyama, S.Ishiguro, E. Miyaoka, T. Sobue and T. Kakizoe. 2005. Randomized trial of dietary fiber and *Lactobacillus casei* administration for prevention of colorectal tumors. **Int. J. Cancer.** 116: 762-767.

Isolauri E., Y. Sutas, P. Kankaanpaa, H. Arvilommi and S. Salminen. 2001. Probiotics: effects on immunity. **Am. J. of Clin.Nutr**. 73: 444S-450S.

_____, Salminen, S., Mattila-Sandholm, T. 1999. New functional foods in the treatment of food allergy. **Ann. Med**. 31: 299-302.

Iwasaki, A. and R. Medzhitov. 2004. Toll-like receptor control of the adaptative immune response. **Nat. Immunol**. 5: 987-995.

Jaichumjai, P., R. Valyasevi, A. Assavanig and P. Kurdi. 2011. Isolation and characteri-zation of acid-sensitive *Lactobacillus plantarum* with application as starter culture for Nham production. **Food. Microbiol**. 27: 741-748.

Janeway, C. A. and R. Medzhitov. 2002. Innate immune recognition. **Annu. Rev. Immunol**. 20: 197-216.

Jang, M. H., M.-N. Kweon, K. Iwatani, M. Yamamoto, K. Terahara, Ch.Sasakawa, T. Suzuki, T. Nochi, Y. Yokota, P. D. Rennert, T. Hiroi, H. Tamagawa, H. Iijima, J. Kunisawa, Y. Yuki, and H. Kiyono. 2004. Intestinal villous M cells: an antigen entry site in the mucosal epithelium. Proc. **Natl. Acad. Sci**. USA 101: 6110–6115.

Janse I., J. Bok and G. Zwart. 2004. A simple remedy against artifactual double bands in denaturing gradient gel electrophoresis. **J. Microbiol. Meth**. 57: 279-281

_____, M. Meima, W.E.A. Kardinaal, and G. Zwart. 2003. Highresolution differentiation of cyanobacteria by using rRNA-Internal Transcribed Spacer Denaturing Gradient Gel Electrophoresis. **Appl. Environ. Microbiol**. 69: 6634-6643.

Jiménez-colmenero, F., J. Carballo and S. Cofrades. 2001. Healthier meat and meat products: their role as functional foods. **Meat Sci**. 59: 5-13.

Jian-Kui L., H. Xi-Lin, W. Chun-Hua, Y. Li-Yun, H. Xiao-Jie, W. Gui-Hua, L. Jong-Soo and K. Chul-Joong. 2009**.** Induction of immune responses in mice after oral immunization with recombinant *Lactobacillus casei* Strains Expressing Enterotoxigenic *Escherichia coli* F41 Fimbrial Protein. **Appl. Environ. Microbiol**. 75(13): 4491-4497.

Jin, L. Z., Y. W. Ho, N. Abdullah, and S. Jalaludin. 1998. Acid and bile tolerance of Lactobacillus isolated from chicken intestine. **Lett. Appl. Microbiol**. 27: 183-185.

Johnson, L.R. 1977. Regulation of gastric secretion. **Gastrointestinal Physiology**. The C.V. Mosby, Saint Lowis.

Johnson-Henry, K. C., K.E. Hagen, M. Gordonpour, T.A. Tompkins and P.M. Sherman. 2007. Surface-layer protein extracts from *Lactobacillus helveticus* inhibit enterohaemorrhagic *Escherichia coli* O157:H7 adhesion to epithelial cells. **Cell Microbiol**. 9:356-367.

Kado-Oka Y, S. Fujiwara and T. Hirota. 1991. Effects of bifidobacteria cells on mitogenic response of splenocytes and several functions of phagocytes. **Milchwissenshaft**. 46: 626.

Kagnoff, M.F. 1993. Immunology of the intestinal tract. **Gastroenterol**.105: 1275-1280.

Kahala, M., K. Savijoki and A. Palva. 1997. In vivo expression of the *Lactobacillus brevis* S-layer gene. **J Bacteriol**. 179:284-286.

Kaila M., E. Isolauri, M. Saxelin, H. Arvilommi and T. Veskari. 1995. Viable versus inactivated *Lactobacillus* strain GG in acute rotavirus diarrhoea. **Arch. Dis. Childhood**. 72: 51-53.

Kailasapathy, K. and C. James. 2000. Surviaval and therapeutic potential of probiotic organisms with reference to *Lactobacillus acidophilus* and *Bifidobacterium* spp. **Immunol. and Cell Biol**. 78: 80-88.

Kashket, E. R. 1987. Bioenergetics of lactic acid bacteria: cytoplasmic pH and osmotolerance. **FEMS Microbiol. Lett.** 46 (3): 233-244.

Kato I., T. Yokokura and M. Mutal. 1984. Augmentation of mouse natural killer cell activity by *Lactobacillus casei* and its surface antigens. **Microbiol. Immunol**. 28: 209-217.

Kato, I., K. Tanaka and T. Yokokura. 1999. Lactic acid bacterium potently induces the production of interleukin-12 and interferon-gamma by mouse splenocytes. **Int. J. Immunopharmacol**. 21: 121–131.

Kawther, E.L.S., N.F. Tawfik, M.A.D. Nadia, O.M. Sharaf and B.A. Effat. 2011. In vitro assessment of gastrointestinal viability of potentially probiotic Lactobacilli. **J. of American Sci.** 357-367.

Kelly, D., S. Conway and R. Aminov. 2005. Commensal gut bacteria: mechanisms of immune modulation. **Trends Immunol**. 26: 326-333.

Ketema, B., M. Tetemke and A. Mogessie. 2009. In-Vitro Probiotic Potential of Lactic Acid Bacteria Isolated from 'Wakalim', a traditional Ethiopian fermented beef sausage. **Ethiop J Health Sci**. 19 (1): 21-27.

Kim, W.J. 1993. Bacteriocins of lactic acid bacteria: their potentials as food bio-preservative. **Food Rev. Int**. 9: 299-313.

Kirjavainen P.V., H.S. El-Nezami, S.J. Salminen, J.T. Ahokas and P.F.A. Wright. 1999. The effect of orally administered viable probiotic and dairy lactobacilli on mouse lymphocyte proliferation. **FEMS Immunol. Med Microbiol**. 26: 131-135.

Kirjavainen, P.V., H.S. Elnezami, S.J. Salminen, J.T. Ahokas and P.F.Wright. 1999. Effects of orally administered viable Lactobacillus rhamnosus GG and Propionibacterium freudenreichii subsp. shermanii JS on mouse lymphocyte proliferation. **Clin. Diagn. Lab. Immunol**. 6: 799-802.

Klaenhammer T.R. and M. Kullen. 1999. Selection and design of probiotics. **Int. J. Food Microbiol**. 50: 45-57.

Klettner, P. and P. Baumgartner. 1980. The technology of raw dry sausage manufacture. **Food Technol. Austral**. 32: 380-384.

Klingberg, T.D., L. Axelsson, K. Naterstad, D. Elsser and B. B. Budde. 2006. Identification of potential probiotic starter cultures for Scandinavian- type fermented sausages. **Int. J. of Food Microbiol**. 105: 419-431.

Kneifel, W., S. Halub and M. Wirthman. 1989. Monitoring of B-complex vitamin in yoghurt during fermentation. **J. Dairy Res**. 56: 651-656.

Kopp-Hoolihan, L. 2001. Prophylactic and therapeutic uses of probiotics: a review. **J. Am. Diet. Assoc.** 101: 229-238.

Krahenbuhl, J. P. and M. R. Neutra. 2000. Epithelial M cells: differentiation and function. **Annu. Rev. Cell Dev. Biol**. 16: 301-332.

Kramlich, W.E. 1960. The science of meat and meat products .pp.484-512. *In* J.F. Price and B.S. Schweigert,eds. **Sausage Products**. 2nd ed. W.H. Freeman and Company, San Francisco.

Kullen, M. J. and T. R. Klaenhammer. 1999. Identification of the pH inducible, proton-translocating F1F0-ATPase (atpBEFHAGDC) operon of Lactobacillus acidophilus by differential display: gene structure, cloning and characterization. **Mol. Microbiol**. 33: 1152-1161.

Kumprecht, I. 1984. The effect of single and continuos administration of Streptococcus faecium M-74 germs on the growth of broilers and on metabolic process in broiler. **Zivoc. Vyr**. 29: 945-955.

Kunawasen S.,V. Chavasit and R. Valyasevi. 2000. Molecular typing of lactic acid bacteria isolated during Nham fermentation. **Annual abstract**. Mahidol University.

Kunimoto, D.Y., R.P. Nordan and W. Strober. 1989. IL-6 is a potent cofactor of IL-1 in IgM synthesis and of IL-5 in IgA synthesis. **J. Immunol**. 143: 2230-2235.

Kwanmuang, P. 2003. Isolation of Lactic acid bacteria from Nham sample in Thailand for use as starter culture. **Ph D Thesis**, Kasetsart University. Bangkok. Thailand.

Lamm, M. 2003. IgA function. **Mucosal Immunol**. **Update.** 11: 7-8.

Lankapurtha, W.E.V and N.P. Shah. 1995. Survival of *Lactobacillus acidophilus and Bifidobacterium* spp in the presence of acid and bile salts. **Cult. Dairy Prod. J**. 30: 2-7.

La Ragione, R.M., A. Narbad, M.J.Gasson and M.J. Woodward. 2004.In vivo characteriza-tion of *Lactobacillus johnsonii* FI9785 for use as a defined competitive exclusion agent against bacterial pathogens in poultry. **Lett. Appl. Microbiol**.38: 197–205.

Lee, Y.K. and S. Salminen. 1995. The coming of age of probiotics. **Trends in Food Sci. and Technol**. 6: 241-245.

Lee, J.W., J.G. Shin, E.H. Hee, H.E. Kang, I.B. Yim, J.Y. Kim, H.G. Joo and H.J. Woo. 2004. Immunomodulatory and antitumor effects *in vivo* by the cytoplasmic fraction of *Lactobacillus casei* and *Bifidobacterium longum*. **J. Vet. Sci. 5**(1): 41-48.

Lee, S.J., S. Evers, D. Roeder, A.F. Parlow, J. Risteli, L. Risteli, Y.C. Lee, T. Feizi, H. Langen and M. C. Nussenzweig. 2002. Mannose receptormediated regulation of serum glycoprotein homeostasis. **Sci**. 295: 1898-1901.

Lefrancois, L. 1994. Basic aspects of intraepithelial lymphocyte immunobiology, pp. 287-290. *In*: P.L. Ogra, J. Mestecky, M.E. Lamm, W. J.R Strober McGhee., J. Bienenstock, eds. **Handbook of Mucosal Immunology**. Academic Press, San Diego.

Levis S., R. Goodlad, G. Stamp, C. Lee, M. Walport and N. Wright. 1992. Effects of non-steroidal anti-inflammatory drugs and misoprostol on gastric and duodenal epithelial proliferation in patients with arthritis. **Gastroenterol**. 102: 1605-1611.

Lilly, D.M. and R.H. Stillwell. 1965. Probiotics : growth promoting factors produced by microorganisms. **Sci**. 147: 747-748.

Lise, L. and F. Audibert. 1989. Immunoadjuvants and analogs of immunomodulatory bacterial structures. **Curr. Opin. in Immunol**. 2: 269-274.

Lortal, S., J. van Heijenoort, K. Gruber and U. B. Sleytr. 1992. S-layer of *Lactobacillus helveticus* ATCC 12046: isolation, chemical, characterization and re-formation after extraction with lithium chloride. **Microbiol.** 138 (3): 611-618.

Luck, E. and M. Jager. 1997. **Antimicrobial Food Additives: Characteristics, Use and Effects**. Springer-Verlag, New York.

Lücke, F. K. 2000. Utilization of microbes to process and preserve meat. **Meat Sci**. 56: 105-115.

Luo, X. M., G.. Fosmire and R.M. Leach. 2002. Chicken keel cartilage as a source of chondroitin sulfate. **Poultry Sci**. 8: 1086-1089.

Maassen C.B.M., C. van Holten-Neelen, F. Balk, M.J. Heijne den Bak-Glashouwer, R.J. Leer, J.D. Laman, W.J.A. Boersma and E. Claassen. 2000. Strain dependent induction of cytokine profiles in the gut by orally administered Lactobacillus strains. **Vaccine**. 18: 2613-1623.

MacFarlane G.T. and J.H. Cummings. 2002. Probiotics, infection and immunity. **Curr Opin Infect Dis**. 15: 501-506.

Macpherson, A.J. and T. Uhr. 2004. Compartmentalization of the mucosal immune response to commensal intestinal bacteria. **Ann. N. Y. Acad. Sci**. 1029: 36-43.

Macpherson, A.J. and N.L. Harris. 2004. Interaction between commensal intestinal bacteria and the immune system. **Nat. Rev. Immunol**. 4:478-485.

Macpherson, A.J. and T. Uhr. 2004. Induction of protective IgA by intestinal dendritic cells carrying commensal bacteria. **Science**. 303: 1662-1665.

Majamaa, H. and E. Isolauri. 1997. Probiotics: a novel approach in the management of food allergy. **J. Allergy Clin. Immunol**. 99: 179-185.

Maldonado Galdeano, C., A. De Moreno de LeBlanc, G. Vinderola, M.E. Bibas Bonet and G. Perdigon. 2007. Proposed Model: Mechanisms of immunomodulation induced by probiotic bacteria. **Clin. Vaccine Immunol**. 14 (5): 485-492.

Maldonado Galdeano, C. and G. Perdigon. 2004. Role of viability of probiotic strains in their persistence in the gut and in mucosal immune stimulation. **J. Appl. Microbiol**. 97: 673-681.

Maldonado Galdeano, C. and G. Perdigon. 2005. The probiotic bacteria *Lactobacillus casei* induces activation of the gut mucosal immune system through the innate immunity. **Clin. Vaccine Immunol**. 13: 219-226.

Maldonado Galdeano, C. and G. Perdigon. 2006. The probiotic bacteria *Lactobacillus casei* induces activation of the gut mucosal immune system through the innate immunity. **Clin. Vaccine Immunol**. 13: 219-226.

Males, R.J. and B. Johnson. 1990. Probiotics-What are they ? What do they do?. **J. Animal Sci.** 68: 505-508.

Maneerat, S., S. Phanyosaranya, W. Rojanawongchai. 2000. Probiotic lactic acid bacteria from traditional fermented foods: isolation and inhibition of pathogenic bacteria. Available Source: http://www.clib.psu.ac.th/acad_43/msup2.htm, September 8, 2001.

Martin, B., M. Garriga, M. Hugas and T. Aymerich. 2005. Genetic diversity and safety aspects of enterococci from slightly fermented sausages. **J. of Appl. Microbiol**. 98(5): 1177-1190.

Masuda, K. and T. Kawata. 1985. Reassembly of a regularly arranged protein in the cell wall of Lactobacillus buchneri and its reattachment to cell walls: chemical modification studies. **Microbiol. Immunol**. 29: 927-938.

Marin B.L., M.V. Tejada-Simon, J.G. Lee, J. Murtha, L. Ustunol and J.J. Pestka. 1998. Stimulationof cytokine production in clonal macrophage and T-cell models by *Streptococcus thermophilus* : Comparison with *Bifidobacterium* sp. And *Lactobacillus bulgaricu*. **J. Food. Prot**. 61: 859-864.

Marin, M., A. Karis, P. Visser, F. Grosveld and S. Philipsen. 1997. Transcription factor Sp1 is essential for early embryonic development but dispensable for cell growth and differentiation. **Cell**. 89: 619-628.

Marteau P., P. Seksik and R. Jian. 2002. Probiotics and health: new facts and ideas. **Curr. Opin. in Biotechnol**. 13: 486-489.

Matsumoto M., H. Ohishi and Y. Benno. 2004. H+-ATPase activity in Bifidobacterium with special reference to acid tolerance. **Int. J. of Food Microbiol**. 93: 109-113.

Mattila-Sandholm, T., J. Matto and M. Saarela. 1999. Lactic acid bacteria with health claims-interactions and interference with gastrointestinal flora. **Int. Dairy J.** 91: 25-35.

Mc Auliffe, O., R. J. Cano and T. R. Klaenhammer. 2005. Genetic analysis of two bile salt hydrolase activities in Lactobacillus acidophilus NCFM. **Appl. Environ. Microbiol**. 71: 4925-4929.

McCracken, A. and P. Timms. 1999. Efficiency of transcription from promoter sequence variants in Lactobacillus is both strain and context dependent. **J Bacteriol**. 181: 6569-6572.

McCracken, V.J. and H.R. Gaskins. 1999. Probiotics and the immune system, pp. 85-111. *In* G.W. Tannock, ed. **Probiotics: A Critical Review**. Horizon Scientific Press, Norfolk UK.

McCracken, V.J. and R.G. Lorenz. 2001. The gastrointestinal ecosystem: a precarious alliance among epithelium, immunity and microbiota. **Cell. Microbiol**. 3: 1-11.

McGhee J. and H. Kiyono. 1992. Mucosal immunity to vaccines: Current concepts for Vaccine development and immune response analysis, pp. 3-12. *In*: Ciardi J. *et al*., eds. **Genetically Engineered Vaccines**. Plenum Press, New York.

McMullen, L. M. and M. E. Stiles, ME. 1996. Potential for use of bacteriocin producing lactic-acid bacteria in the preservation of meats. **J. of Food Protect** Supplement: 6.C71.

Meghrous, J., C. Lacroix, and R. E. Simard. 1999. The effects on vegetative cells and spores of three bacteriocins from lactic acid bacteria. **Food Microbiol**. 16: 105-114.

Mestecky J., Z. Moldoveanu, M. Novak, W. Huang, R. Gilley, J. Stass, D. Schafer and R. Compans. 1994. Biodegradable microspheres for the delivery of oral vaccines. **J. Controlled Release.** 28: 131-141.

Michaela S., W.K.R. Gerhard and M.E. Christine. 2009. Cultivation of anaerobic and facultatively anaerobic bacteria from spacecraft-associated clean rooms. **Appl. Environ. Microbiol**. 11 (75): 3484-3491.

Michalek S., J. Eldridge, R. Curtiss III and K. Rosenthal. 1994. Antigen delivery systems: New approaches to mucosal immunization. pp. 373-390. *In* P. Ogra, J. Mestecky, M. Lamm, W. Strober, J. McGhee and J. Bienenstock, eds. **Handbook of Mucosal Immunology**. Academic Press, San Diego, California..

Michalek S., J. Mc Ghee, H. Kiyono, D. Colwell, J. Eldridge, Wannenmuehler M. and W. Koopman. 1983. The IgA response: inductive aspects, regulatory cells and effector functions. **Ann. N. Y. Acad. Sci**. 409: 48.

Miettinen M., A. Lehtonen, I. Julkunen and S. Maitkainen. 2000. Lactobacilli and Streptococci activate NF-kB and STAT signalling pathways in humanm macrophages. **J. of Immunol**. 164: 3733-3740.

Miettinen, M., J. Vuopio-Varkila, J. Pirhonen, K. Varkila, M. Korimoto and I. Julkunen. 1998. Lactobacilli and streptococci induce interleukin 12 (IL-12), IL-18, and gamma interferon production in human peripheral blood mononuclear cells. **Infect. Immun**. 66: 6058-6062.

Miettinen, M., S. Matikainen, J. Vuopio-Varkila, and K. Varkila. 1996. Production of tumor necrosis factor alpha, interleukin-6, and interleukin-10 is induced by lactic acid bacteria. **Infect. Immun**. 64: 5403-5405.

Milling, S.W.F., L. Cousins and G. MacPherson. 2005. How do dendritic cells interact with intestinal antigens?. **Trends. Immunol**. 26: 349-352.

Mishra, V. and D. Prasad. 2005. Application of in vitro methods for selection of *Lactobacillus casei* strains as potential probiotics. **Int. J. Food. Microbiol.** 103: 109-115.

Montes, R.G., T.M. Bayless, J.M. Saavedra and j.A. Perman. 1995. Effect of milk inoculated with *L. acidophilus* or a yoghurt starter culture in lactose-maldigesting children. **J. Dairy Sci**.78: 1657-1664.

Mosmann T. 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cyto-toxicity assays. **J. Immunol. Meth**. 65: 55-63.

Mowat, A. M. 2003. Anatomical basis of tolerance and immunity to intestinal antigens. **Nat. Rev. Immunol**. 3: 331-341.

Mowat A. 1987. The regulation of immune responses to dietary protein antigens**.** **Immunol. Today**. 8: 93-98.

Muyzer, G., E.C. de Waal and A.G. Uitterlinden. 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. **Appl. Environ. Microbiol**. 59: 695-700.

Nader De Macias M.E., M.C. Apella, N.C. Romero, S.N. Gonzales, G. Oliver. 1992. Inhibition of *Shigella sonnei* by *Lactobacillus casei* and *Lactobacillus acidophilus*. **J. Appl. Bacteriol**.73: 407-411.

Naidu, A.S.,W.R. Bidlack and R.A. Clemens. 1999. Probiotic spectra of lactic acid bacteria (LAB). **Crit. Rev. Food Sci. Nutr**. 39: 13-126.

Navarre, W.W. and O. Schneewind. 1999. Surface proteins of gram positive bacteria and mechanisms of their targeting to the cell wall envelope. **Microbiol. Mol. Biol. Rev.** 63: 174-229.

Netea, M.G., C. van der Graaf, J.W.M. Van der Meer and B. Jan Kullberg. 2004. Toll-like receptors and the host defense against microbial pathogens: bringing specificity to the innate-immune system. **J. Leukoc. Biol**. 75: 749-755.

Neuhaus, F.C. and J. Baddiley. 2003. A continuum of anionic charge: structures and functions of D-alanyl-teichoic acids in gram-positive bacteria. **Microbiol. Mol. Biol. Rev.** 67: 686-723.

Neutra, M. R., N. J. Mantis and J. P. Kraenhenbuhl. 2001. Collaboration of epithelial cells with organized mucosal lymphoid tissues. **Nat. Immunol**. 2: 1004-1009.

Newton, I. 2003. **Influence of different capsule materials on the physiological properties of microencapsulated *L. acidophilus*.** Inaugural-Dissertation. University of Bonn.

Nicaise, P., A. Gleizes, C.Sandre, R. Kergot, H. Lebrec, F. Forestier and C. Labarre. 1999. The intestinal microflora regulates cytokine production positively in spleen-derived macro-phages but negatively in bone marrow-derived macrophages. **Eur. Cytokine Netw**. 10: 365-372.

Noh, D.O. and S.E. Gilland. 1994. Influence of bile on β-galactosidase activity of component species of yoghurt starter cultures. **J. Dairy Sci**. 77: 3532-3537.

Nomchit, K. and L. Vichien. 1998. Screening of lactic acid bacteria for yoghurt starter, pp. 25-27. *In* **Abstract Biotechnology for a self-sufficient economy**. November 1998. Bangkok.

Noonpakdee, W., C. Santivarangkna, P. Jumriangrit a, K. Sonomoto and S. Panyim. 2003. Isolation of nisin-producing *Lactococcus lactis* WNC 20 strain from nham, a traditional Thai fermented sausage. **Int. J. of Food Microbiol**. 81: 137-145.

Noriega L., M. Gueimonde, B. Sanchez, A. Margolles, C.G. de los Reyes-Gavilan. 2004. Effect of the adaptation to high bile salts concentrations on glycosidic activity, survival at low pH and cross-resistance to bile salts in Bifidobacterium. **Int J Food Microbiol**. 94: 79-86.

Noriega, L., I. Cuevas, A. Margolles and C. de los Reyes. 2006. Deconjugation and bile salts hydrolase activity by Bifidibacterium strain with acquired resistance to bile. **Int. Dairy J.** 16: 850-855.

Nousiainen, J. and J. Setala. 1998. Lactic acid bacteria as animal probiotics, pp. 431-4713. *In* S. Salminen and A. von Wright, eds. **Lactic acid bacteria**. 2nd ed., Mercel Dekker Inc., New York.

O´ May G.A. and G.T. Macfarlane. 2005. Probiotic efficacy: are the claims justified?, pp. 138–166. *In*: A.Y. Tamine, ed. **Probiotic Dairy Products**. Blackwell Publishing, London.

Oberman, H. and Z. Libudzisz. 1998. Fermented milk, pp. 308-350. *In* B.J.B. Wood, ed. **Microbiology of fermented foods**. 2nd ed. Blackie Academic & Professional,Glasgow.

Olejnik A., M. Lewandowska, M. Obarska and W. Grajek. 2005. Tolerance of Lactobacillus and Bifidobacterium strains to low pH, bile salts and digestive enzymes. **EJPAU**. Available Source: http://www.ejpau.media.pl/volume8/issue1/art-05.htm., September 27, 2005.

Opperdoes, F. 1997. Construction of a distance tree using clustering with the Unweighted Pair Group Method with Arithmetic Mean (UPGMA). Available Sourse: http://www.icp.ucl.ac.be/~opperd/private/upgma.html., December 23, 2013.

Ouellette, A.J., R.M. Greco, M. James, D. Frederick, J. Naftilan and J.T. Fallon. 1989. Developmental regulation of cryptdin, a corticostatin/defensin precursor mRNA in mouse small intestinal crypt epithelium. **J. Cell Biol**. 108: 1687-1695.

Ouwehand, A.C. and S. Salminen. 1998. The health effects of cultured milk products with viable and non-viable bacteria. **Int. Dairy J**. 8: 749-758.

Ouwehand, A.C., S. Salminen and E. Isolauri. 2002. Probiotics: an overview of beneficial effects. **Antonie Leeuwenhoek**. 82: 279-289.

Ouwehand, A. 1998. Antimicrobial components from lactic acid bacteria, pp. 139-160. *In* S. Salminen, A. von Wright, eds, **Lactic Acid Bacteria**. Marcel Dekker, New York.

Papamanoli, E., N. Tzanetakis, E. Litopoulou-Tzanetaki, P. Kotzekidou. 2003. Characterization of lactic acid bacteria isolated from a Greek dry fermented sausage in respect of the technological and probiotic properties. **Meat Sci.** 65: 859-867.

Parada, J.L., C.R. Caron, C.R., Medesiros, B.P. and C.R. Soccol. 2007. Bacteriocins from lactic acid bacteria: purification, properties and use as biopreservatives. **Brazilian archives of Biol.Technol**. 521-542.

Parente, E., M. Martuscelli, F. Gardini, S. Grieco, M.A. Crudele and G.Suzzi. 2001. Evolution of microbial populations and biogenic amine production in dry sausages produced in Southern Italy. **J. of Appl. Microbiol**. 90: 882-891.

Park, Y.S., J.Y. Lee, Y.S. Kim and D.H. Shin. 2002. Isolation and characterization of lactic acid bacteria from feces of newborn baby and from dongchimi, **J. of Agri. Food Chem**. 50: 2531-2536.

Paubert-Braquet M., X.H. Gan, C. Gaudichon, N. Hedef, A. Serikoff, C. Bouley, B. Bonavida and P. Braquet. 1995. Enhancement of host resistance against *Salmonella typhimurium* in mice fed a diet supplemented with yoghurt or milks fermented with various *Lactobacillus casei* strains. **Int. J. Immunother**. 11: 153-161.

Pelto, L., E. Isolauri, E. M. Lilius, J. Nuutila and S. Salminen. 1998. Probiotic bacteria down-regulate the milk-induced inflammatory response in milk-hypersensitive subjects but have an immunostimulatory effect in healthy subjects. **Clin. Exp. Aller**. 28: 1474-1479.

Pena, J.A. and J. Versalovic. 2003. *Lactobacillus rhamnosus* GG decreases TNF-alpha production in lipopolysaccharide-activated murine macrophages by a contact-independent mechanism. **Cell. Microbiol**. 5: 277-285.

Pennacchia, C., D. Ercolini, G. Blaiotta, O. Pepe, G. Mauriello and F. Villani. 2004. Selection of Lactobacillus strains from fermented sausages for their potential use as probiotics. **Meat Sci**. 62: 309-317.

Perdigon G. and S. Alvarez. 1992. Probiotics and the immune state, pp. 146-180. *In* R. Fuller**,** eds. **Probiotics : The Scientific Basis**. Chapman and Hall, London.

Perdigón G., S. Alvarez, M.E. Nader de Macías and M. Medici. 1989. The oral administration of lactic acid bacteria increases the mucosal intestinal immunity in response to enteropa-thogens. **J. Food Protect**. 53: 404-410.

Perdigon, G., A. de Moreno de LeBlanc, J. Valdez and M. Rachid. 2002. Role of yoghurt in the prevention of colon cancer. **Eur. J. Clin. Nutr**. 56 (Suppl. 3): S65-S68.

Perdigon, G., C. Maldonado Galdeano, C. G. Vinderola, A. de Moreno, M. Medici, and M. E. Bibas Bonet. 2004. Immunomodulation of mucosal immune response by probiotics. **Curr. Trends Immunol**. 6: 69-85.

Perdigon, G., C. Maldonado Galdeano, J. C. Valdez and M. Medici. 2002. Interaction of lactic acid bacteria with the gut immune system. **Eur. J. Clin.Nutr**. 56 (Suppl.4): S21-S26.

Perdigon, G., M. E. Nader de Macias, S. Alvarez, G. Oliver and A. Pescede Ruiz Holgado. 1990. Prevention of gastrointestinal infection using immunobiological methods with milk fermented with *Lactobacillus casei* and *Lactobacillus acidophilus*. **J. Dairy Res**. 57: 255-264.

Perdigón G., S. Alvarez and A. Pesce De Ruiz Holgado A. 1991. Oral immunoadjuvant activity of *Lactobacillus casei* influence of dose on the secretory immune response and protective capacity in intestinal infections. **J. Dairy Res**. 58: 485-496.

Perdigon, G., M. Medina, E. Vintini and J.C. Valdez. 2001. Intestinal pathway of internalization of lactic acid bacteria and gut mucosal immunostimulation. **Int. J. Immunol. Pharmacol**. 13: 141-150.

Perdigon, G., R. Fuller and R. Raya. 2001. Lactic acid bacteria and their effect on the immune system. **Curr. Iss. Intest. Microbiol**. 2: 27-42.

Perdigon, G., R. Fuller and M. Medina. 2005. The influence of the lactic acid bacteria and other resident microflora on the immune system of the growing animal, pp. 351-375. *In* W.H. Holzapfel and P.J. Naughton, eds, **Microbial ecology in growing animals**. Elsevier Publishing Inc., London, United Kingdom.

Perdigon, G., S. Alvarez, M. Medina, E. Vintini, and E. Roux. 1999. Influence of the oral administration of lactic acid bacteria on IgA producing cells associated to bronchus. **Int. J. Immunol. Pharmacol**. 12: 97-102.

Philip, C.D. and K. Samantha. 2002. The immune system: a target for functional foods?. **Brit. J. Nutr**. 88: 165-176.

Phillips J., M. Everson and Z. Moldoveanu. 1990. Synergistic effect of IL-4 and IFN-gamma on the expression of polymeric Ig receptor (secretory component) and IgA binding by human epithelial cells. **J. Immunol**. 145: 1740-1744.

Phillips-Quagliata, J. M., M. E. Roux, M. Arny, P. Kelly-Hatfield, M. Mc-Williams and M. E.Lamm. 1983. Migration and regulation of B cells in the mucosal immune system, pp. 194–202. *In* J. R. McGhee and J. Mestecky,eds. **The secretory immune system**. The New York Academy of Science, New York, NY.

Phromraksa, P., P. Wiriyacharee, L. Rujanakraikarn and P. Pathomrungsiyungkul. 2003. Identification of main factors affecting quality of Thai fermented pork sausage (Sai Krok Prew). **CMU. Journal**. 2 (2): 89-96.

Pidcock, K., G.M. Heard, A. Henriksson. 2002. Application of nontraditional meat starter cultures in production of Hungarain salami. **Int. J. of Food Microbiol**. 76: 75-81.

Pilar C.M., A. Samuel, B. Karola, M. Trinidad, P. Ananias and B.V. Jorge. 2008. Current applications and future trends of lactic acid bacteria and their bacteriocins for the biopreservation of aquatic food products. **Food and Bioproc. Technol**. 1 (1): 43-63.

Playne, M. 1995. Probiotic microorganisms. **Res. Adv. Microbiol**. 3: 215-254.

Podolak, P.K., J.F. Zayas, C. L. Kastner and D.Y.C. Fung. 1996. Inhibition of *Listeria monocytogenes* and *Escherichia coli* O157:H7 on beef by application of organic acids. **J. Food Protect.** 59: 370-373.

Popova, P., G. Guencheva, G. Davidkova, A. Dogdanov, E. Pacelli, G. Opalchenova , T. Kutzalova and C. Koychev. 1993. Stimulating effect of DEODAN (an oral preparation from *Lactobaciffus bulgaricll*s "LB51 ") on monocytes/macrophages and host resistance to experimental infections. ***Int.*** **J. Immunophamacol**. 15: 25-37.

Prasad, J., H.S. Gill, J. Smart, J. and P.K. Gopal. 1998. Selection and characterization of Lactobacillus and Bifidobacterium strains for use as probiotics. **Int. Dairy J**. 8: 993-1002.

Raibaud, P. 1992. Bacterial interactions in the gut, pp. 9-28. *In* R. Fuller, ed. **Probiotics**. Chapman & Hall London, England.

Rakoff-Nahoum, S., J. Paglino, F. Eslami-Varzaneh, S. Edberg and R. Medzhitov. 2004. Recognition of commensal microflora by Toll-like receptors is required for intestinal homeostasis. **Cell**. 118: 229-241.

_____, L. Hao and R. Medzhitov. 2006. Role of toll-like receptors in spontaneous commensal-dependent colitis. **Immunity**. 25: 319-329.

Ramare, F., J. Nicoli, J. Dabard, T.Corring, M. Ladire, A.M. Gueugneau and P. Raibaud. 1993. Trypsin-dependent production of an antibacterial substance by a human *Peptostrepto-coccus* strain in gnotobiotic rats and in vitro. **Appl.Environ. Microbiol**. 59: 2876-2883.

Ramirez-Chavarin, M.L., C. Wacher, C. A. Eslava-Campos and M. L. Perez-Chabela. 2013. Probiotic potential of thermotolerant lactic acid bacteria strains isolated from cooked meat products. **Int. Food. Res. J.** 20 (2): 991-1000.

Rangavajhyala, N., K.M. Shahani, G.Sridevi and S. Srikumaran. 1997. Nonlipopoly-saccharide component(s) of *Lactobacillus acidophilus* stimulate(s) the production of interleukin-1 alpha and tumor necrosis factor-alpha by murine macrophages. **Nutr. Cancer**. 28: 130-134.

Rantsiou, K., R. Urso, L. Iacumin, C. Cantoni, P. Cattaneo, G. Comi and L .Cocolin**.** 2005. Culture-dependent and -Independent methods to investigate the microbial mcology of Italian fermented sausages**. Appl. Environ. Microbiol**. 71: 1977-1986**.**

Rault, A., M. Bouix and C. Beal. 2008. Dynamic analysis of *Lactobacillus delbrueckii* subsp. *bulgaricus* CFL1 physiological characteristics during fermentation. **Appl. Microbiol. Biotechnol**. 81: 559-570.

_____, _____, and _____. 2009. Fermentation pH Influences the physiological state dynamics of *Lactobacillus bulgaricus* CFL1 during pH-controlled culture. **Appl. Environ. Microbiol.** 4374- 4381.

Reddy, G.V., B.A. Friend, K.M. Shahani and R.E. Farmer. 1983. Antitumer activity of yoghurt components. **J. Food protect**.46: 98-110.

Reddy, B. S. 1999. Possible mechanisms by which pro-and prebiotics influence colon carcinogenesis and tumor growth. **J. Nutr**. 129: 1478S-1482S.

Reid, G. and A. W. Bruce. 2006. Probiotics to prevent urinary tract infections: the rationale and evidence. **World J. Urol**. 24: 28-32.

Rescigno, M., M. Urbano, B. Valzasina, M. Francolini, G. Rotta, R. Bonasio, F. Granucci, J. P. Kraehenbuhl and P. Ricciardi-Castagnoli. 2001. Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. **Nat. Immunol**. 2: 361-367.

Rhee, S.J., J.E. Lee and C.H. Lee. 2001. Importance of lactic acid bacteria in Asian fermented foods. **10th Symposium on Lactic Acid Bacterium**. Egmond aan Zee, the Netherlands. 28 August - 1 September 2011.

Rook, G.A. and L.R. Brunet. 2005. Microbes, immunoregulation and the gut. **Gut**. 54: 317–320.

Roos, N. M. and M. B. Katan. 2000. Effects of probiotic bacteria on diarrhea, lipid meta-bolism and carcinogenesis: a review of papers published between 1988 and 1998. **Am. J. Clin. Nutr**. 71: 405-411.

Roughley, P.J. and E.R. Lee. 1994. Cartilage proteoglycans: structure and potential function. **Microsc. Res. Technol**. 28: 385-397.

Rumbo, M., P. Anderle, A. Didierlaurent, F. Sierra, N. Debard, J. C. Sirard,D. Finke and J. P. Kraenhenbuhl. 2004. How the gut link innate and adaptative immunity. **Ann. N. Y. Acad. Sci**. 1029: 16-21.

Sai, S., T. Tanaka, R. A. Kosher, and M. L. Tanzer. 1986. Cloning and sequence analysis of a partial cDNA for chicken cartilage proteoglycan core protein. **Proc. Natl. Acad. Sci.** 83: 5081-5085.

Saito I., K. Sato and Y. Horikawa. 1983. Enhanced humoral antibody production and delayed type hypersensitivity responce in mice by *Lactobacillus casei*. **Hiroshima J. Med. Sci**., 32: 223-225.

Salminen, S. J., Isolauri E. and Salminen E. 1996. Probiotics and stabilization of the gut mucosal barrier. **Asia Pac. J. Clin. Nutr**. 5: 53-56.

Salminen, S. J., A. Ouwehand, Y. Benno and Y.K. Lee. 1999. Probiotics: how should they be defined? **Trends Food Sci. Techn**. 10: 107-110.

Salminen S.J., C. Bouley, M.C. Boutron-Ruault, J. Cummings, A. Franck, G. Gibson, E. Isolauri, M.C. Moreau, M. Roberfroid and I. Rowland. 1998. Functional food science and gastrointestinal physiology and function. **Brit. J. Nutr**.80: 147-171.

Salminen, S., E. Isolauri and E. Salminen. 1996a. Clinical uses of probiotics for stabilizing the gut mucosal barrier: successful strains and future challenges. **Antonie van Leeuwenhoek**. 70: 347-358.

_____, _____and _____. 1996b. Probiotics and stabilization of the gut mucosal barrier. **Asia Pac. J. Clin. Nutr**. 5: 53-56.

Sameshima, T., C. Magome, K. Takeshita, K. Arihara, M. Itoh and Y. Kondo. 1998. Effect of intestinal Lactobacillus starter cultures on the behavior of *Staphylococcus aureus* in fermented sausage. **Int. J. of Food Microbiol**. 41: 1-7.

Sanders, M. and J.Huis in't Veld. 1999. Bringing a probiotic-containing functional food to the market: microbiological, product, regulatory and labeling issues. **Antonie van Leeuwenhoek**. 76: 293-315.

Santos, E.M., I. Jaime, J. Rovira, U. Lyhs, H. Korkeala, J. Bjorkroth. 2005. Characterization and identification of lactic acid bacteria in morcilla de Burgos. **Int. J. Food Microbiol**. 95: 285-296.

Sanz, Y., Fadda, S., Vignolo, G., Aristoy, M., Oliver, G., Toldra , F. 1999. Hydrolysis of muscle myofibrillar proteins by *Lactobacillus curvatus* and *Lactobacillus sake*. **Int. J. of Food Microbiol**. 53: 115-125.

Sara, M. 2001. Conserved anchoring mechanisms between crystalline cell surface S-layer proteins and secondary cell wall polymers in Gram-positive bacteria. **Trends Microbiol**. 9: 47-49.

Sara, M. and U. B. Sleytr. 2000. S-layer proteins. **J. Bacteriol**. 182: 859-868.

Sascha S. and K. Magdalena. 2010. Mass spectrometry tools for the classification and identification of bacteria. **Nat. Revs. Microbiol**. 8: 74-82.

Schell, M.A., M. Karmirantzou, B. Snel, D. Vilanova, B. Berger,, G. Pessi, M.C. Zwahlen, F. Desiere, P. Bork, M. Delley, R.D. Pridmore and F. Arigoni. 2002. The genome sequence of *Bifidobacterium longum* reflects its adaptation to the human gastrointestinal tract. **Proc. Natl. Acad. Sci**. USA 99: 14422–14427.

Schiffrin, E. J., D. Brassart, A. L. Servin, F. Rochat and A. Donnet-Hughes. 1997. Immune modulation of blood leukocytes in humans by lactic acid bacteria: criteria for strain selection. **Am. J. Clin. Nutr**. 66: 515S-520S.

Schiffrin, E. J., F. Rochat, H. Link-Amster,J. M. Aeschlimann and A. Donnet-Hughes. 1995. Immunomodulation of human blood cells following the ingestion of lactic acid bacteria. **J. Dairy Sci**. 78: 491-497.

Schleifer K. and W.Ludwig. 1995. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. **Microbiol.Revs**. 59: 143-169.

Schultz, M. 2000. Probiotics and inflammatory bowel disease. **Am. J. Gastroenterol**. 1(Suppl.): S19-S21.

Scicchitano R. Stanisz A., Ernst P. and Bienenstock J. 1988. A Common Mucosal Immune System Revisited. pp. 1-34. *In*. A. Husband, ed. **Migration and Homing of Lymphoid Cells**. CRC Press, Boca Raton, Florida.

Sebald, W., P. Friedl, H. U. Schairer, and J. Hoppe**.** 1982. Structure and genetics of the H^+-conducting F_0 portion of the ATP synthase. **Ann. N. Y. Acad. Sci**. 402: 28–44.

Senok, A.C., A.Y.Ismaeel and Botta G.A. 2005. Probiotics: facts and myths. **Clin Microbiol Infect**. 11: 958-966.

Servin A.L. 2004. Antagonistic activities of lactobacilli and bifidobacteria against microbial pathogens. **FEMS Microbiol. Rev**. 28: 405-440.

Shimizu, M., R. C. Fitzsimmons and S. Nakai. 1988. Anti-E. coli immunoglobulin Y isolated from egg yolk of immunized chickens as a potential food ingredient. **J. Food Sci**. 53:1360-1366.

Sillanpaa, J., B. Martinez, J. Antikainen, T. Toba, N. Kalkkinen and S. Tankka. 2000. Characterization of the collagen-binding S-layer protein CbsA of *Lactobacillus crispatus.* **J. of Bacteriol**. 182 (22): 6440-6450.

Silva, A. M., E. A. Bambirra, A. L. Oliveira, P. P. Souza, D. A. Gomes, E. C.Vieira and J. R. Nicoli. 1999. Protective effect of bifidus milk on the experimental infection with Salmonella enteritidis subsp. Typhimurium in conventional and gnotobiotic mice. **J. Appl. Microbiol**. 86: 331-336.

Simmering,R., B. Kleessen and M. Blaut. 1999. Quantification of the flavonoid-degrading bacterium Eubacterium ramulus in human fecal samples with a species-specific oligonucleotide hybridization probe. **Appl. Environ.Microbiol**. 65: 3705-3709.

Simpson W.J. and H. Taguchi. 1995. The genus *Pediococcus*, with notes on the genera*Tetratogenococcus* and *Aerococcus*, pp. 125-172. *In* B.J.B. Wood. and W.H. Holzapfel, eds. **The Genera of Lactic Acid Bacteria**. Chapman & Hall, London.

Sleytr, U.B. and T.J. Beveridge. 1999. Bacterial S-layers. **Trends in Microbiol**. 7: 253-260.

Smit, E., F. Oling, R. Demel, B. Martinez and P. H. Pouwels. 2001. The S-layer protein of *Lactobacillus acidophilus* ATCC 4356: identification and characterization of domains responsible for S-protein assembly and cell wall binding. **J. Mol. Biol**. 305: 245–257.

Smith, T. 1995. The digestive system, pp.150-173. *In* **The Human body: An Illustrated Guide to its structure, Function and Disorders**. Ken Fin Books. Collingwood. Victoria.

Snijders, J.M., J. G. van Logtestijn, D. A. Mossel and F. J. Smulders. 1985. Lactic acid as a decontaminant in slaughter and processing procedures. **Veterinary Quarterly**. 7: 277-282.

Solis Pereyra, B. and D. Lemonnier. 1993. Induction of human cytokines by bacteria used in dairy foods. **Nutr. Res**. 13: 1127-1140.

Spyropoulou, K.E., N. G. Chorianopoulos, P. N. Skandamis and G. Nychas. 2001. Control of *Escherichia coli* O157:H7 during the fermentation of Spanish-style greentable olives (Conservolea variety) supplemented with different carbon sources. **Int. J. Food Microbiol**. 66: 3-11.

Sobrino, O. J., J. M. Rodriguez, W. L. Moreira, M. F. Fernandez, B. Sanz and P. E. Hernandez. 1991. Antibacterial activity of *Lactobacillus sake* isolated from dry fermented sausages. **Int. J. of Food. Microl**. 13: 1-10.

Stanton, C., G. Gardiner, H. Meehan, K. Collins, G. Fitzgerald, P. B. Lynch, and R. P. Ross. 2001. Market potential for probiotics. **Am. J. Clin. Nutr**. 73(Suppl.): 476S-483S.

Stiles M.E. 1996. Biopreservation by lactic acid bacteria. **Antonie Leeuwenhoek**.70: 331–345.

Stiles, M.E. and J.W. Hastings. 1991. Bacteriocin production by lactic acid bacteria: potential for use in meat preservation. **Trends Food Sci. Technol**. 2: 247-251.

Stiles, M.E. and W.H. Holzapfel. 1997. Lactic acid bacteria of foods and their current taxonomy. **Int.J. Food Microbiol**. 36: 1-29.

Strobel, S. 1995. Oral tolerance. In: Mucosal Immunity and the Gut Epithelium: Interactions in Health and Disease. **Dyn. Nutr. Res.Basel Karger**. 4: 65-75.

Succi, M., P. Tremont, A. Reale, E. Sorrentino, L. Grazia, S. Pacifico and R. Coppola. 2005. Bile salt and acid tolerance of Lactobacillus rhamnosus strains isolated from Parmigiano Reggiano cheese. **FEMS Microbiol**. 244: 129-137.

Sullivan, A. and C.E. Nord. 2002. The place of probiotics in human intestinal infections. **Int. J. Antimicrob. Agents**. 20: 313-319.

Suskovic, J., Brkic, B., Matisic, S. and V. Maric. 1997. *Lactobacillus acidophilus* M92 as potential probiotic strain. **Milchwissenschaft**. 52: 430-435.

Suzuki, M.T. and S. J. Giovannoni. 1996. Bias caused by template annealing in the amplification of mixture of 16S rRNA genes by PCR. **Appl. Environ. Microbiol**. 62: 625-630.

Tagg, J.R. and A.R. McGiven, 1971. Assay system for bacteriocins. **Applied Microbiology**. 21: 943.

Taguchi T., W.K. Aicher, K. Fujihashi, M. Yamamoto, J.R., McGhee, J.A. Bluestone and H. Kiyono. 1991. Novel function for intestinal intraepithelial lymphocytes: murine CD+, γ/δ TCR+ T cells produce IFN-γ and IL-5. **J. Immunol**. 147: 3736-3744.

Tejada., M.V. Simon, Z. Ustunol and J. Pestka. 1999. Effects of lactic acid bacteria ingestion of basal cytokine mRNA and immunoglobulin levels in the mouse. **J. Food Protect**. 62: 287-291.

Takasugi M., Y. Tamura, H. Tachibana, M. Sugano and K. Yamada. 2001. Development of assay system for immunoglobulin production regulatory factors using whole cell cultures of mouse splenocytes. **Biosci. Biotechnol.Biochem**. 65(1): 143-149.

Tancrede, C. 1992. Role of human microflora in health and disease. **Eur. J.Microbiol. Infect. Dis**. 11: 1012-015.

Tani, K., K. Kurokawa, and M. Nasu. 1998. Development of a direct in situ PCR method for detection of specific bacteria in natural environments. **Appl. Environ. Microbiol**. 64: 1536-1540.

Tannock G.W. 2002. **Probiotics and prebiotics. Where are we going?.** Caister Acad Press. Norfolk. UK.

Tannock, G. 1999. Introduction, pp. 1-14. *In* G. Tannock, ed. **Probiotics: A critical review.** Horizon Scientific press, Norfolk, UK.

Tejada., Simon M.V., Z. Ustunol, J.J. Pestka. 1999. Effects of lactic acid bacteria ingestion of basal cytokine mRNA and immunoglobulin levels in the mouse. **J. Food Protect**. 62: 287-291.

The University of Arizona. 2000. **Immunology**. Available Source: http://www.biology.arizona.edu/immunology/tutorials/immunology/09t.html, March 25, 2006.

Thiravattanamontri, P., S. Tanasupawat, W. Noonpakdee, R. Valyasevi. 1998. Catalases of bacteria isolated from Thai fermented foods. **Food Biotechnol**. 12: 221-238.

Tizard R.I. 2000. **Veterinary Immunology**. WB, Saunders Company.

Trentham, D.E., R.A. Dynesius-Trentham, E.J. Orav, D. Combitchi , C.Lorenzo, K.L. Sewell, D.A. Hafler and H. L. Weiner. 1993. Effects of oral administration of type II collagen on rheumatoid arthritis. **Science**. 261: 1727-1730.

Tsai, C.C., H.Y. Hsih, H.H. Chiu, Y.Y. Lai, J.H. Liu, B. Yu and H.Y. Tsen. 2005. Antagonistic activity against Salmonella infection in vitro and in vivo for two Lactobacillus strains from swine and poultry. **Int J. Food. Microbiol.** 102: 185-194.

Tuomola, E.M. and S.J. Salminen. 1998. Adhesion of some probiotic and dairy Lactobacillus strains to Caco-2 cells cultures. **Int. J. Food Microbiol**. 41: 45-51.

Tyopponen, S., E. Petaja, T. Mattila-Sandholm. 2003. Bioprotectives and probiotics for dry sausages. **Int. J. of Food Microbiol**. 83: 233-244.

Tzortzis, G., M. L. Baillon, G. R. Gibson and R. A. Rastall. 2004. Modulation of anti-pathogenic activity in caninederived Lactobacillus species by carbohydrate growth substrate. **J. Appl. Microbiol.** 96: 552-559.

Uwe, B. S. and T.J. Beveridge. 1999. Bacterial S-layers. **Trends in Microbiol**. 7(6): 253-260.

Vaarala O. 2003. Immunological effects of probiotics with special reference to lactobacilli. **Clin Exp Allergy**. 33: 1634-1640.

van Hannen, E.J., M. P. von Agterveld, H. J. Gons and H. J. Laanbroek. 1998. Revealing genetic diversity of eukaryotic microorganisms in aquatic environments by denaturing gradient gel electrophoresis. **J. Phycol**. 34. 206-213.

Vanam, A.H. and M. Sutberland. 1995. **Meat and meat products:Technology, Chemistry and Microbiology**. Chapman and Hall, London.

Vaughan, E. E., B. Mollet, and W.M. Vos. 1999. Functionality of probiotics and intestinal lactobacilli: light in the intestinal tract tunnel. **Curr. Opin.Biotechnol**. 10: 505-510.

Venkatesh, K.V., M.R. Okos and P.C. Wankat. 1993. Kinetic model of growth and lactic acid production from lactose by *Lactobacillus bulgaricus*. **Process Biochem**. 28: 231-241.

Ventura, M., I. Jankovic, D.C. Walker, R.D. Pridmore and R. Zink. 2002. Identification and characterization of novel surface proteins in *Lactobacillus johnsonii* and *Lactobacillus gasseri.* **Appl. Environ. Microbiol.** 68(12): 6172-6181.

Verluyten J., F. Leroy and L. de Vuyst. 2004. Effects of different spices used in production of fermented sausages on the growth of and curvacin A production by *Lactobacillus curvatus* LTH 1174 **. Appl. Environ. Microbiol**. 70(8): 4807-4813.

Vidgren, G., I. Palva, R. Pakkanen, K. Lounatmaa and A. Palva. 1992. S-layer protein gene of Lactobacillus brevis: cloning by polymerase chain reaction and determination of the nucleotide sequence. **J. Bacteriol**. 174: 7419-7427.

Vinderola, C.G., C. Matar and G. Perdigon. 2005. Role of the epithelial cells in the immune effects mediated by gram-positive probiotic bacteria. Involvement of Toll-like receptors. **Clin. Diagn. Lab. Immunol**. 12: 1075-1084.

Vinderola C.G. and J.A. Reinheimer. 2003. Lactic acid starter and probiotic bacteria: a comparative "in vitro" study of probiotic characteristics and biological barrier resistance. **Food. Res. Int**. 36(9): 895-904.

Vintini, E., S. Alvarez, M. Medina, M. Medici, M. V. de Budeguer and G. Perdigon. 2000. Gut mucosal immunostimulation by lactic acid bacteria. **Biocell** 24: 223-232.

Vintini, E., V. Ocana and M. E. Nader de Macıas. 2004. Effect of lactobacilli administration in the vaginal tract of mice: evaluation of side effects and local immune response by local administration of selected strains. **Methods Mol.Biol**. 268: 401-410.

Visessanguana, W., B. Soottawat, R. Siriporn and T. Preenapha. 2004**.** Changes in composition and functional properties of proteins and their contributions to Nham characteristics. **Meat Science**. 66: 579-588.

Vizoso Pinto M.G., C.M. Franz, U. Schillinger and W.H. Holzapfel. 2006. Lactobacillus spp. with in vitro probiotic properties from human faeces and traditional fermented products. **Int. J. Food Microbiol**. 109**:** 205-214.

Vizoso Pinto, M. G., M. Rodriguez Gómez, S. Seifert , B. Watzl , W.H. Holzapfel and C. M. Franz. 2009. Lactobacilli stimulate the innate immune response and modulate the TLR expression of HT29 intestinal epithelial cells in vitro**. Int. J. Food. Microbiol.** 133(1-2): 86-93.

Von Wright, A., Salminen, S.. 1999. Probiotics: established effects and open questions. **Eur. J. Gastroenterol. Hepatol**. 11: 1195-1198.

Wang, X., L.L. Brown, A.J. Evans and P.L. Conway. 1999. The protective effects of high amylase maize starch granules on the survival of *Bifidobacteium* spp. In the mouse intestinal tract. **J. Appl. Microbiol.** 87: 631-639.

Ward, D. M., M. J. Ferris, S. C. Nold, and M. M. Bateson. 1998. A natural view of microbial biodiversity within hot spring cyanobacterial mat communities. **Microbiol. Mol. Biol. Rev.** 62: 1353-1367.

Ward, L.J.H. and M.J. Timmins. 1999. Differentiation of *Lactobacillus casei*, *Lactobacillus paracasei* and *Lactobacillus rhamnosus* by polymerase chain reaction. **Lett. Appl. Microbiol**. 29: 90-92.

Waterman, S.R and P.L Small. 1998. Acid-sensitive enteric pathogens are protected from killing under extremely acidic conditions of pH 2-5 when they are inoculated onto certain solid food sources. **Appl. Environ. Microbiol**.64: 3882–3886.

Weisz-Carrington P., M.E. Roux, M. Mc Williams, J.M. Phillips-Quagliata and M.E. Lamm. 1979. Organ and isotype distribution of plasma cells producing specific antibody after oral immunization: Evidence for a generalized secretory immune system. **J. Immunol**. 123: 1705-1710.

Wells, J.M., K. Robinson, L.M. Chamberlain, K.M. schofield and R.W.F. LePage. 1996**. Lactic acid bacteria as vaccine delivery vehicles**. Antonie va Leeuwenhoek. 70: 317-330.

Whitehead, K., J. Versalovic, S. Roos and R.A. Britton. 2008. Genomic and genetic characterization of the bile stress response of probiotic *Lactobacillus reuteri* ATCC 55730. **Appl. Environ. Microbiol**. 1812-1819.

Wilson, I.G. 1997. Inhibition and facilitation of nucleic acid amplification. **Appl. Environ. Microbiol**. 63: 3741-3751.

Wiriyacharee, P., L.Rujanakraikarn and A. Kuntiya. 1993. Nham product development using mixed bacterial starter cultures 1. Suitable source of carbohydrate for Nham production. **Journal of Agriculture**. 9(1): 51-60.

Wongkhalaung, C. and M. Boonyaratanakornkit. 1986. **Fermented foods in Thailand and similar products in ASEAN and elsewhere**. Institute of Food Research and Product Development ,Bangkok.

Wostmann B and Pleasants J. 1991. The germfree animal fed chemically defined diet: A unique tool. **Proc. Soc. Exp. Biol. Med**. 198: 539-546.

Wright, N. 1995. Growth factors in gastrointestinal mucosal inflammation. In: Mucosal Immunity and the Gut Epithelium. Interactions in Health and Disease. **Dyn. Nutr. Res**.Basel Karger. 4: 50-64.

Yan F. and D.B. Polk. 2011. Probiotics and immune health. **Curr. Opin. Gastroenterol**. 27: 496-501.

Yasui, H., K. Shida, T. Matsuzaki and T. Yokokura. 1999. Immunomodulatory function of lactic acid bacteria**. Antonie Leeuwenhoek** 76: 383-389.

Yukuchi, H., T. Goto and S. Okonogi. 1992. The nutritional and physiological value of fermented milk and lactic acid milk drink. pp. 217-245. *In* Y. Nakazawa and Hosono, eds. Translated by B.W. Howells. **Functions of fermented milk**. Elsevier Appl. Sci.USA.

Zalacian, I., M. J. Zapelena, M.P. de Pena, I. Astiasaran and J. Bello. 1997. Lipid fractions of dry fermented sausages change when starter culture and/or Aspergillus lipase are added**. J. Food Sci**. 62: 1076-1079.

Zarate, G., A. Perez-Chaia, S. Gonzalez and G. Oliver. 2000. Viability and B-galactosidase activity of dairy propionibacteria subjected to digestion by artificial gastric and intestinal fluids. **J. Food Prot.** 63: 1214-1221.

Zeljka, C.F., S. Vladimir, K. Lidija, N. Bela, Z. Nevijo and F.Ivana. 2012. Identification of lactic acid bacteria isolated from dry fermented cation of lactic acid bacteria isolated from dry fermented sausages. **Veterinarski Arhiv**. 82(3): 265-272.

APPENDICES

Appendix A

Chemicals preparation

Chemicals preparation

1. De Man Rogosa and Sharpe (MRS)

Dextrose	20.0 g.
Peptone	10.0 g.
Yeast extract	4.0 g.
Beef extract	8.0 g.
Sodium acetate	5.0 g.
Tri-Ammonium citrate	2.0 g.
Di-potassium hydrogen phosphate	2.0 g.
Magnesium sulphate	0.2 g.
Manganese sulphate	0.04 g.
Tween 80	1.0 ml.
Distilled water	1,000 ml.

Mixed all substances and stirred thoroughly, then autoclaved at 118 ^{o}C, 15 lb/inch^{2} pressure for 15 min.

2. De Man Rogosa and Sharpe Ox bile (MRS - Ox bile)

Dextrose	20.0 g.
Peptone	10.0 g.
Yeast extract	4.0 g.
Beef extract	8.0 g.
Sodium acetate	5.0 g.
Ammonium citrate	2.0 g.
Di-potassium hydrogen phosphate	2.0 g.
Magnesium sulphate	0.2 g.
Manganese sulphate	0.04 g.
Tween 80	1.0 ml.
Ox bile	3.0 g.

Distilled water 1,000 ml.

Mixed all substances and stirred thoroughly, then autoclaved at 118 ^{o}C, 15 lb/inch^2 pressure for 15 min.

3. Phosphate buffer saline (PBS)

KH_2PO_4
$Na_2HPO_4.12H_2O$
KCl
NaCl

Mix 100 ml Ca^{2+}/Mg^{2+} free 10X PBSA with 800 ml of distilled water. Separately, dissolve 0.1 g of magnesium chloride and 0.1 g of anhydrous calcium chloride to a final volume of 100 ml with water. With constant stirring, slowly add the magnesium/calcium chloride solution to the diluted PBSA. If a precipitate forms, start over, and slowly add with continuous stirring. Autoclaved at 118 ^{o}C, 15 lb/inch^2 pressure for 15 min.

Ca^{2+}/Mg^{2+} fr ee 10X PBSA (10X PBSA)

Dissolve 80 g of NaCl, 2.0 g of KCl, 15.0 g of Dibasic sodium phosphate and 2.0 of Monobasic potassium phosphate in 1 liter of distilled water. This makes a 10X solution of Ca^{2+}/Mg^{2+} free phosphate buffered saline.Dilute 1:10 prior to use. Store in refrigerator.

0.01M Potassium phosphate, monobasic (KH_2PO_4 MW 136.09)

Dissolve 1.36 g of monobasic potassium phosphate to a final volume of 1,000 mL with water.

0.01M Potassium phosphate, dibasic (K_2HPO_4 MW 174)

Dissolve 1.74 g of dibasic potassium phosphate to a final volume of 1,000 mL with water.

4. Phosphate buffered saline-Tween 20

Mix PBS and add 0.1% (v/v) Tween 20.

5. 0.85% (w/v) Saline (NaCl)

Saline refers to a solution of NaCl, with the most common usage for that which is isotonic to mammalian blood cells, notable a 0.85% or 0.9% solution. To mix, dissolve 8.5 g. of NaCl to a final volume of 1 liter with water.

6. Acrylamide Solutions

Acrylamide solutions for PAGE are given as total concentration of acrylamide (acrylamide + bisacrylamide) and the amount of cross linker (bisacrylamide). This is listed as the T:C ratio. For example, a 10% gel (10%T:5%C) would contain a total of 10 g of acrylamide per 100 ml, and would be composed of 5 g. of acrylamide and 5 g. of bis- acrylamide. Usually, a stock solution of 30% acrylamide is produced containing 0.8% bis-acrylamide. Many investigators use 30 g. of acrylamide plus 0.8 gram s of bis-acrylamide per 100 ml of water, but 29.2 g. of acrylamide plus 0.8 g. of bis would be technically correct. In practice, it makes little difference since the gels are diluted to 10% or less. The 30% stock solution is filtered through a 0.45 µ filter and stored at 4 ° C in the dark. For use, the stock solution is diluted with an appropriate buffer (usually a 2X Tris-HCl). The stock solution is stable for about one month. Discard after this period.

7. 0.1M Ammonium acetate (MW 77.08)

Add 7.708 grams of Ammonium acetate to a final volume of 1 liter of water.

8. n-Amyl alcohol (Pentanol $C_5H_{11}OH$ MW 88.15) Density = 0.8144 g/ml

The amyl alcohol can be weighed (33.5 grams) or measured volumetrically by using the density. That is, 33.5 g/0.8144 g/ml or 41.1 ml of n-amyl alcohol. Weigh or measure the appropriate amount and dilute to a final volume of 1 liter with water.

9. 1% (w/v) Bovine Serum Albumin (BSA)

There are many grades of BSA available and care should be taken when using this protein. For routine protein concentration standards, a 96-99% pure fraction (Sigma # A 2153) may be used. For tissue culture, RIA, or molecular weight standardization, BSA should be optained which is extracted and purified specifically for that purpose. Dissolve 0.5 g of BSA to a final volume of 50 ml in water or buffer.

10. Bromophenol blue (Sodium salt, MW 692.0)

Dissolve 1 mg of Bromophenol blue, sodium salt (Sigma # B7021) to a final volume of 100 ml with either water or buffer to obtained 0.001 (w/v).

11. 0.001M Citrate buffer (Sodium phosphate-Citrate buffer)

pH 4.8 Add 493 ml of 0.2 M Na_2HPO_4 to 507 ml of 0.1 M citric acid.

pH 3.6 Add 322 ml of 0.2 M Na_2HPO_4 to 678 ml of 0.1 M citric acid.

pH 4.2 Add 414 ml of 0.2 M Na_2HPO_4 to 586 ml of 0.1 M citric acid.

pH 5.4 Add 557.5 ml of 0.2 M Na_2HPO_4 to 442.6 ml of 0.1 M citric acid.

pH 6.0 Add 631.5 ml of 0.2 M Na_2HPO_4 to 368.5 ml of 0.1 M citric acid.

pH 6.6 Add 727.5 ml of 0.2 M Na_2HPO_4 to 272.5 ml of 0.1 M citric acid.

pH 7.2 Add 869.5 ml of 0.2 M Na_2HPO_4 to 130.5 ml of 0.1 M citric acid.

pH 7.8 Add 957.5 ml of 0.2 M Na_2HPO_4 to 42.5 ml of 0.1 M citric acid.

0.1 M Citric acid ($H_3C_6H_5O_7 \cdot H_2O$ MW 210.14)

Dissolve 21.01 g of citric acid to a final volume of 1 L

12. Fetal Calf Serum (FCS)

While it is possible to prepare your own serum from whole blood, it is easier (and safer) to purchase FCS from a reputable supplier. Commercial sources are free of mycoplasma, pre-sterilized and controlled for the presence of antibodies. There are a number of serum substitutes available on the market and these may be less expensive when storage is considered. Suppliers include Gibco, Flow Laboratories, KC Biological and Sigma Chemical Co.

13. 18.4 mg% Dinitrophenol (DNP MW 184.11)

Dissolve 18.4 mg of 2,4-dinitrophenol to a final volume of 100 ml with water or buffer.

14. 1 M EDTA (Ethylenediaminetetraacetic acid MW 292.24)

Dissolve 292.24 grams of EDTA, free acid to a final volume of 1 liter. If the more soluble disodium salt of EDTA is used, adjust the weight accordingly. The pH can be adjusted with acetic acid or NaOH. For corresponding concentration dilutions, multiply the weight in grams by the desired molarity. For example, for 10 mM EDTA, multiply 292.24 X 0.010 to obtain 2.92 grams of EDTA per liter.

15. Glycerol (MW 92.09)

10% (v/v)

10 ml of glycerol (glycerine) add enough water to make a final volume of 100 ml.

8 M

Weigh 73.67 grams of glycerol and add to a final volume of 100 ml. Alternatively, measure 499.1 ml of glycerol and make to a final volume of 1 liter (the density of glycerol at room temperature is 1.476) with water or buffer. For 8 M glycerol in MT buffer, make a 2X MT buffer for use as the diluents.

16. Hydrochloric Acid (HCl MW 36.46)

Concentrated HCl has a molarity of approximately 11.6.HCl is a gas, which is soluble in water and which comes in the form of concentrated reagent grade HCl. This solution is approximately 36-38% (w/v) HCl. To make a 1 N solution, add 86 ml of concentrated HCl to 800 ml of water and dilute to a final volume of 1 liter. For 0.1 N, dilute the 1 N by a factor of 10. For % solutions, note that liquid HCl is only 38% HCl, thus a 1% solution would require 2.6 ml of concentrated HCl (1/.38) per final volume of 100 ml.

Appendix B

ELISA assays and chemicals

ELISA assays and chemicals

Mouse IgA ELISA assay

Solution

All buffer solutions should be freshly prepared or use within 7 days of preparation, with 4-8°C storage.

Coating buffer:

50 mM Carbonate-bicarbonate buffer **,** pH 8.5

Washing solution : Tris buffer solution/Tween 20 (TBST)

8.00 g NaCl, 0.2 g KCl, 6.1 g Tris base (THAM), q. s. to 1 L; pH to 8.0. Add Tween20 0.05 %.

Sample diluents :

TBST + 1 % BSA

Materials

1. Microwell plates; 96-well
2. Microplate reader capable of measuring absorbance at 450 nm
3. Precision pipettes/ Micropipettes: 10 µl ,100 µl and 200-1,000µl single channel, 50-200 µl multichannel , and tips
4. Graduated cylinder
5. Deionized water
6. Incubator
7. Automated washer/Microplate washer

8. Microplate reader
9. Eppendorf tubes
10. Laboratory timer
11. Parafilm

Method

Coat with Capture antibody:

1. Dilute the capture antibody[#1] to 1:500 with coating buffer (50 mM Sodium Carbonate (pH 8.5). Add 100 µl of diluted antibody per well.
2. Store overnight at 4 °C.

Blocking:

1. Bring the plate to RT, wash 4 times. Tap plate on paper towels to remove excess buffer.
2. Block non-specific binding by adding 200 µl of blocking solution.
3. Incubate at 37 °C for 1 h.
4. Wash the plate 4 times.

Apply Standards and Samples:

1. Add standards[#2] or samples (dilute in sample diluents) 100 µl per well.
2. Incubate at 37 °C for 2 h.
3. Wash the plate 4 times.

Detection/Av-HRP:

1. Dilute the detection antibody to 1:500. with sample diluents (detection antibody 40 µl + sample diluent 20 ml). Add 100 µl /well
2. Incubate it for 1 h at 37 °C.
3. Wash 4 times.
4. Dilute Av-HRP to 1:2000 with sample diluents. (Av-HRP 10 □l + sample diluents 20 ml). Add 100 µl /well.
5. Incubate it for 30 min at 37 °C.
6. Wash 4 times.

Color Development:

1. Add 100 µl of Substrate Solution (TMB) to each well.
2. Incubate it at 37 °C for 30-120 min* in the dark.
3. Add 100 µl of Stop Solution (0.5 M H_2SO_4) to each well.
4. A_{450} nm.
5.

Capture antibody :

Purified anti-mouse IgA antibody (#556969, BD)

Standard :

Purified mouse IgA isotype control (#553476, BD, 0.5 mg/ml)

Detection antibody :

Biotin-conjugated rat anti-mouse IgA detection antibody (#556978, BD)

Av-HRP:

Avidin-Horseradish Peroxidase Conjugates (#554058, BD)

TMB:

3,3',5,5'-Tetramethylbenizidine Liquid Substrate System (T8665, Sigma)

MouseTotal IgG ELISA assay

Solution

Coating buffer : 50 mM Carbonate-bicarbonate buffer , pH 8.5

Washing solution : Phosphate buffer solution/Tween 20 (PBST)

8.00 g NaCl, 1.16 g Na_2HPO_4, 0.2 g KH_2PO_4, 0.2 g KCl, q. s. to 1 L; pH to 7.0. Add Tween20 0.05 %.

Sample diluents : PBST + 1 % BSA**.**

Substrate Buffer for pNPP : 28.*84 ml diethanolamine (Fisher, 85%), 0.025 g* $MgCl_2$*, 0.05 g* NaN_3*. Adjust pH to 9.8, q. s. to 250 ml.*

Method

Coat with Capture antibody:

1. Dilute the capture antibody[#1] to **1:2000** with 0.05 M Sodium Carbonate (pH 8.5).
2. Store overnight at 4 °C.

Blocking:

1. Bring the plate to RT, wash 4 times with PBST. Tap plate on paper towels to remove excess buffer.
2. Block non-specific binding by adding 200 µl of blocking solution per well.
3. Incubate at 37 °C for 1 h.
4. Wash the plate 4 times.

Apply Standards and Samples:

1. Add standards[#2] or samples, diluted in sample diluents, at 100 µl per well. Dilute sample 4-8 times .
2. Incubate at 37 °C for 2 h.
3. Wash the plate 4 times.

Incubation with Detection Antibody:

1. Dilute alkaline phosphatase conjugated rabbit anti-mouse IgG[#3] to 1:2000 with sample diluents. (alkaline phosphatase conjugated rabbit anti-mouse IgG[#3] 10 µl + sample diluents 20 ml). Add 100 µl per well.
2. Incubate at 37 °C for 1 h.
3. Wash 4 times.

Color Development:

1. Prepare substrate solution prior to use (1 mg/ml pNPP). p-Nitrophenol phosphate (pNPP)[#4] is 5 mg (4 tablet) , and mix with 20 ml substrate buffer[#5] in dark.
2. Add 100 µl of Substrate Solution (pNPP) per well.
3. Incubate it at 37 °C for 5-15 min in the dark.
4. Add 100 µl of stop solution (3N NaOH) per well.
5. A_{405} nm.

#1: Anti-mouse IgG monoclonal antibody (Calbiochem # 401223, 2 mg/ml), 4 ^{o}C Fridge

#2: Recombinant mouse IgG (Serotec # PMP01, 1 mg/ml), 4 ^{o}C Fridge
Prepare a 20 ng/ml from the stock standard and dilute it in a series of several two-fold dilutions, in 1% BSA in PBST.

#3: Alkaline phosphatase conjugated rabbit anti-mouse IgG (Sigma # A-1293), 4 ^{o}C Fridge

#4: Phosphate substrate (Sigma # S0942), -30 ^{o}C Freezer

#5: Substrate buffer, 4^{o}C Fridge.

Appendix C

Data of Fermented chicken cartilage pH value during 84 hour of fermentation process

Appendix Table C1 Data of Fermented chicken cartilage pH value during 84 hour of fermentation process.

Treatment/Time	0 h	12 h	24 h	36 h	48 h	60 h	72 h	84 h
control	6.54	5.76	5.33	5.10	4.92	4.81	4.74	4.63
control	6.32	5.84	5.55	5.23	5.03	4.98	4.84	4.18
control	6.65	5.88	5.73	5.48	4.78	4.78	4.67	4.68
Average of replication 1	6.50	5.83	5.54	5.27	4.91	4.86	4.75	4.50
control	6.57	6.11	5.68	5.44	5.13	4.89	4.79	4.63
control	6.62	5.96	5.38	5.21	5.17	5.06	4.62	4.68
control	6.55	6.08	5.48	5.22	5.05	4.82	4.56	4.38
Average of replication 2	6.58	6.05	5.51	5.29	5.12	4.92	4.66	4.56
control	6.61	6.48	6.08	5.78	5.12	4.81	4.44	4.11
control	6.52	6.13	5.92	5.57	5.09	4.71	4.46	4.44
control	6.45	6.08	5.86	5.03	4.83	4.63	4.14	4.21
Average of replication 3	6.53	6.23	5.95	5.46	5.01	4.72	4.35	4.25
Lb.johnsonii KUNN19-2	6.54	6.04	5.67	5.34	5.01	4.83	4.58	4.44
Lb.johnsonii KUNN19-2	6.34	5.57	4.51	4.46	3.88	3.61	3.8	4.11
Lb.johnsonii KUNN19-2	6.42	5.56	4.63	4.42	4.02	3.92	3.93	3.93
Average of replication 1	6.68	5.68	4.61	4.38	4.09	3.76	3.77	3.77

Appendix Table C1 (continued)

Treatment/Time	0 h	12 h	24 h	36 h	48 h	60 h	72 h	84 h
Lb.johnsonii KUNN19-2	6.48	5.60	4.58	4.42	4.00	3.76	3.83	3.94
Lb.johnsonii KUNN19-2	6.24	5.55	4.51	4.43	4.07	3.81	3.78	3.66
Lb.johnsonii KUNN19-2	6.22	5.43	4.58	4.43	4.11	3.84	3.82	3.85
Average of replication 3	6.55	5.11	4.6	4.58	4.3	3.77	3.72	3.89
P.pentosaceus KUNNE6-1	6.32	5.33	4.59	4.53	4.17	3.79	3.77	3.83
P.pentosaceus KUNNE6-1	6.36	5.49	4.59	4.48	4.10	3.78	3.80	3.83
P.pentosaceus KUNNE6-1	6.28	5.61	5.38	4.91	4.02	3.93	3.82	3.71
Average of replication 1	6.52	5.73	5.36	5.02	4.14	3.95	3.85	3.66
P.pentosaceus KUNNE6-1	6.55	5.88	5.41	4.93	4.21	4.06	3.83	3.67
P.pentosaceus KUNNE6-1	6.45	5.74	5.38	4.95	4.12	3.98	3.83	3.68
P.pentosaceus KUNNE6-1	6.46	5.95	5.23	4.91	4.31	4.08	3.91	3.89
Average of replication 2	6.52	5.97	5.24	4.91	4.25	4.01	3.86	3.71
P.pentosaceus KUNNE6-1	6.31	5.99	5.77	5.07	4.26	4.00	3.94	3.83
P.pentosaceus KUNNE6-1	6.43	5.97	5.41	4.96	4.27	4.03	3.90	3.81
P.pentosaceus KUNNE6-1	6.26	5.95	5.23	4.89	4.32	4.03	3.86	3.89
Average of replication 3	6.61	5.97	5.24	5.02	4.19	3.89	3.81	3.64

Appendix Table C1 (continued)

Treatment/Time	0 h	12 h	24 h	36 h	48 h	60 h	72 h	84 h
Mixed starter cuture	6.31	5.99	5.77	5.07	4.29	4.03	3.75	3.71
Mixed starter cuture	6.39	5.97	5.41	4.99	4.27	3.98	3.81	3.75
Mixed starter cuture	6.42	5.89	5.40	4.97	4.22	4.00	3.85	3.75
Average of replication 1	6.36	5.67	4.55	4.47	4.01	3.67	3.61	3.55
Mixed starter cuture	6.51	5.70	4.62	4.38	4.00	3.91	3.88	3.83
Mixed starter cuture	6.28	5.68	4.62	4.33	3.99	3.86	3.78	3.72
Mixed starter cuture	6.38	5.68	4.60	4.39	4.00	3.81	3.76	3.70
Average of replication 2	6.38	5.76	4.64	4.44	4.04	3.68	3.56	3.51
Mixed starter cuture	6.49	5.81	4.51	4.33	3.95	3.81	3.78	3.84
Mixed starter cuture	6.48	5.62	4.51	4.27	4.09	3.96	3.78	3.67
Mixed starter cuture	6.45	5.73	4.55	4.35	4.03	3.82	3.71	3.67
Average of replication 3	6.66	5.86	4.59	4.46	4.0	3.63	3.61	3.41

*Mixed starter culture**:** *Lb.johnsonii* KUNN19-2 + *P.pentosaceus* KUNNE6-1

Appendix D

Data of cell counts of Fermented chicken cartilage by total plate count on MRS agar at 12 hour of fermentation process

Appendix Table D1 Data of cell counts of Fermented chicken cartilage by total plate count on MRS agar at 12 hour of fermentation process.

Treatment	Cell count 1	Cell count 2	Cell count 3	Average	Log CFU
control	9.20E+08	8.50E+08	9.20E+07	7.02E+08	8.846
control	9.90E+07	8.50E+08	2.30E+09		
control	9.80E+08	9.90E+07	1.28E+08		
control	8.90E+07	9.50E+08	9.80E+08	1.01E+09	9.002
control	9.90E+08	9.80E+08	9.80E+08		
control	1.80E+08	1.27E+09	8.70E+08		
control	3.20E+08	9.80E+08	3.80E+08	7.28E+08	8.862
control	9.60E+07	8.80E+08	1.05E+09		
control	6.80E+08	9.80E+08	9.90E+07		
Lb.johnsonii KUNN19-2	8.80E+09	4.60E+09	9.40E+09	7.25E+09	9.861
Lb.johnsonii KUNN19-2	1.98E+10	1.50E+09	7.30E+09		
Lb.johnsonii KUNN19-2	8.90E+09	1.08E+09	3.90E+09		
Lb.johnsonii KUNN19-2	7.50E+09	4.80E+09	9.70E+09	7.03E+09	9.847
Lb.johnsonii KUNN19-2	1.41E+09	9.30E+09	8.90E+08		
Lb.johnsonii KUNN19-2	4.80E+09	6.90E+09	1.80E+10		
Lb.johnsonii KUNN19-2	2.24E+09	6.80E+09	9.10E+09	7.35E+09	9.866
Lb.johnsonii KUNN19-2	8.20E+09	1.15E+10	9.30E+08		
Lb.johnsonii KUNN19-2	1.08E+10	9.80E+09	6.80E+09		
P.pentosaceus KUNNE6-1	1.60E+09	1.30E+10	2.60E+09	3.16E+09	9.500
P.pentosaceus KUNNE6-1	1.80E+09	1.80E+09	4.50E+08		
P.pentosaceus KUNNE6-1	6.70E+09	1.04E+09	9.80E+07		
P.pentosaceus KUNNE6-1	1.23E+09	8.90E+09	6.90E+09	4.13E+09	9.616
P.pentosaceus KUNNE6-1	9.10E+09	7.30E+09	1.06E+09		
P.pentosaceus KUNNE6-1	1.08E+09	2.70E+08	3.30E+08		
P.pentosaceus KUNNE6-1	4.90E+09	9.50E+08	9.90E+07	1.68E+09	9.225
P.pentosaceus KUNNE6-1	1.09E+09	1.18E+09	1.08E+08		

Appendix Table D1 (continued)

Treatment	Cell count 1	Cell count 2	Cell count 3	Average	Log CFU
P.pentosaceus KUNNE6-1	1.18E+09	7.50E+09	2.43E+08		
Mixed starter cuture	1.90E+09	1.50E+09	6.90E+09	3.05E+09	9.484
Mixed starter cuture	3.80E+09	6.80E+09	9.80E+08		
Mixed starter cuture	1.08E+10	1.07E+09	1.04E+09		
Mixed starter cuture	1.08E+09	8.90E+08	2.90E+09	2.07E+09	9.317
Mixed starter cuture	9.10E+08	1.70E+09	9.50E+08		
Mixed starter cuture	9.80E+08	2.70E+09	3.30E+09		
Mixed starter cuture	6.30E+09	6.80E+08	3.60E+09	3.70E+09	9.568
Mixed starter cuture	2.80E+09	3.80E+09	4.40E+09		
Mixed starter cuture	8.90E+08	6.80E+09	2.90E+09		

*Mixed starter culture: *Lb.johnsonii* KUNN19-2 + *P.pentosaceus* KUNNE6-1

Appendix Table D2 Data of cell counts of Fermented chicken cartilage by total plate count on MRS agar at 24 hour of fermentation process

Treatment	Cell count1	Cell count2	Cell count3	Average	Log CFU
control	9.20E+08	1.05E+09	9.80E+09	6.74E+09	9.828
control	9.90E+08	9.80E+09	9.80E+09		
control	9.80E+09	8.70E+09	1.27E+09		
control	9.90E+08	9.50E+09	9.20E+08	3.90E+09	9.591
control	9.90E+08	1.05E+10	1.03E+10		
control	1.80E+10	9.90E+09	9.80E+09		
control	8.20E+08	9.80E+09	4.80E+09	8.93E+09	9.951
control	9.60E+09	8.80E+09	1.05E+10		
control	6.80E+09	9.80E+09	9.90E+09		
Lb.johnsonii KUNN19-2	9.80E+11	8.60E+11	1.04E+12	8.66E+11	11.937
Lb.johnsonii KUNN19-2	9.80E+11	7.50E+11	8.80E+11		
Lb.johnsonii KUNN19-2	7.30E+11	6.80E+11	8.90E+11		
Lb.johnsonii KUNN19-2	8.50E+11	3.40E+12	9.90E+10	8.66E+11	11.938
Lb.johnsonii KUNN19-2	6.10E+11	8.80E+11	7.50E+11		
Lb.johnsonii KUNN19-2	4.80E+11	9.90E+10	6.30E+11		
Lb.johnsonii KUNN19-2	6.80E+11	6.80E+11	2.90E+12	9.32E+11	11.969
Lb.johnsonii KUNN19-2	8.20E+11	1.25E+12	9.90E+10		
Lb.johnsonii KUNN19-2	9.80E+11	9.80E+10	8.80E+11		
P.pentosaceus KUNNE6-1	4.70E+10	6.50E+10	2.10E+10	2.75E+10	10.439
P.pentosaceus KUNNE6-1	4.90E+10	1.80E+09	1.80E+10		
P.pentosaceus KUNNE6-1	1.08E+11	3.50E+10	2.40E+10		
P.pentosaceus KUNNE6-1	1.03E+11	8.90E+09	4.90E+09	2.31E+10	10.363
P.pentosaceus KUNNE6-1	4.50E+10	7.70E+10	1.60E+10		
P.pentosaceus KUNNE6-1	8.00E+10	2.70E+10	4.60E+09		
P.pentosaceus KUNNE6-1	1.00E+11	7.90E+10	6.90E+09	3.91E+10	10.592
P.pentosaceus KUNNE6-1	4.60E+10	1.18E+11	6.30E+09		

Appendix Table D2 (continued)

Treatment	Cell count1	Cell count2	Cell count3	Average	Log CFU
P.pentosaceus KUNNE6-1	3.20E+10	1.05E+10	1.40E+10		
Mixed starter cuture	8.90E+10	8.30E+10	6.90E+10	1.02E+11	11.007
Mixed starter cuture	3.80E+10	7.08E+10	1.80E+11		
Mixed starter cuture	1.80E+10	1.23E+11	8.40E+10		
Mixed starter cuture	1.08E+11	7.90E+09	8.90E+10	7.27E+10	10.862
Mixed starter cuture	8.10E+10	1.70E+11	9.50E+10		
Mixed starter cuture	9.80E+10	6.70E+10	7.30E+09		
Mixed starter cuture	8.30E+10	4.80E+11	3.60E+10	1.29E+11	11.112
Mixed starter cuture	2.80E+11	9.80E+09	4.40E+10		
Mixed starter cuture	8.10E+09	1.28E+11	7.90E+10		

*Mixed starter culture: *Lb.johnsonii* KUNN19-2 + *P.pentosaceus* KUNNE6-1

Appendix Table D3 Data of cell counts of Fermented chicken cartilage by total plate count on MRS agar at 36 hour of fermentation process

Treatment	Cell count1	Cell count2	Cell count3	Average	Log CFU
control	9.90E+09	9.50E+10	4.20E+09	4.55E+10	10.658
control	9.90E+09	5.10E+10	3.30E+10		
control	9.80E+10	9.90E+10	9.80E+09		
control	8.20E+09	9.80E+09	8.80E+09	2.75E+10	10.439
control	1.06E+10	1.48E+10	1.95E+10		
control	8.80E+09	9.80E+10	6.90E+10		
control	9.20E+08	1.65E+09	9.80E+09	5.86E+09	9.768
control	9.90E+08	9.80E+09	9.80E+09		
control	9.80E+09	8.70E+09	1.27E+09		
Lb.johnsonii KUNN19-2	7.80E+11	8.80E+11	5.30E+12	1.46E+12	12.165
Lb.johnsonii KUNN19-2	8.80E+11	1.55E+12	9.30E+11		
Lb.johnsonii KUNN19-2	9.80E+11	9.80E+11	8.90E+11		
Lb.johnsonii KUNN19-2	7.70E+11	4.10E+12	1.09E+11	1.13E+12	12.053
Lb.johnsonii KUNN19-2	7.80E+11	8.70E+11	8.50E+11		
Lb.johnsonii KUNN19-2	8.80E+11	9.90E+11	8.30E+11		
Lb.johnsonii KUNN19-2	1.08E+12	8.80E+11	1.04E+12	1.29E+12	12.110
Lb.johnsonii KUNN19-2	9.80E+11	1.07E+12	9.80E+11		
Lb.johnsonii KUNN19-2	8.70E+11	3.80E+12	8.90E+11		
P.pentosaceus KUNNE6-1	1.27E+11	9.50E+11	8.20E+11	1.55E+12	12.189
P.pentosaceus KUNNE6-1	1.39E+12	2.80E+12	6.80E+11		
P.pentosaceus KUNNE6-1	1.38E+11	3.50E+12	3.50E+12		
P.pentosaceus KUNNE6-1	8.20E+11	8.90E+11	8.90E+11	2.14E+12	12.330
P.pentosaceus KUNNE6-1	6.70E+12	9.70E+11	8.80E+11		
P.pentosaceus KUNNE6-1	8.00E+11	2.70E+12	4.60E+12		
P.pentosaceus KUNNE6-1	6.40E+12	8.80E+11	8.90E+11	2.72E+12	12.435
P.pentosaceus KUNNE6-1	9.60E+11	1.38E+12	1.28E+12		

Appendix Table D3 (continued)

Treatment	Cell count1	Cell count2	Cell count3	Average	Log CFU
P.pentosaceus KUNNE6-1	9.20E+11	5.40E+12	6.40E+12		
Mixed starter cuture	8.90E+11	6.30E+11	8.90E+11	1.13E+12	12.054
Mixed starter cuture	1.80E+12	7.80E+11	1.80E+12		
Mixed starter cuture	1.80E+12	6.60E+11	9.40E+11		
Mixed starter cuture	1.38E+12	9.90E+11	8.90E+11	1.09E+12	12.037
Mixed starter cuture	8.60E+11	2.70E+12	1.15E+12		
Mixed starter cuture	9.80E+11	7.70E+11	7.30E+10		
Mixed starter cuture	7.30E+11	7.80E+12	3.60E+11	1.33E+12	12.125
Mixed starter cuture	7.08E+11	9.80E+10	6.40E+11		
Mixed starter cuture	8.10E+11	1.48E+11	7.10E+11		

*Mixed starter culture: *Lb.johnsonii* KUNN19-2 + *P.pentosaceus* KUNNE6-1

Appendix Table D4 Data of cell counts of Fermented chicken cartilage by total plate count on MRS agar at 48 hour of fermentation process

Treatment	Cell count1	Cell count2	Cell count3	Average	Log CFU
control	4.20E+10	8.50E+09	9.80E+09	5.66E+10	10.753
control	2.90E+10	1.38E+11	9.80E+10		
control	9.80E+10	8.90E+09	7.70E+10		
control	9.90E+09	2.50E+11	8.20E+09	9.17E+10	10.962
control	9.90E+10	8.10E+10	8.80E+10		
control	1.80E+11	9.90E+10	9.80E+09		
control	9.80E+10	1.28E+11	8.80E+10	1.02E+11	11.009
control	8.60E+10	7.80E+10	1.15E+11		
control	1.09E+11	9.80E+10	1.19E+11		
Lb.johnsonii KUNN19-2	8.90E+11	9.30E+11	9.90E+11	1.22E+12	12.087
Lb.johnsonii KUNN19-2	1.80E+12	8.80E+11	1.80E+12		
Lb.johnsonii KUNN19-2	1.80E+12	9.60E+11	9.40E+11		
Lb.johnsonii KUNN19-2	1.38E+12	9.90E+11	8.90E+11	1.16E+12	12.065
Lb.johnsonii KUNN19-2	8.60E+11	2.70E+12	1.15E+12		
Lb.johnsonii KUNN19-2	9.80E+11	7.70E+11	7.30E+11		
Lb.johnsonii KUNN19-2	7.30E+11	7.80E+12	6.60E+11	2.24E+12	12.351
Lb.johnsonii KUNN19-2	7.08E+12	9.80E+11	6.40E+11		
Lb.johnsonii KUNN19-2	8.10E+11	7.80E+11	7.10E+11		
P.pentosaceus KUNNE6-1	9.70E+11	9.50E+11	8.20E+11	1.66E+12	12.220
P.pentosaceus KUNNE6-1	1.39E+12	2.80E+12	8.80E+11		
P.pentosaceus KUNNE6-1	1.38E+11	3.50E+12	3.50E+12		
P.pentosaceus KUNNE6-1	8.20E+11	8.90E+11	8.90E+11	2.49E+12	12.395
P.pentosaceus KUNNE6-1	6.70E+12	9.90E+11	9.80E+11		
P.pentosaceus KUNNE6-1	8.00E+11	5.70E+12	4.60E+12		
P.pentosaceus KUNNE6-1	6.40E+12	8.80E+11	8.90E+11	2.99E+12	12.476
P.pentosaceus KUNNE6-1	9.60E+11	3.80E+12	1.28E+12		
P.pentosaceus KUNNE6-1	9.20E+11	5.40E+12	6.40E+12		

Appendix Table D4 (continued)

Treatment	Cell count1	Cell count2	Cell count3	Average	Log CFU
Mixed starter cuture	7.80E+11	8.80E+11	5.30E+12	1.46E+12	12.165
Mixed starter cuture	8.80E+11	1.55E+12	9.30E+11		
Mixed starter cuture	9.80E+11	9.80E+11	8.90E+11		
Mixed starter cuture	7.70E+11	4.10E+12	1.09E+11	1.13E+12	12.053
Mixed starter cuture	7.80E+11	8.70E+11	8.50E+11		
Mixed starter cuture	8.80E+11	9.90E+11	8.30E+11		
Mixed starter cuture	1.08E+12	8.80E+11	1.04E+12	1.29E+12	12.110
Mixed starter cuture	9.80E+11	1.07E+12	9.80E+11		
Mixed starter cuture	8.70E+11	3.80E+12	8.90E+11		

*Mixed starter culture: *Lb.johnsonii* KUNN19-2 + *P.pentosaceus* KUNNE6-1

Appendix Table D5 Data of cell counts of Fermented chicken cartilage by total plate count on MRS agar at 60 hour of fermentation process

Treatment	Cell count1	Cell count2	Cell count3	Average	Log CFU
control	9.80E+10	4.80E+11	7.30E+11	4.86E+11	11.687
control	5.80E+11	8.90E+10	8.80E+11		
control	8.60E+10	7.80E+11	6.50E+11		
control	9.90E+10	9.50E+10	8.80E+10	2.66E+11	11.425
control	1.09E+12	9.80E+10	1.19E+11		
control	6.20E+11	8.50E+10	9.80E+10		
control	9.90E+10	4.50E+11	7.80E+11	5.09E+11	11.707
control	1.80E+12	9.90E+10	9.80E+11		
control	1.09E+11	9.80E+10	1.69E+11		
Lb.johnsonii KUNN19-2	9.00E+05	5.30E+06	3.90E+05	2.11E+06	6.324
Lb.johnsonii KUNN19-2	2.80E+06	3.70E+06	7.30E+05		
Lb.johnsonii KUNN19-2	3.30E+06	1.80E+06	3.60E+04		
Lb.johnsonii KUNN19-2	1.38E+06	9.00E+04	9.00E+05	1.32E+06	6.121
Lb.johnsonii KUNN19-2	8.60E+05	2.70E+05	1.50E+05		
Lb.johnsonii KUNN19-2	8.10E+06	1.48E+05	7.00E+03		
Lb.johnsonii KUNN19-2	1.80E+04	7.80E+03	1.80E+02	5.46E+05	5.737
Lb.johnsonii KUNN19-2	7.80E+05	3.80E+04	6.40E+02		
Lb.johnsonii KUNN19-2	3.80E+06	2.60E+05	9.40E+03		
P.pentosaceus KUNNE6-1	9.20E+05	5.40E+07	6.40E+04	6.25E+06	6.796
P.pentosaceus KUNNE6-1	8.00E+05	2.70E+05	4.60E+04		
P.pentosaceus KUNNE6-1	1.30E+04	2.80E+04	6.80E+04		
P.pentosaceus KUNNE6-1	9.60E+06	1.30E+04	2.60E+04	1.34E+06	6.126
P.pentosaceus KUNNE6-1	2.70E+04	7.50E+05	6.20E+04		
P.pentosaceus KUNNE6-1	1.38E+04	1.50E+06	3.50E+04		
P.pentosaceus KUNNE6-1	6.70E+07	9.70E+04	8.00E+03	8.26E+06	6.917
P.pentosaceus KUNNE6-1	6.40E+06	8.80E+03	8.90E+03		
P.pentosaceus KUNNE6-1	8.20E+05	8.90E+02	6.70E+03		

Appendix Table D5 (continued)

Treatment	Cell count1	Cell count2	Cell count3	Average	Log CFU
Mixed starter cuture	7.80E+04	5.30E+05	8.80E+05	3.30E+05	5.518
Mixed starter cuture	4.10E+05	8.50E+04	1.55E+04		
Mixed starter cuture	8.70E+05	8.30E+02	9.80E+04		
Mixed starter cuture	7.70E+06	9.80E+05	9.80E+05	3.21E+06	6.507
Mixed starter cuture	7.80E+05	8.80E+04	9.30E+06		
Mixed starter cuture	8.80E+04	9.80E+04	8.90E+06		
Mixed starter cuture	1.08E+05	1.04E+03	8.80E+05	5.12E+05	5.709
Mixed starter cuture	1.09E+06	9.90E+04	1.07E+06		
Mixed starter cuture	8.70E+04	8.90E+05	3.80E+05		

*Mixed starter culture: *Lb.johnsonii* KUNN19-2 + *P.pentosaceus* KUNNE6-1

Appendix Table D6 Data of cell counts of Fermented chicken cartilage by total plate count on MRS agar at 72 hour of fermentation process

Treatment	Cell count1	Cell count2	Cell count3	Average	Log CFU
control	9.90E+05	9.80E+03	7.00E+00	2.07E+05	5.316
control	5.80E+02	1.10E+01	8.80E+02		
control	8.60E+05	9.80E+01	6.50E+02		
control	9.90E+05	9.80E+03	8.80E+01	2.16E+05	5.335
control	4.80E+04	9.80E+02	6.20E+02		
control	8.90E+05	8.50E+03	9.00E+00		
control	7.80E+04	4.50E+02	1.80E+01	1.00E+04	4.001
control	9.50E+03	7.80E+02	1.09E+02		
control	1.09E+02	9.80E+02	1.69E+02		
Lb.johnsonii KUNN19-2	9.00E+02	9.00E+02	9.00E+02	9.91E+05	5.996
Lb.johnsonii KUNN19-2	6.40E+02	1.30E+04	2.60E+03		
Lb.johnsonii KUNN19-2	8.80E+02	9.80E+02	8.90E+06		
Lb.johnsonii KUNN19-2	1.38E+02	1.50E+02	3.50E+02	1.15E+04	4.060
Lb.johnsonii KUNN19-2	8.60E+02	2.70E+02	1.50E+03		
Lb.johnsonii KUNN19-2	1.09E+03	9.90E+04	7.00E+01		
Lb.johnsonii KUNN19-2	8.70E+02	8.90E+03	1.80E+02	2.64E+03	3.422
Lb.johnsonii KUNN19-2	7.80E+03	3.80E+02	6.40E+02		
Lb.johnsonii KUNN19-2	3.80E+03	2.60E+02	9.40E+02		
P.pentosaceus KUNNE6-1	9.20E+03	5.40E+03	9.80E+03	3.59E+03	3.555
P.pentosaceus KUNNE6-1	8.00E+02	2.70E+02	4.60E+03		
P.pentosaceus KUNNE6-1	1.30E+03	2.80E+02	6.80E+02		
P.pentosaceus KUNNE6-1	9.60E+02	8.50E+02	5.50E+02	1.68E+03	3.225
P.pentosaceus KUNNE6-1	2.70E+02	8.30E+02	3.60E+02		
P.pentosaceus KUNNE6-1	1.38E+03	9.80E+03	9.80E+01		
P.pentosaceus KUNNE6-1	6.70E+03	9.70E+01	8.00E+02	1.21E+03	3.084
P.pentosaceus KUNNE6-1	6.40E+02	8.80E+02	8.90E+01		
P.pentosaceus KUNNE6-1	8.20E+02	8.90E+02	6.00E+00		

Appendix Table D6 (continued)

Treatment	Cell count1	Cell count2	Cell count3	Average	Log CFU
Mixed starter cuture	7.80E+01	5.30E+02	8.80E+02	4.21E+02	2.625
Mixed starter cuture	4.10E+01	5.30E+02	3.90E+01		
Mixed starter cuture	8.70E+02	8.10E+02	1.40E+01		
Mixed starter cuture	7.70E+02	1.80E+02	7.00E+00	5.19E+02	2.716
Mixed starter cuture	7.80E+02	8.80E+02	9.30E+02		
Mixed starter cuture	2.80E+01	3.70E+02	7.30E+02		
Mixed starter cuture	1.08E+03	1.04E+02	8.80E+02	9.34E+02	2.971
Mixed starter cuture	3.30E+03	1.80E+03	1.07E+03		
Mixed starter cuture	7.50E+01	6.20E+01	3.80E+01		

*Mixed starter culture: *Lb.johnsonii* KUNN19-2 + *P.pentosaceus* KUNNE6-1

Appendix E

Data of acid and bile tolerance of *P.pentosaceus* KUNNE6-1and *Lb.johnsonii* KUNN19-2

Appendix Table E1 Acid tolerance of *P. pentosaceus* KUNNE6-1

Replication I

Treatment	Cell count 1	Cell count 2	Cell count 3	Average	Log CFU	Average log	% Survival
control	7.40E+08	4.30E+08	1.00E+08	4.23E+08	8.6267	8.7333	100
control	3.60E+08	5.80E+08	7.70E+08	5.70E+08	8.7559		
control	8.20E+08	9.20E+08	2.30E+08	6.57E+08	8.8173		
0.3M NaCl, pH 1.5	1.80E+06	1.00E+06	1.30E+06	1.37E+06	6.1357	6.0046	68.756
0.3M NaCl, pH 1.5	1.30E+06	7.00E+05	1.40E+06	1.13E+06	6.0544		
0.3M NaCl, pH 1.5	3.00E+05	1.40E+06	3.00E+05	6.67E+05	5.8239		
0.3M NaCl,pH 1.5, 0.3% pepsin	3.90E+05	2.30E+05	1.80E+05	2.67E+05	5.4260	5.0826	58.198
0.3M NaCl,pH 1.5, 0.3% pepsin	5.00E+04	3.20E+04	2.80E+05	1.21E+05	5.0816		
0.3M NaCl,pH 1.5, 0.3% pepsin	9.00E+03	7.00E+04	8.60E+04	5.50E+04	4.7404		

Appendix Table E1 (continued)

Replication II

Treatment	Cell count 1	Cell count 2	Cell count 3	Average	Log CFU	Average log	% Survival
control	6.40E+08	5.60E+08	9.40E+07	4.31E+08	8.6348	8.6819	100
control	5.30E+08	4.40E+08	3.40E+08	4.37E+08	8.6402		
control	8.70E+08	6.30E+08	2.70E+08	5.90E+08	8.7709		
0.3M NaCl, pH 1.5	2.20E+06	1.20E+06	2.00E+06	1.70E+06	6.2304	5.8840	67.773
0.3M NaCl, pH 1.5	1.30E+06	9.00E+05	2.10E+06	1.10E+06	6.0414		
0.3M NaCl, pH 1.5	1.80E+05	3.00E+05	4.00E+05	2.40E+05	5.3802		
0.3M NaCl,pH 1.5, 0.3% pepsin	2.70E+05	1.70E+05	1.30E+05	1.90E+05	5.2788	5.1236	59.015
0.3M NaCl,pH 1.5, 0.3% pepsin	7.20E+04	2.30E+04	2.90E+05	1.28E+05	5.1083		
0.3M NaCl,pH 1.5, 0.3% pepsin	8.80E+04	9.00E+04	1.11E+05	9.63E+04	4.9838		

$$\% \text{ Survival} = \frac{\text{Colonies grown on MRS agar at detection time}}{\text{The initial bacterial cell counts}} \times 100$$

Appendix Table E2 Bile tolerance of *P.pentosaceus* KUNNE6-1

Condition	Cell count 1	Cell count 2	Cell count 3	Average	log average	Average log	% Survival
control	1.48E+08	8.90E+08	6.90E+08	5.76E+08	8.76E+00	8.760	100.000
control	1.10E+08	7.10E+08	1.50E+08	3.23E+08	8.51E+00		100.000
control	6.80E+07	2.70E+08	3.30E+08	2.23E+08	8.35E+00		100.000
control	8.90E+08	5.50E+08	1.10E+08	5.17E+08	8.71E+00	8.713	100.000
control	8.90E+08	7.30E+08	8.80E+07	5.69E+08	8.76E+00		100.000
control	8.80E+08	7.70E+08	2.40E+08	6.30E+08	8.80E+00		100.000
Intestinal 1h	4.60E+05	3.70E+05	3.20E+05	3.83E+05	5.58E+00	5.584	63.736
Intestinal 1h	5.80E+05	3.80E+05	6.10E+05	5.23E+05	5.72E+00		67.203
Intestinal 1h	1.30E+05	4.50E+05	3.30E+05	3.03E+05	5.48E+00		65.670
Intestinal 1h	3.40E+05	6.60E+05	4.50E+05	4.83E+05	5.68E+00	5.684	65.237
Intestinal 1h	5.40E+05	4.50E+05	8.10E+05	6.00E+05	5.78E+00		65.996
Intestinal 1h	3.80E+05	1.60E+05	3.60E+05	3.00E+05	5.48E+00		62.245
Intestinal 2h	1.70E+03	1.14E+03	1.90E+03	1.58E+03	3.20E+00	3.199	36.513
Intestinal 2h	1.58E+02	4.90E+02	2.80E+02	3.09E+02	2.49E+00		29.266
Intestinal 2h	8.90E+02	2.30E+02	1.34E+03	8.20E+02	2.91E+00		34.906
Intestinal 2h	9.90E+02	2.16E+03	1.03E+03	1.39E+03	3.14E+00	3.144	36.084

Appendix Table E2 (continued)

Condition	Cell count 1	Cell count 2	Cell count 3	Average	log average	Average log	% Survival
Intestinal 2h	1.39E+02	1.83E+02	2.50E+02	1.91E+02	2.28E+00		26.044
Intestinal 2h	1.83E+02	6.80E+02	2.60E+02	3.74E+02	2.57E+00		29.244
Intestinal 4h	7.60E+04	2.50E+05	1.10E+05	1.45E+05	5.16E+00	5.162	58.928
Intestinal 4h	3.80E+04	8.80E+05	2.40E+04	3.14E+05	5.50E+00		64.596
Intestinal 4h	2.40E+04	3.00E+04	2.40E+05	9.80E+04	4.99E+00		59.792
Intestinal 4h	1.10E+05	2.80E+05	7.40E+04	1.55E+05	5.19E+00	5.189	59.558
Intestinal 4h	7.40E+04	8.40E+05	3.80E+04	3.17E+05	5.50E+00		62.836
Intestinal 4h	1.40E+05	7.00E+04	2.60E+04	7.87E+04	4.90E+00		55.638
Gastric and intestinal 1h	1.03E+02	1.34E+02	3.90E+01	9.20E+01	1.96E+00	1.964	22.417
Gastric and intestinal 1h	1.14E+02	6.70E+01	1.43E+02	1.08E+02	2.03E+00		23.896
Gastric and intestinal 1h	8.40E+01	1.40E+02	2.60E+01	8.33E+01	1.92E+00		23.010
Gastric and intestinal 1h	7.10E+01	1.80E+02	2.10E+02	1.54E+02	2.19E+00	2.187	25.095
Gastric and intestinal 1h	1.40E+02	1.30E+02	4.60E+02	2.43E+02	2.39E+00		27.254
Gastric and intestinal 1h	6.60E+01	2.30E+01	9.70E+01	6.20E+01	1.79E+00		20.370
Gastric and intestinal 2h	8.00E+04	9.80E+04	2.70E+04	6.83E+04	4.83E+00	4.835	55.187
Gastric and intestinal 2h	4.40E+04	7.70E+04	6.20E+04	6.10E+04	4.79E+00		56.234

Appendix Table E2 (continued)

Condition	Cell count 1	Cell count 2	Cell count 3	Average	log average	Average log	% Survival
Gastric and intestinal 2h	8.80E+04	1.80E+04	3.50E+04	4.70E+04	4.67E+00		55.969
Gastric and intestinal 2h	8.20E+04	4.70E+04	1.80E+04	4.90E+04	4.69E+00	4.690	53.829
Gastric and intestinal 2h	6.40E+04	6.20E+04	1.70E+04	4.77E+04	4.68E+00		53.433
Gastric and intestinal 2h	7.40E+04	3.50E+04	7.80E+03	3.89E+04	4.59E+00		52.167
Gastric and intestinal 4h	3.40E+01	3.40E+01	4.30E+01	3.70E+01	1.57E+00	1.568	17.901
Gastric and intestinal 4h	6.60E+01	2.40E+01	3.40E+01	4.13E+01	1.62E+00		18.994
Gastric and intestinal 4h	4.20E+01	5.70E+01	5.60E+01	5.17E+01	1.71E+00		20.523
Gastric and intestinal 4h	4.30E+01	5.50E+01	6.70E+01	5.50E+01	1.74E+00	1.740	19.974
Gastric and intestinal 4h	3.80E+01	3.20E+01	1.01E+02	5.70E+01	1.76E+00		20.055
Gastric and intestinal 4h	1.80E+01	5.60E+01	6.50E+01	4.63E+01	1.67E+00		18.932

$$\% \text{ Survival} = \frac{\text{Colonies grown on MRS agar at detection time}}{\text{The initial bacterial cell counts}} \times 100$$

Appendix Table E3 Acid tolerance of *Lb.johnsonii* KUNN19-2

Replication I

Treatment	Cell count 1	Cell count 2	Cell count 3	Average	Log CFU	Average log	% Survival
control	8.80E+08	1.80E+09	9.00E+08	1.19E+09	9.0768	9.077	
control	6.00E+08	9.80E+08	7.20E+08	7.67E+08	8.8846		
control	7.80E+08	8.00E+08	9.40E+08	8.40E+08	8.9243		
0.3M NaCl, pH 1.5	8.00E+06	6.80E+06	1.30E+06	5.37E+06	6.7297	6.730	74.142
0.3M NaCl, pH 1.5	1.08E+07	9.40E+06	7.10E+06	9.10E+06	6.9590		
0.3M NaCl, pH 1.5	8.00E+06	1.30E+07	5.00E+06	8.67E+06	6.9379		
0.3M NaCl,pH 1.5, 0.3% pepsin	1.60E+06	9.00E+05	1.40E+06	1.30E+06	6.1139	6.114	67.358
0.3M NaCl,pH 1.5, 0.3% pepsin	7.00E+05	1.15E+06	7.90E+05	8.80E+05	5.9445		
0.3M NaCl,pH 1.5, 0.3% pepsin	6.00E+05	1.20E+06	1.60E+06	1.13E+06	6.0544		

Appendix Table E3 (continued)

Replication II

Treatment	Cell count 1	Cell count 2	Cell count 3	Average	Log CFU	Average log	% Survival
control	9.90E+08	6.30E+08	1.17E+09	9.30E+08	8.9685	8.968	
control	6.60E+08	7.80E+08	8.20E+08	7.53E+08	8.8770		
control	7.70E+08	6.50E+08	9.90E+08	8.03E+08	8.9049		
0.3M NaCl, pH 1.5	4.50E+06	1.46E+06	1.80E+06	2.59E+06	6.4127	6.413	71.503
0.3M NaCl, pH 1.5	8.80E+06	3.10E+06	3.40E+06	5.10E+06	6.7076		
0.3M NaCl, pH 1.5	1.12E+07	2.00E+06	6.70E+06	6.63E+06	6.8217		
0.3M NaCl,pH 1.5, 0.3% pepsin	8.30E+05	1.23E+06	8.30E+05	9.63E+05	5.9838	5.984	66.720
0.3M NaCl,pH 1.5, 0.3% pepsin	8.00E+05	1.50E+05	3.20E+05	4.23E+05	5.6267		
0.3M NaCl,pH 1.5, 0.3% pepsin	3.20E+05	8.40E+05	4.40E+05	5.33E+05	5.7270		

$$\% \text{ Survival} = \frac{\text{Colonies grown on MRS agar at detection time}}{\text{The initial bacterial cell counts}} \times 100$$

Appendix Table E4 Bile tolerance of *Lb.johnsonii* KUNN19-2

Condition	Cell count 1	Cell count 2	Cell count 3	Average	log average	Average log	% Survival
control	1.30E+09	1.60E+09	1.20E+08	1.01E+09	9.00E+00	9.003	100.000
control	1.80E+09	4.50E+08	3.30E+08	8.60E+08	8.93E+00		100.000
control	8.00E+08	9.00E+07	6.80E+08	5.23E+08	8.72E+00		100.000
control	1.25E+08	1.27E+09	1.06E+09	8.18E+08	8.91E+00	8.913	100.000
control	1.30E+09	1.80E+09	5.50E+08	1.22E+09	9.09E+00		100.000
control	1.08E+09	8.80E+08	9.90E+07	6.86E+08	8.84E+00		100.000
Intestinal 1h	1.07E+06	1.80E+06	1.30E+06	1.39E+06	6.14E+00	6.143	68.234
Intestinal 1h	7.00E+05	1.10E+06	3.00E+05	7.00E+05	5.85E+00		65.422
Intestinal 1h	1.40E+06	6.50E+05	4.30E+05	8.27E+05	5.92E+00		67.869
Intestinal 1h	1.16E+06	4.80E+05	8.60E+05	8.33E+05	5.92E+00	5.921	66.430
Intestinal 1h	1.19E+06	1.80E+06	1.37E+06	1.45E+06	6.16E+00		67.829
Intestinal 1h	4.90E+05	9.30E+05	7.50E+05	7.23E+05	5.86E+00		66.308
Intestinal 2h	5.90E+05	6.40E+05	9.70E+04	4.42E+05	5.65E+00	5.646	62.710
Intestinal 2h	4.50E+05	5.30E+05	8.80E+05	6.20E+05	5.79E+00		64.832
Intestinal 2h	3.60E+05	8.80E+04	7.30E+05	3.93E+05	5.59E+00		64.161
Intestinal 2h	4.40E+05	9.10E+05	7.60E+05	7.03E+05	5.85E+00	5.847	65.603

Appendix Table E4 (continued)

Condition	Cell count 1	Cell count 2	Cell count 3	Average	log average	Average log	% Survival
Intestinal 2h	1.20E+05	3.70E+05	8.00E+04	1.90E+05	5.28E+00		58.103
Intestinal 2h	1.30E+05	2.90E+05	6.40E+04	1.61E+05	5.21E+00		58.934
Intestinal 4h	1.80E+05	1.50E+05	1.70E+05	1.67E+05	5.22E+00	5.222	58.002
Intestinal 4h	8.40E+04	9.60E+04	1.60E+05	1.13E+05	5.05E+00		56.571
Intestinal 4h	5.50E+04	2.10E+05	2.30E+05	1.65E+05	5.22E+00		59.842
Intestinal 4h	1.18E+05	1.30E+05	1.54E+05	1.34E+05	5.13E+00	5.127	57.524
Intestinal 4h	8.40E+04	1.10E+05	9.60E+04	9.67E+04	4.99E+00		54.873
Intestinal 4h	2.40E+04	1.40E+05	1.03E+05	8.90E+04	4.95E+00		56.011
Gastric and intestinal 1h	3.30E+04	1.50E+04	3.70E+04	2.83E+04	4.45E+00	4.452	49.454
Gastric and intestinal 1h	3.70E+04	1.40E+04	1.70E+04	2.27E+04	4.36E+00		48.748
Gastric and intestinal 1h	1.60E+04	3.80E+04	2.80E+04	2.73E+04	4.44E+00		50.887
Gastric and intestinal 1h	1.18E+04	1.50E+04	1.12E+04	1.27E+04	4.10E+00	4.103	46.030
Gastric and intestinal 1h	5.40E+04	1.90E+04	2.40E+04	3.23E+04	4.51E+00		49.637
Gastric and intestinal 1h	2.60E+04	1.40E+04	2.80E+04	2.27E+04	4.36E+00		49.288
Gastric and intestinal 2h	1.60E+03	3.50E+03	1.80E+03	2.30E+03	3.36E+00	3.362	37.341
Gastric and intestinal 2h	2.20E+03	2.80E+03	3.60E+03	2.87E+03	3.46E+00		38.697

Appendix Table E4 (continued)

Condition	Cell count 1	Cell count 2	Cell count 3	Average	log average	Average log	% Survival
Gastric and intestinal 2h	1.60E+03	3.40E+03	4.70E+03	3.23E+03	3.51E+00		40.254
Gastric and intestinal 2h	1.20E+03	4.50E+03	2.60E+03	2.77E+03	3.44E+00	3.442	38.618
Gastric and intestinal 2h	1.40E+03	3.50E+03	4.20E+03	3.03E+03	3.48E+00		38.325
Gastric and intestinal 2h	2.60E+03	4.00E+03	3.10E+03	3.23E+03	3.51E+00		39.717
Gastric and intestinal 4h	3.60E+02	5.40E+02	5.60E+02	4.87E+02	2.69E+00	2.687	29.849
Gastric and intestinal 4h	3.80E+02	4.70E+02	9.80E+02	6.10E+02	2.79E+00		31.175
Gastric and intestinal 4h	3.00E+02	1.03E+03	4.40E+02	5.90E+02	2.77E+00		31.780
Gastric and intestinal 4h	5.00E+02	1.00E+03	1.04E+03	8.47E+02	2.93E+00	2.928	32.848
Gastric and intestinal 4h	3.20E+02	5.60E+02	7.70E+02	5.50E+02	2.74E+00		30.163
Gastric and intestinal 4h	1.90E+02	4.40E+02	5.10E+02	3.80E+02	2.58E+00		29.195

$$\% \text{ Survival} = \frac{\text{Colonies grown on MRS agar at detection time}}{\text{The initial bacterial cell counts}} \times 100$$

Appendix F

Data set details of phylogenetic dendrograms of the bacterial population isolated from natural fermented chicken cartilage (control samples) in every 12 h interval during the fermen-tation process, based on the UPGMA linkage of Dicecoefficients

Appendix Table F1 Data set details of phylogenetic dendrograms of the bacterial population isolated from natural fermented chicken cartilage (control samples) in every 12 h interval during the fermentation process, based on the UPGMA linkage of Dice coefficients.

Database name:-	Natural fermented chicken cartilage (control samples)
Data set:-	Control
Cluster Analysis Parameters	
Match type:-	Match all tracks to all tracks
Match basis:-	RF
% Tolerance:-	0.50
Similarity measure:-	Dice
Linkage method:-	UPGMA

Index	Column Name 1	Column Name 2	Column Name 3	Column Name 4	Column Name 5	Experiment	Gel ID	Description
1						Control	1386584051	3
2						Control	1386584051	4
3						Control	1386584051	5
4						Control	1386584051	1

Appendix Table F1 (continued)

Index	Column Name 1	Column Name 2	Column Name 3	Column Name 4	Column Name 5	Experiment	Gel ID	Description
6						Control	1386584051	6
7						Control	1386584051	10
8						Control	1386584051	12
9						Control	1386584051	11
10						Control	1386584051	9
11						Control	1386584051	7
12						Control	1386584051	8
13						Control	1386584051	13
14						Control	1386584051	14
15						Control	1386584051	15
16						Control	1386584051	16

Similarity Matrix Parameters	
Match type:-	Match all tracks to all tracks
Match basis:-	RF
% Tolerance:-	0.50
Similarity measure:-	Dice

Appendix Table F1. (continued)

Similarity Matrix Parameters

Match type:- Match all tracks to all tracks

Match basis:- RF

% Tolerance:- 0.50

Similarity measure:- Dice

Index	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	1.00	0.88	0.77	0.87	0.75	0.66	0.33	0.00	0.20	0.21	0.40	0.30	0.11	0.23	0.00	0.11
2	0.88	1.00	0.88	0.75	0.62	0.66	0.33	0.10	0.10	0.10	0.40	0.30	0.23	0.35	0.00	0.11
3	0.77	0.88	1.00	0.75	0.62	0.77	0.22	0.20	0.10	0.00	0.50	0.30	0.23	0.35	0.00	0.11
4	0.87	0.75	0.75	1.00	0.85	0.62	0.37	0.22	0.44	0.23	0.55	0.55	0.13	0.13	0.00	0.12
5	0.75	0.62	0.62	0.85	1.00	0.75	0.25	0.22	0.33	0.00	0.22	0.33	0.13	0.13	0.00	0.12
6	0.66	0.66	0.77	0.62	0.75	1.00	0.44	0.60	0.60	0.31	0.70	0.60	0.11	0.23	0.13	0.11
7	0.33	0.33	0.22	0.37	0.25	0.44	1.00	0.50	0.40	0.52	0.50	0.40	0.23	0.35	0.00	0.11
8	0.00	0.10	0.20	0.22	0.22	0.60	0.50	1.00	0.54	0.47	0.54	0.54	0.31	0.42	0.00	0.20
9	0.20	0.10	0.10	0.44	0.33	0.60	0.40	0.54	1.00	0.47	0.54	0.63	0.10	0.21	0.00	0.10
10	0.21	0.10	0.00	0.23	0.00	0.31	0.52	0.47	0.47	1.00	0.66	0.57	0.11	0.11	0.00	0.00
11	0.40	0.40	0.50	0.55	0.22	0.70	0.50	0.54	0.54	0.66	1.00	0.72	0.21	0.31	0.00	0.10

Appendix Table F1 (continued)

Similarity Matrix Parameters

Match type:- Match all tracks to all tracks

Match basis:- RF

% Tolerance:- 0.50

Similarity measure:- Dice

Index	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
12	0.30	0.30	0.30	0.55	0.33	0.60	0.40	0.54	0.63	0.57	0.72	1.00	0.00	0.10	0.00	0.20
13	0.11	0.23	0.23	0.13	0.13	0.11	0.23	0.31	0.10	0.11	0.21	0.00	1.00	0.87	0.00	0.11
14	0.23	0.35	0.35	0.13	0.13	0.23	0.35	0.42	0.21	0.11	0.31	0.10	0.87	1.00	0.00	0.00
15	0.00	0.00	0.00	0.00	0.00	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.40
16	0.11	0.11	0.11	0.12	0.12	0.11	0.11	0.20	0.10	0.00	0.10	0.20	0.11	0.00	0.40	1.00

Appendix G

Data set details of phylogenetic dendrograms of the bacterial population isolated from fermented chicken cartilages inoculated with *Lb. johnsonii* KUNN19-2 in every 12 h interval during the fermentation process, based on the UPGMA linkage of Dice coefficients

Appendix Table G1 Data set details of phylogenetic dendrograms of the bacterial population isolated from fermented chicken cartilages Inoculated with *Lb. johnsonii* KUNN19-2 in every 12 h interval during the fermentation process, based on the UPGMA linkage of Dice coefficients

Data Set Details

File:-

Database name:- Fermented chicken cartilage inoculated with *Lb. johnsonii* KUNN19-2

Data set:- P1

Cluster Analysis Parameters

Match type:- Match all tracks to all tracks

Match basis:- RF

% Tolerance:- 0.50

Similarity measure:- Dice

Linkage method:- UPGMA

Index	Column Name 1	Column Name 2	Column Name 3	Column Name 4	Column Name 5	Experiment	Gel ID	Description
1						P1	1386579336	8
2						P1	1386579336	9
3						P1	1386579336	11
4						P1	1386579336	10

238

Appendix Table G1 (continued)

Index	Column Name 1	Column Name 2	Column Name 3	Column Name 4	Column Name 5	Experiment	Gel ID	Description
5						P1	1386579336	6
6						P1	1386579336	7
7						P1	1386579336	2
8						P1	1386579336	16
9						P1	1386579336	17
10						P1	1386579336	14
11						P1	1386579336	15
12						P1	1386579336	12
13						P1	1386579336	13
14						P1	1386579336	3
15						P1	1386579336	4
16						P1	1386579336	5

Appendix Table G1 (continued)

Similarity Matrix Parameters

Match type:-	Match all tracks to all tracks
Match basis:-	RF
% Tolerance:-	0.50
Similarity measure:-	Dice

Index	1	2	3	4	5	6	7	8	9	10	11	12	13
1	1.00	0.87	0.61	0.53	0.55	0.44	0.42	0.33	0.11	0.21	0.47	0.13	0.14
2	0.87	1.00	0.72	0.61	0.37	0.37	0.33	0.37	0.26	0.35	0.53	0.30	0.33
3	0.61	0.72	1.00	0.60	0.46	0.46	0.44	0.46	0.33	0.28	0.33	0.40	0.44
4	0.53	0.61	0.60	1.00	0.26	0.40	0.18	0.53	0.42	0.37	0.28	0.66	0.54
5	0.55	0.37	0.46	0.26	1.00	0.77	0.42	0.22	0.11	0.10	0.11	0.00	0.00
6	0.44	0.37	0.46	0.40	0.77	1.00	0.28	0.22	0.00	0.10	0.11	0.00	0.00
7	0.42	0.33	0.44	0.18	0.42	0.28	1.00	0.14	0.00	0.00	0.15	0.00	0.00
8	0.33	0.37	0.46	0.53	0.22	0.22	0.14	1.00	0.82	0.52	0.47	0.80	0.57

Appendix Table G1 (continued)

Similarity Matrix Parameters	1	2	3	4	5	6	7	8	9	10	11	12	13
		0.26	0.33	0.42	0.11	0.00	0.00	0.82	1.00	0.44	0.50	0.57	0.46
Match type:-		0.35	0.28	0.37	0.10	0.10	0.00	0.52	0.44	1.00	0.77	0.62	0.66
Match basis:-	RF	0.53	0.33	0.28	0.11	0.11	0.15	0.47	0.50	0.77	1.00	0.71	0.61
% Tolerance:-	0.50	0.30	0.40	0.66	0.00	0.00	0.00	0.80	0.57	0.62	0.71	1.00	0.72
Similarity measure:-	Dice	0.33	0.44	0.54	0.00	0.00	0.00	0.57	0.46	0.66	0.61	0.72	1.00
14	0.14	0.00	0.44	0.00	0.28	0.28	0.20	0.00	0.00	0.13	0.00	0.00	0.00
15	0.00	0.00	0.00	0.00	0.30	0.30	0.22	0.15	0.16	0.00	0.00	0.00	0.00
16	0.15	0.18	0.25	0.00	0.46	0.30	0.22	0.15	0.16	0.14	0.00	0.00	0.00

CIRRICULUM VITAE

NAME : Miss. Panward Prommadee

BIRTH DATE : March 25, 1975

BIRTH PLACE : Sakol Nakorn, Thailand

EDUCATION :

YEAR	INSTITUTE	DEGREE/DIPLOMA
1997	Khon Kaen University, Thailand.	B.Sc. (Food Technology)
2002	Khon Kaen University, Thailand.	M.Sc (Food Technology)

POSITION/TITLE : Lecturer

WORKPLACE Faculty of Natural Resource and Agro-Industry
Kasetsart University
Chalermphrakiat Sakon Nakhon Province
Campus

SCHOLARSHIP/AWARDS : -