Research Article

Efficacy of Isolated Probiotic Bacteria from Piglet Nostrils in Fattening Pigs

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Abstract

Probiotics applications in animal husbandry can increase feed intake, growth and immune responses. The aim of this study was to isolate the probiotic bacteria from piglet nostrils and test their efficacy in fattening pigs. Nasal swabs collected from 110 healthy piglets were processed on Man, Rogosa and Sharpe (MRS) agar plates for lactic acid bacteria isolation. The biochemical standard tests were used for identification of isolates organisms including: Gram staining, catalase production, tolerance to bile salts, acid and base and the utilization of proteins, starch and fat. The antimicrobial activity and resistance against antibiotics were also determined. Comparative 16S rRNA analysis confirmed the identity of selected strains as *Enterococcus italicus.* Thirty weaned piglets (mixed sex and 35-day old) were divided into three groups (five replicates with two animals) consisting of a control group, a positive control group fed with commercial feed and antibiotic, and an experimental group fed with commercial feed and probiotics. Porcine reproductive and respiratory syndrome (PRRS) vaccination were performed twice. Administration of *Enterococcus italicus* had no impact on average daily gain, whereas it affected feed intake and feed conversion ratio ($p < 0.05$). The experimental and positive control groups were differed significantly from the control group regarding the level of immunity ($p > 0.05$).

***Key Words*:** *Enterococcus italicus*; growth performance; nasal swab; PRRS

Introduction

Probiotics are beneficial microorganisms and their application in animal husbandry increases feed intake, promotes growth (Lessard and Brisson, 1987), immune responses (Isolauri et al., 1995), and inhibits the growth of pathogenic bacteria (Xuan et al., 2001; Deprez et al., 1986). To develop effective probiotics for pigs, the identification of suitable microbial strains with specific effects on pork production is very important. The efficacy of a probiotic depends on the selected strain and the physiology of the particular animals. The selection of an appropriate probiotic is the most time-consuming step during the development of a probiotic feed additive specifically used in animals, particularly pigs (Jens and Hansan, 2006).

The aim of the present study was to isolate new probiotic lactic acid bacteria from piglet nostrils with protective activity against respiratory diseases.

Materials and Methods

Nasal swabs were collected from 110 healthy piglets from four provinces in the western region of Thailand (Nakhon Pathom, Ratchaburi, Phetchaburi and Prachuap Khiri Khan). Serial dilutions of the nasal samples were performed on Man, Rogosa and Sharpe (MRS) agar plates (Himedia, India) using the streak plate method. All colonies were Gram stained and examined for catalase production. Tolerance to bile salts (0.3% w/v) (Biomark, India), acids, bases (Gilliland et al., 1984), and different pH values (pH 2–10) (Conway et al., 1987), and the

utilization of proteins, starch and fats (Larpent and Larpent, 1985) were determined. The antimicrobial activity of these probiotics against pathogenic indicator bacteria, such as *Escherichia coli, Salmonella typhimurium, Staphylococcus aureus, Streptococcus agalactiae* and *Pasteurella multocida,* was determined by the agar spot method (Reque et al., 2000; Timbuntam et al., 2001). Resistance against ampicillin (10 µg), penicillin (10 IU), colistin (10 µg), norfloxacin (10 µg) and sulphamethoxazole plus trimethoprim (25 µg) was also examined. Selected strains were identified by 16S rRNA analysis at the Mahidol University-Osaka University Collaborative Research Center, Faculty of Science, Mahidol University, for template preparation and DNA sequencing. The Basic Local Alignment Search Tool (BLASTN 2.2.24) was used to detect regions of local similarity between sequences obtained from The National Center for Biotechnology Information for strain identification.

For *in vivo* experiments, 30 mixed sex weaned piglets (35 days of age) were raised in the open house farm of a small scale farmer who had never raised pigs before. The piglets were divided into three groups, with five replications for each group and two piglets per replication. A completely randomized design (CRD) was used for all experiments. The piglets were fed *ad libitum* in which the control group received commercial feed and the positive control group received the same commercial feed supplemented with the antibiotic Doxycycline (0.5 kg per ton of feed). The experimental group received commercial feed supplemented with at least 10^6 cfu/dose of specific probiotics, which were sprayed on the feed twice daily (in the morning and in the evening). Animals were vaccinated against PRRS twice. The live attenuated vaccine contained 103.5 TCID/g of live PRRS Virus VP046 Bis Strain. The serum collected at the Betagro Science Center was examined to determine the titer using an ELISA kit (IDEXX; X3). The results were evaluated using the sample to positive ratio (S/P ratio)

S/P ratio = [Sample mean (mean of optical absorbance) – negative control mean] / [Positive control mean – negative control mean]

All results were statistically analyzed by analysis of variance, and the mean values obtained from each group were compared using Duncan's multiple range tests.

Results

Among the 650 isolated bacterial strains, 218 isolates (33.54%) were Gram-positive and catalase-negative, 12 isolates (1.85%) were tolerant to bile salts, six isolates (0.93%) utilized protein, six isolates (0.93%) decomposed fat and only 1 isolate (0.15%) (P2-23) degraded starch and was also tolerant to extreme pH levels of 2–9. The antimicrobial activity of P2-23 against the pathogenic indicator bacteria (*E. coli, S. typhimurium, Sta. aureus, Str. agalactiae* and *P. multocida*) was determined by measuring the diameter of the inhibition zones (2.0, 2.6, 1.5, 3.0 and 1.0 mm, respectively). Antibiotic resistance was tested and confirmed by measuring the inhibition zones for ampicillin, penicillin, colistin, norfloxacin and sulphamethoxazole plus trimethoprim (11.2, 3.6, 3.2, 2.8 and 2.6 mm, respectively). The selected strains were identified by 16s rRNA sequencing, which confirmed that P2-23 had 100% query coverage to *Enterococcus italicus*.

No statistically significant differences in average daily gain were observed between the groups ($p > 0.05$). However, significant differences in feed intake and feed conversion ratio were observed (Table 1).

Significant differences in the level of immunity against PRRS were observed between the groups ($p < 0.01$). The level of immunity in each group increased significantly, starting on the seventh day after the second vaccine booster. No significant difference in immunity level was observed between the experimental group and the positive control group. However, these two groups differed significantly from the control group (Table 2).

Discussion

The selection of effective probiotic bacterial strains is crucial because it affects biosafety, administration, sites of activity of probiotic organisms, probiotic survival, colonization in the host, tolerance to bile and low pH, antimicrobial activity and viability during storage. These factors need to be investigated because they affect

probiotic efficacy and usage (Gilliland and Walker, 1990; Fuller, 1992; Reque et al., 2000 cited in Kasornpikul et al., 2008), and they can be evaluated *in vitro* to select effective strains (Salminen et al., 1998).

E. italicus is a Gram-positive, catalase-negative and acid- and bile-resistant organism that uses proteins, lipids and starch as nutrients, leading to improved digestive function. Furthermore, *E. italicus* exhibits antimicrobial activity, and can therefore confer disease resistance to swine.

Fortina et al. (2008) showed that *E. italicus* strains isolated from cheese did not associate with potentially virulent profiles compared with the clinical strains. Their presence poses a low health risk and might have potential for use as probiotic feed supplements for swine.

Among the selected probiotics, *E. italicus,* had no effect on average daily gain, whereas it affected feed intake and feed conversion ratio ($p < 0.05$). This could be explained by the temperature and humidity conditions used for raising piglet, which was performed in an open house. In a tropical climate, swine eat less in the cold climates, and the feed conversion ratio is higher in the hot climate. This can be attributed to the body temperature of the animal in the hot climate which does not lose energy to the surrounding environment (Sittigool, 2014). In the present study, the piglets did not eat much, but their feed conversion ratio was higher than normal.

Probiotics might increase growth rate. Davis et al. (2008) studied the effect of *Bacillus* species on the growth of fattening swine by mixing the bacterium into their feed. This increased the feed conversion ratio and the average daily gain ($p < 0.05$) but decreased death rate. Administration of *Lactobacillus* probiotics increased the growth rate of milk feeding and weaning piglets, and fattening pigs. This can be explained by the ability of probiotics to stimulate feed fermentation and digestion and thus enhance the growth rate in swine. *Lactobacillus* probiotics induce lactic acid and enzyme production, improving digestion and nutrient absorption in the host's intestine. In addition, the pigs that received probiotics had better digestion of protein and, as a result, had more energy than those that did not (Sirichokchatchawan et al., 2014). However, Strompfová et al 2006) reported that *E. faecium* EK13 did not enhance the average daily gain in piglets.

After vaccination against PRRS, the results were positive in the three groups after two vaccination doses. The experimental group showed the highest level of immunity, which is not consistent with the results reported by Kritas and Robert (2006), who analyzed the effect of intranasal/oral administration of probiotics on preventing PRRS in swine vaccinated against this disease. These authors showed that swines receiving *L. casei* and vaccination had higher weight than those that did not receive the bacterium and vaccination. Among the vaccinated animals, viremia was detected at 4, 11 and 17 days after a PRRS challenge, and its incidence was lower than that in non-vaccinated swine. Administration of probiotics had no effect on the incidence of viremia. The authors concluded that orally administered *L. casei* has no effect on stimulating immunity against PRRS. In the present study, the experimental pigs were tested to determine the level of immunity against PRRS. Nevertheless, our study indicates that *E. italicus* probiotics stimulate immunity against PRRS.

Table 1 The average daily gain, the feed intake and the feed conversion ratio of the experimental pigs

Results/group	Control group	Positive control group	Experimental group
Average daily gain (kg./pig/day)	0.667 ± 0.079^{a}	0.696 ± 0.043^{a}	0.734 ± 0.082^{a}
Feed Intake (kg./pig/day)	1.576 ± 0.010^{b}	1.422 ± 0.003^{a}	1.428 ± 0.006^{a}
Feed conversion ratio	2.412^{a}	2.029^{b}	1.962^{b}

^{a,b} The same letters in a row indicate no significant differences ($p > 0.05$)

Table 2 The level of immunity against PRRS (S/P Ratio)

Day after the first vaccination	Control group	Positive control group	Experimental group
0	0.0507 ± 0.0679^{c}	0.0454 ± 0.0196^{c}	0.0488 ± 0.0239^{c}
7	0.0359 ± 0.0167^{c}	0.3725 ± 0.7281^{c}	0.1971 ± 0.4836^{c}
14	0.0380 ± 0.0177^{c}	0.0473 ± 0.0244^{c}	0.1548 ± 0.2491^{c}
28	0.8380 ± 0.6580^{b}	1.2558 ± 0.7149^{a}	1.561 ± 0.4727^{a}

[a,b,c] The same letters in a row indicate no significant differences ($p > 0.05$)

Conclusion

Among the 650 bacterial strains, the selected probiotic *En. italicus* is a Gram-positive, catalase-negative and acid- and bile-resistant bacterium with the capacity to utilize proteins, lipids and starch as nutrients. *En. italicus* also exhibits antimicrobial activity. This bacterium has no effect on average daily gain but it decrease feed intake and feed conversion ratio. However, bacterial application stimulates immunity against PRRS ($p < 0.05$).

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