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## Preliminary Screening of Appropriate Probiotic Bacteria for Producing Health Drink from Mature Coconut Juice

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### Abstract

Mature coconut juice, waste from the coconut processing industries, is rarely utilized and discarded in the area causing environmental pollution. This study proposed to solve this problem and, at the same time, add value to mature coconut juice by producing "health drink". Therefore, the aim of study was to identify lactic acid bacteria suitable for producing "health drink" from mature coconut juice. Four types of inocula were used, including *Lactobacillus acidophilus*, *L. casei*, *L. delbrueckii* and drinking yogurt commercially available in the market. The results revealed that mature coconut juice supplemented with 5% sucrose could satisfactorily support growth of the adopted inocula providing that viable cell count of as high as 7 log CFU/mL could be achieved within 12 hrs post inoculation at 37°C. However, only mature coconut juice fermented with *L. casei* whose qualities expressed in terms of viable cell count (9.16 log CFU/mL), pH (3.9) and acidity expressed as lactic acid (0.37%) conformed well with fermented milk and plant bioextract standard specified by the Ministry of Public Health of Thailand (2005) and Thai Industrial Standards Institute (2004), respectively.

**Keywords:** health drink, lactic acid bacteria, mature coconut juice

### Introduction

Thailand is one of the top ten exporters of coconut (FAO, 2010) with the total coconut production of approximately 1.51 MT in 2008 given that Prachuab kirikhan is the largest coconut producer with 0.53 MT coconut produced (Agricultural Extension Office of Prachuap Khiri Khan Province, 2008). Most of coconuts were further processed

commercially leading to large amount of wastes generated such as coir pith, coconut shell and coconut juice (Thongthieng et al., 2005). Even though mature coconut juice was employed as raw material for the production Nata de coco, compost and bioextract preparation, large quantity was inattentively discarded causing in turn adverse effect to environment (Fuangworawong et al., 2008). Since



coconut juice contains several beneficial nutritional elements such as sugar (0.1%), protein (0.1%) and trace elements, potassium (247 mg%) in particular (Krishnankutty, 1987; Mekvichitsaeng et al., 1999; Thoboonyanonth, 2002), it is a raw material of high potential for the production of health drink which effectively reduces wastes generated from coconut processing industry and, at the same time, lessens environmental problem.

Probiotics are live microbial dietary Supplement, which beneficially affects the hosts by improving its intestinal microflora balance (Fuller, 1989). Probiotic microorganisms also improve digestion and produce nutrients such as amino acids, lactic acid and vitamins K and B. Most probiotic products available in the market are diary-base in nature, e.g., yogurt, fermented milk and kefir (Siuta-Cruce and Goulet, 2001). Presently, probiotics supplemented fruit and vegetable juice rich in mineral, vitamin and fiber are as well available commercially for health conscious but lactose intolerant consumers (Luckow and Delahunty, 2004; Yoon et al., 2005; Sheehan et al., 2007; Prachyakij et al., 2008).

Microorganisms bestowing probiotics effect were *Bifidobacteria* and *Lactobacilli* including *Lactococcus*, *Enterococcus*, *Saccharomyces* and *Propionibacteria* (Rivera-Espinoza and Gallardo-Navarro, 2010). Qualities of probiotics supplemented health drink relate directly to amount of live microorganisms present in the product. According to the fermented milk standard of Thailand (Ministry of Public Health of Thailand, 2007), the live probiotic bacteria present in

the product should be at least  $7 \log \text{CFU/mL}$ . As several factors, i.e., types of microorganism, pH and the presence of inhibitory substance, play significant role on probiotics viability in growth medium (Taweesaeng et al., 2005; Yoon et al., 2005; Sheehan et al., 2007), therefore, growth as well as viability of probiotic bacteria varied from one type of growth medium to another (Taweesaeng et al., 2005). For instance, *Lactobacillus acidophilus*, *L. casei* and *L. delbrueckii* grew comparatively well in beet juice, however, solely *L. casei* remained viable at the detectable level of more than  $1 \times 10^7 \text{CFU/mL}$  after storing at  $4^\circ\text{C}$  for 4 weeks (Yoon et al., 2005). This study aimed at selecting appropriate probiotic bacteria for the production of health drink from mature coconut juice.

## Materials and Methods

### Materials

Mature coconut juice was collected from coconut processing plant. Chemical characteristics of mature coconut juice were provided in Table 1. Mature coconut juice supplemented with 5% sucrose was sterilized at 2000 psi and  $121^\circ\text{C}$  for 15 minutes.

Table 1 Chemical characteristics of mature coconut juice

Characteristics	Mature coconut juice
Moisture content (%)	96.82±0.02
Ash (%)	16.58±0.52
pH	5.77±0.02
Acidity (%as lactic acid)	0.05±0.01
Brix (°)	3.6±0.1
Total sugar (µg/ml)	21.87±3.05

### Probiotics Inocula

Four inocula were employed as follows: (i) commercially available drinking yogurt, (ii) *L. acidophilus* TISTR 450, (iii) *L. casei* TISTR 390 and (iv) *L. delbrueckii* TISTR326. The three lactobacilli were cultivated at 37°C for 24 hrs in MRS broth (Himedia Laboratories, PVT LTD.).

### Preparation of health drink using mature coconut juice

Experiments consist of 4 treatments according to the types of inocula specified previously in probiotics inocula section. 5% inoculum was employed. Each treatment was then incubated at 37°C for 72 hrs. Small sample was collected at 0, 6, 12, 18, 24, 48 and 72 hrs.

### Analytical

Total acidity expressed as % lactic acid was determined by titrating against 0.1 N NaOH using phenolphthalein as indicator (AOAC, 1995). Cell viability was enumerated by total plate count for 48 hrs at 37°C (Johnson and Case, 1989).

### Statistical Analysis

All experiments were carried out in triplicate. Results were expressed as mean±standard deviation. Analysis of variance (ANOVA) and least significant digit (LSD) were conducted using Minitab 14 (Minitab Inc., USA).

## Results and Discussion

### pH and acidity

Thai Industrial Standards Institute (2004) and the Ministry of Public Health of Thailand (2005) have specified, respectively, that pH of less than 4.3 and

acidity (as % lactic acid) of higher than 0.3% should be attained for plant bioextract and fermented milk product in order for the product to be considered acceptable. It is obvious from Figure 1 that both pH and acidity conformed well to those of the standard when *L. acidophilus* and *L. casei* were employed as inocula at 12 and 18 hrs post inoculation, respectively. However, both pH and acidity of the product prepared using commercial drinking yogurt and *L. delbrueckii* as inocula took longer to reach standard at 24 and 48 hrs post inoculation, respectively, which may be due to the fact that longer lag phase was observed (Figure 2).

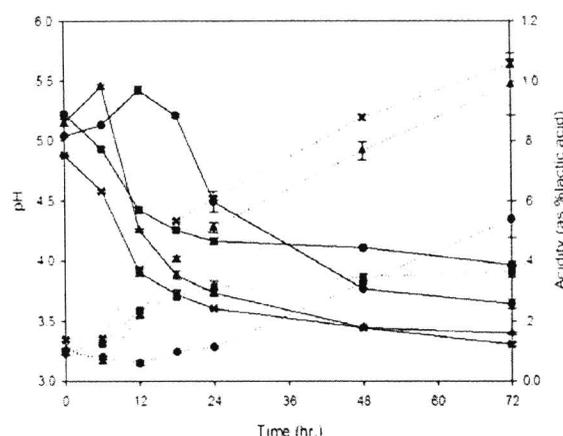


Figure 1 Changes of pH (—) and acidity (....) during fermentation of health drink from mature coconut juice using commercial drinking yogurt (●), *L. acidophilus* (▲), *L. casei* (×), *L. delbrueckii* (■) as inocula

Further, fermentation with commercial drinking yogurt and *L. delbrueckii* as inocula yielded much lower lactic acid production than those obtained with *L. acidophilus* and *L. casei* ( $p < 0.05$ ) providing that the lowest lactic acid

concentration (0.36%) was detected when *L. delbrueckii* was used as inoculum. On the contrary, fermentation with *L. casei* led to the highest lactic acid concentration and the lowest pH of approximately 1.06% and 3.31, respectively, which was significantly different from other preparation ( $p < 0.05$ ).

### Growth and survival of probiotic bacteria

Results obtained in Figure 2 show that mature coconut juice supplemented with 5% sucrose could satisfactorily support growth of all inocula, namely, commercial drinking yogurt, *L. acidophilus*, *L. casei* and *L. delbrueckii*, exceeding 7 log CFU/mL. Both *L. acidophilus* and *L. casei* grew well during the first 6 hrs post inoculation whereas high growth of drinking yogurt inoculum and *L. delbrueckii* noted after 6 hrs of inoculation. At 12 hrs post inoculation, product prepared with *L. casei* yielded the highest viable count, approximately  $9.16 \pm 0.03$  log CFU/mL ( $p < 0.05$ ), whereas the viable counts accomplished with commercial drinking yogurt, *L. acidophilus*, and *L. delbrueckii* were, respectively,  $8.56 \pm 0.07$ ,  $7.98 \pm 0.05$  and  $7.83 \pm 0.03$  log CFU/mL.

As can be observed from Figure 2 that, at 24 hrs of fermentation, except for the commercial drinking yogurt inoculums, viable count declined accordingly due probably to an increase in acidity of the medium, which is inappropriate for growth (Sheehan et al., 2007). Considering microbial acid resistance and survivability at 72 hrs post inoculation, coconut juice fermented with *L. acidophilus* and *L. delbrueckii* showed the reduction rate relative to that of 24 hrs of 0.84 and 0.38

log CFU/mL/day, respectively. When *L. casei* was employed as inoculums the lowest reduction rate of 0.27 log CFU/mL/day was achieved indicating that *L. casei* is more acid resistant than *L. acidophilus* and *L. delbrueckii* (Figure 2), thus, remained more viable.

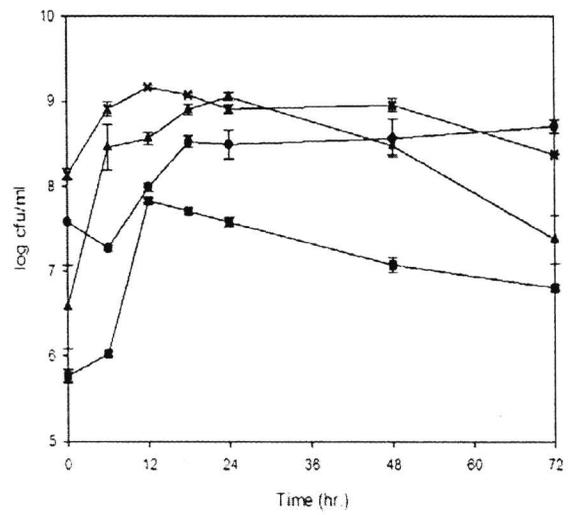


Figure 2 Viable cell counts during fermentation of health drink from mature coconut juice using commercial drinking yogurt (●), *L. acidophilus* (▲), *L. casei* (×), *L. delbrueckii* (■) as inocula

### The quality of health drink from mature coconut juice

According to plant bioextract and fermented milk standards specified by the Thai Industrial Standards Institute (2004) and The Ministry of Public Health of Thailand (2005), respectively, the preparation using *L. casei* as inoculums reached standard the fastest with the highest viable cell count ( $9.16$  log CFU/mL) within 12 hrs of fermentation ( $p < 0.05$ ). Additionally, medium inoculated with *L. delbrueckii* led to the lowest viable cell count of  $7.57$  log CFU/mL at 24 hrs of fermentation (Table 2).

Table 2 Qualities of health drink from mature coconut juice at various inoculum

Inocula	Time (hr.)	Viable cell count (log CFU/mL)	pH	Acidity (% as lactic acid)
Drinking Yoghurt	48	8.56±0.23 <sup>c</sup>	3.77±0.01 <sup>c</sup>	0.33±0.00 <sup>c</sup>
<i>L. acidophilus</i>	18	8.90±0.06 <sup>b</sup>	3.88±0.04 <sup>b</sup>	0.41±0.01 <sup>a</sup>
<i>L. casei</i>	12	9.16±0.03 <sup>a</sup>	3.90±0.03 <sup>b</sup>	0.37±0.01 <sup>b</sup>
<i>L. delbrueckii</i>	24	7.57±0.06 <sup>d</sup>	4.16±0.03 <sup>a</sup>	0.31±0.02 <sup>d</sup>
Standards		7 <sup>**</sup>	4.3 <sup>*</sup>	0.3 <sup>**</sup>

Note: \* Thai Industrial Standards Institute (2004); \*\*The Ministry of Public Health of Thailand (2005); Values within a column followed by different letters are significantly different ( $p < 0.05$ ).

Results found were in good agreement with that of Chewpreecha (1977) who reported that *L. casei* grew comparatively well on coconut juice supplemented with peptone, yeast extract, sodium acetate, ammonium citrate and Tween 80 at 1, 0.5, 0.5, 0.2 and 0.1% in comparison with MRS broth given that viable cell count of *L. delbrueckii* was also the lowest even when cultivated on MRS medium.

### Conclusions

Four types of inocula, *Lactobacillus acidophilus*, *L. casei*, *L. delbrueckii* and commercial drinking yogurt, were examined for their growth and acid resistance capability on mature coconut juice. *L. casei* was the most suitable in terms of growth and acid tolerance. The qualities of the health drink, both the amount of viable count, pH and lactic acid production, conformed well to the fermented milk and plant bioextract standards specified by the Ministry of Public Health of Thailand (2005) and Thai Industrial Standards Institute (2004), respectively, within 12 hours of fermentation.

### Acknowledgement

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