Isolation of probiotics Lactobacillus acidophilus from commercial yoghurt

Hassan Pyar
Kok-Khiang Peh*

School of Pharmaceutical Sciences, University Sains Malaysia, 11800 Minden, Penang, Malaysia.
Telephone +6046532257
Fax +6046570017
E-mail : kkpeh@usm.my; kkpehken@gmail.com


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ABSTRACT

Probiotic lactic acid bacteria, being the most often used vehicle in microorganism, are commercialized mainly as food enhancement among dairy products. The number and the type of the probiotics are very essential in order to ensure the activity. In this study, a rapid screening method was used to isolate probiotic Lactobacillus strain from commercial product of yoghurt after enrichment in De-Man-Rogosa Sharpe (MRS) medium and nutrient medium. The Lactobacillus strain was then isolated and identified based on morphology identification. The result showed a significantly high yield of pure culture of *Lactobacillus acidophilus* obtained from MRS medium (viable number of the cell in the yoghurt was about 10^7 CFU/ml) than compare to the nutrient medium. In conclusion, MRS medium is suitable for isolation of Lactobacilli compared to nutrient medium.

Keywords: Probiotics, *Lactobacillus acidophilus*, Yoghurt

Abbreviation key: CFU = colony forming unit, **FAO** = Food and Agriculture Organization, **WHO** = The World Health Organization, **MRS** = De-Man-Rogosa Sharpe.

INTRODUCTION

Interest in the role of probiotics for human health goes back at least as far as 1908 when Metchnikoff suggested that human should consume milk fermented with Lactobacilli to prolong life (Hughes and Hoover, 1991, O'Sullivan et al., 1992). Probiotics lactic acid bacteria in food can transiently colonize the intestinal and exhibit positive health benefits effects on the host health beyond its nutritional value (Huggett and Verschuren, 1996, Ferencik M et al., 1999). Many different microorganisms are added to dairy products for their probiotic potential (Fuller, 1997, Gibson and Fuller, 1998). *Lactobacillus* spp. are well known dairy probiotic products produced in many countries as functional dairy products (Moayednia et al., 2009). However, there are many problems and difficulties accompanied theses products. Research has shown that survival and viability of probiotic bacteria are often low in yogurt (Gillilanda and Specka, 1977, Hull et al., 1984). Furthermore, problems of variation in viability or activity of the cells within different preparation , the huge differences in survival rates in preparations contain different types of microorganisms , and obstacles related to shelf-life and bioavailability (Kaur et al., 2002). In many countries, standards have been developed for the necessary numbers of the probiotics bacteria in fermented products, recently , regulations approved by the countries of MERCOSUR

(Argentina, Paraguay, Brazil, and Uruguay) determine 10^6 cfu/g as a minimal content of probiotics in fermented milks preparations (Pagano, 1998). In Japan, a standard has been developed by the Fermented Milks and Lactic Acid Bacteria Beverages Association, which requires a minimum of 10^7 viable probiotic bacteria cells per milliliter to be present in fresh dairy products (Robinson, 1987). Availability should be confirmed, likewise, viability with minimum viable numbers of the probiotic at the end of the shelf life should be 10^6 cfu/g or ml (FAO/WHO, 2001). To maintain confidence in probiotic products, it is important to demonstrate good survival of these bacteria in the final products and should have desirable properties and functions (Chan et al., 2000). As the number and the type of the microorganisms within the diary products play a critical role in activity of the dairy products, this study aims to isolate and enumerate viable cell counts of *Lactobacillus* present in probiotic yoghurts using conventional plating techniques.

MATERIALS AND METHODS

I. Chemicals

De-Man-Rogosa-Sharpe broth was purchased from Criterion (U.S.A). Nutrient broth, nutrient agar, peptone water and carbon dioxide generating system were purchased from Oxoid (U.K). Bacto agar and Gram staining reagents were purchased from Becton, Dickinson and company (U.S.A). The other reagents used in the experiment were analytical grade.

II. Preparation of culture media

A. De-Man-Rogosa–Sharpe (MRS) broth

The ingredients of MRS broth per liter of distilled water as given in the instruction sheet of the supplier (Criterion, U.S.A) were: 20.0 g dextrose, 10.0 g meat peptone, 10.0 g beef extract, 5.0 g yeast extract, 2.0 g sodium acetate, 2.0 g disodium phosphate, 2.0 g ammonium citrate, 1.0 g Tween 80, 0.1 g magnesium sulfate and 0.12 g manganese sulfate. The MRS broth was prepared at 5.5 % w/v in distilled water. The ingredients were boiled until completely dissolved and poured into conical flasks. The pH of the medium was adjusted to 6.0 ± 0.1 using 1 M HCl (Mettler Toledo, Switzerland). The medium was autoclaved at 121 °C for 15 min (Stermite Model SM-230 Hirayama, Tokyo, Japan) (DeMan et al., 1960).

B. De-Man-Rogosa–Sharpe (MRS) agar

The medium was prepared by suspending 55.0 g of MRS broth and 15.0 g of Bacto agar in one liter of distilled water using the same procedure as described in section II (A). The Bacto agar was added to make the medium sufficiently solid and to prevent the movement of bacteria through the medium. The medium after autoclaving was cooled to 50 °C and poured into 20 ml petridishes for 3 hr to allow solidification at room temperature. The plates were then kept in a refrigerator at 4 °C until used (DeMan et al., 1960). MRS-agar (solid) plates were use directly or packed in plastic beg and stored in refrigerator.

C. Nutrient broth

The ingredients of nutrient broth per liter of distilled water were 5.0 g peptone from meat and 3.0 g meat extracts. The nutrient broth medium was prepared at 0.8 % w/v in distilled water. The mixture was heated until completely dissolved. The pH of the medium was adjusted to 6.0 ± 0.1 using 1 M HCl, and autoclaved for 15 min at 121 °C (Bridson, 1990).

D. Nutrient agar

The ingredients of nutrient agar per liter of distilled water were 5.0 g peptone from meat, 3.0 g meat extracts and 12.0 g agar. The medium was prepared by mixing 20 g of

nutrient agar in one liter of distilled water and heated until completely dissolved. The pH of the medium was adjusted to 6.0 ± 0.1 using 1 M HCl, and autoclaved at 121 °C for 15 min (Bridson, 1990)

III. Sample collection and storage

Commercial drinking yoghurt was collected from the local supplier in Penang, Malaysia and taken as a source of Lactobacilli for this study. The sample was stored at 4 ° C before analysis. The analysis was carried out within 24 hr of sampling. The sample was left at room temperature for 30 min before analysis.

IV. Isolation of probiotic bacteria

For the isolation of *Lactobacillus* acidophilus, 5 ml of the yoghurt drink was inoculated into 95 ml of nutrient broth (general medium) as well as MRS broth (selective medium) and incubated at 37 °C for 24 hr (Memmert, Germany). One loopful of the suspension was then streaked on MRS agar and nutrient agar media and the inoculated plates were incubated at 37°C for 24-72 hr. The single isolated colonies formed on both plates were examined.

V. Enumeration of probiotic bacteria

Enumeration of *Lactobacillus* spp was carried out by aseptically mixing yogurt sample 1.0 ml of yoghurt sample was suspended in 9.0 ml of sterile buffered peptone water. The sample was thoroughly mixed and ten fold serial dilutions of the resulting suspension were performed using sterile peptone water solution as the diluents to obtain a suitable dilution. For the viable *Lactobacillus* spp. counts, 0.1 ml of the appropriate dilutions was pour plated on MRS-agar. All the dilutions were plated out in triplicate. Empty petri-dishes were inoculated with 1.0 ml of diluted yogurt, followed by the addition of 15.0 ml melted (45°C) MRS agar. The plates were covered and the contents mixed thoroughly by gentle tilting and swirling. The plates were inverted and incubated in anaerobic condition at 37 °C for 48-72 hr. The anaerobic condition was achieved by use of an anaerobic jar (Oxoid Ltd., England) and gas generating kit BR39 (Oxoid Ltd., England). Gas generating kits bind to oxygen efficiently to create anaerobic condition. After incubation, the number of colonies on the plate was counted and recorded using a colony counter (Technical Lab Instrument, USA). The visible colonies were counted and expressed as colony forming units per milliliter (cfu/ml), representing the number of viable *Lactobacillus* spp. present in yoghurt sample. The numbers of colony were counted and calculated as follows:

cfu/ml = cfu/plate x dilution factor

VI. Morphology Identification

The colonies were selected randomly for Gram staining. All colonies were examined either gram positive or gram negative and the shape of bacteria under microscope (1000X).

RESULTS AND DISCUSSION

I. Isolation of Lactobacilli

The results showed that the bacteria contained in yoghurt produced only one type of colony when isolated in MRS agar (selective medium for Lactobacilli). On the other hand, isolation on nutrient agar (general medium) produced two types of colony as shown in Table (1)

II. Morphology Identification

The colonies yielded were tested for Gram staining. From the results, all the microorganisms from nutrient agar were Gram positive, rod and cocci shape because in nutrient agar growth claimed to contained mix culture. In contrast, rod shape was found in MRS agar because it claimed to contain pure culture of Lactobacilli (Fig 1). The colony morphology of microorganism obtained from the MRS agar was in accordance with (Vinderola et al., 2000).

III. Enumeration of probiotic bacteria

The enumeration of the bacteria was directly counted from the yogurt diluted to ten fold serially in the MRS agar medium and the viability result was about 10^7 cells / ml. The number of probiotics obtained was higher than the minimal number of probiotic bacteria required in a product (above 10^5 or 10^6 per gram or ml) that needed to be consumed to exert a health-promoting effect for the consumers (Speck, 1978, Pennacchia et al., 2004). It is stipulated in the health and food draft regulations that probiotic products should indicate the viable counts of bacteria per milliliter/gram of product at the end of shelf life and also give full scientific name of the probiotic species present in the product (Anonymous, 2002). Earlier reports have already highlighted the importance of documentation of viability, colony counts and species identification of organisms in probiotic products (Marcon, 1997). Although other species such as *L. casei* and *L. paracasei* are increasingly being used in the manufacture of probiotic yogurts (Sarem-Damerdji et al., 1995). *L. acidophilus* is expected to be the main *Lactobacillus* species involved in the manufacture of yogurt and yogurt drinks. This observation has indeed been supported by this study. The numbers of viable Lactobacilli from the yoghurt were greater than the suggested minimum as required by international standards.

CONCLUSION

The bacteria were successfully isolated from a commercial yoghurt drink and identified as *Lactobacillus acidophilus*. MRS medium is the suitable medium for isolating this strain.

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REFERENCES

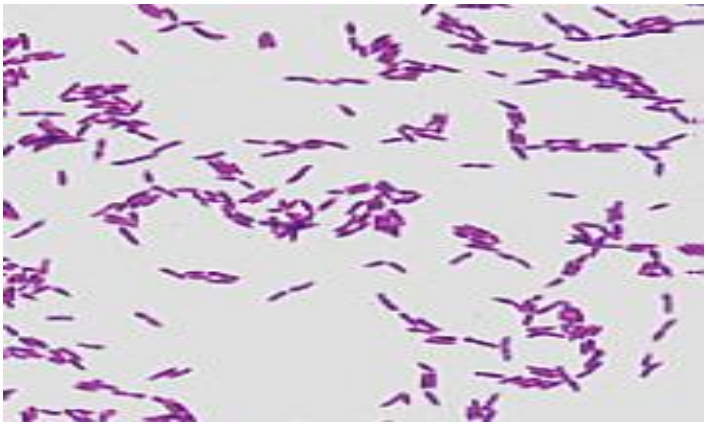

Fig 1: *Lactobacillus acidophilus* as observed under light microscope (Magnification 1000x)

Table 1: Colony morphology on MRS and nutrient media

Media	Microorganism*	Colony			
		Diameter (mm)	Color	Shape	Size
MRS agar	Pure culture	0.9 – 1.5	whitish brown	Circular	Large
Nutrient agar	Mix culture	0.9 – 1.5 0.7 – 1.1	White yellow	Circular Irregular	Large small

* target microorganism of the differential media