Lactic Acid and Probiotic Bacteria from Fermented and Probiotic Dairy Products

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ABSTRACT

Lactic acid and probiotic bacteria were enumerated and isolated from commercially available yoghurt and probiotic milk products. *Lactobacillus delbrueckii* ssp. *bulgaricus* were enumerated and isolated using MRS agar incubated anaerobically at 37°C for 72 hrs. M17 agar was used for the enumeration and isolation of *Streptococcus thermophilus* incubated aerobically at 37°C for 48 hrs. MRS agar and modified MRS agar (MRS + L-cysteine + LiCl + Na propionate) were used for the enumeration and isolation of probiotic bacteria. Both were incubated anaerobically at 37°C for 72 hrs. Morphological, physiological and biochemical reactions were used to characterize the isolates.

Str. thermophilus counts ranged from 2.6 x 10^{11} to 2.9 x 10^{20} CFU/g with Fruit Yoghurt (FY) having the highest count and Yoghurt Natural (YN) with the lowest count. Highest *Lactobacillus delbrueckii* ssp. *bulgaricus* count was obtained in Duo Yoghurt (DY), 1.1 x 10^{9} and lowest in Yoghurt Drink (YD), 8.0 x 10^{7} CFU/g. The highest probiotic bacterial count of 2.3 x 10^{8} was obtained in Yakult (YK) and Neslac (Nes) showed the lowest, 1.6 x 10^{2} CFU/g. The viable counts of all the products examined met the prescribed minimum viable count of 10^{5} to 10^{6}CFU/g for the claimed health benefits for the consumer except for Chamyto Plain (CP), Nes and Nan-2 (Nan).

Morphological, physiological and biochemical characteristics showed that the following genera and species were present *Pediococcus acidilactici* (YN), *P. pentosaceus* (FY), *Lactobacillus delbrueckii delbrueckii* and *L. brevis* in Non Fat High Calcium Yoghurt (NC), *L. acidophilus* and *L. delbrueckii delbrueckii* (DY, YD), *P. damnosus* and *P. pentosaceus* in Chamyto Orange (CO), *L. delbrueckii bulgaricus, L. acidophilus,* and *L. delbrueckii delbrueckii* (CP), *L. para. paracasei* (YK) and *Bifidobacterium* ssp. (Nes and Nan).Of the 28 isolates characterized in this study, 15 were *Lactobacillus* (5 species), 5 were *Pediococcus* (3 species), 6 were *Bifidobacterium* (species not identified), and 2 were *Actinomyces israelii* (1 species).

Key words: LAB, lactic acid bacteria, probiotic, fermented dairy products

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INTRODUCTION

Lactic acid bacteria (LAB), which are primarily used by the dairy industry, are extensively utilized in the fermentation of wide variety of food products and are known for their preservative and therapeutic effects (Gourama, 1995). *Streptococcus thermophilus* and *Lactobacillus bulgaricus* are used for manufacturing of yogurt. *Lactococcus lactis, Lactococcus cremoris, Lactococcus diacetylactis, Leuconostoc cremoris, Leuconostoc lactis, Lactobacillus helveticus, Lactobacillus acidophilus, Lactobacillus casei,* and *Streptococcus sp.* are used for the production of ripened cheese, cultured milk, cream and ripened butter and *L. acidophilus* and *L. casei* are widely used as probiotic bacteria in human and animal health. In meat and fish fermentation, *Lactobacillus plantarum* and *Pediococcus acidilactici* are used. *Lactobacillus sanfrancisco, L. brevis, L. plantarum, L. delbrueckii, L. lechmannii, L. casei* and *L. brevis* are used for the production of soda crackers (Gilliland, 1990).

Metchnikoff first hypothesized the importance of lactobacilli for human health and longevity at the beginning of 19 th century. He considered the gut microbes as detrimental rather than beneficial and suggested that desirable effects might only be expected from their substitution by yogurt bacteria. Since then attempts have been made, especially during the last two to three decades to improve the health status by modulating the indigenous intestinal microflora by live microbial adjunct, now called "probiotics" (Holzapfel et al., 1998).

The word "probiotic" was derived from the Greek word which means "on be half of ". The concept was introduced by Lilly & Stillwell (1965) and was intended to stimulate substances produced by one microorganism to enhance the growth of another. Probiotic therefore is the exact opposite of antibiotic. The word probiotic was used later to refer to animal feed supplements and was defined as a live microbial feed supplement, which beneficially affects the host animal by improving its intestinal microbial balance (Fuller, 1989).

The probiotic bacterial culture upon passage through the upper digestive tract must be capable of surviving and growing in the intestine and maintain their viability and activity in the carrier food before consumption (Gilliland, 1989). The health and nutritional benefits ascribed to probiotics can be generalized under the following categories: maintenance of normal intestinal microflora balance in infant and old age, improvement of lactose tolerance and digestibility of the milk products, antitumorogenic activity, reduction of serum cholesterol levels, synthesis of B-complex vitamins, and absorption of calcium (David & Dauas, 1991). The commonly used probiotic strains in different dairy products are: *L. acidophilus, L. casei Shirota, L. casei immunitas, L. crispatus, L. gasserei, L. johansonii, L. plantarum, L. reuterii, B. adolescentis, B. animalis, B. bifidum, B. breve, B. infantis, B. lactis, B.longum, E. faecium,* and *E. faecalis* (Holzapfel et al., 1998).

This study deals with the isolation and identification of lactic acid and probiotic bacteria from locally available fermented and probiotic dairy products with the ultimate objective of preserving the isolates for their future potential use in the development of new fermented and probiotic foods, feeds, pharmaceuticals, and other applications.

MATERIALS AND METHODS

Lactic Acid Bacteria (LAB)

Locally available fermented dairy products, namely: Nestle Yoghurt Natural (YN), Fruit Yoghurt (FY), Non Fat High Calcium Yoghurt (NC), Yoghurt Drink (YD), and Duo Yoghurt (DY), which were all claimed to contain lactic acid bacteria (LAB), were used in this study as sources of LAB. The products were collected from the supermarkets as fresh as possible. Their manufacturing date, use by date and composition were carefully recorded. Three (3) samples of each product were obtained and as much as possible, it was ensured that the triplicates were within the same or very close manufacturing dates. All samples were maintained at a temperature range of 4 to 60C prior to immediate microbiological examination. Plating and isolation of LAB were done as shown in Figure 1 (IDF, 1988). Anaerobic incubation was done using BBL Gas Pak (Code No.270308, Becton Dickinson and Co., USA). Number of colonies equal to the square root of the total colony count were isolated from the plates counted. Morphological characterization was done by

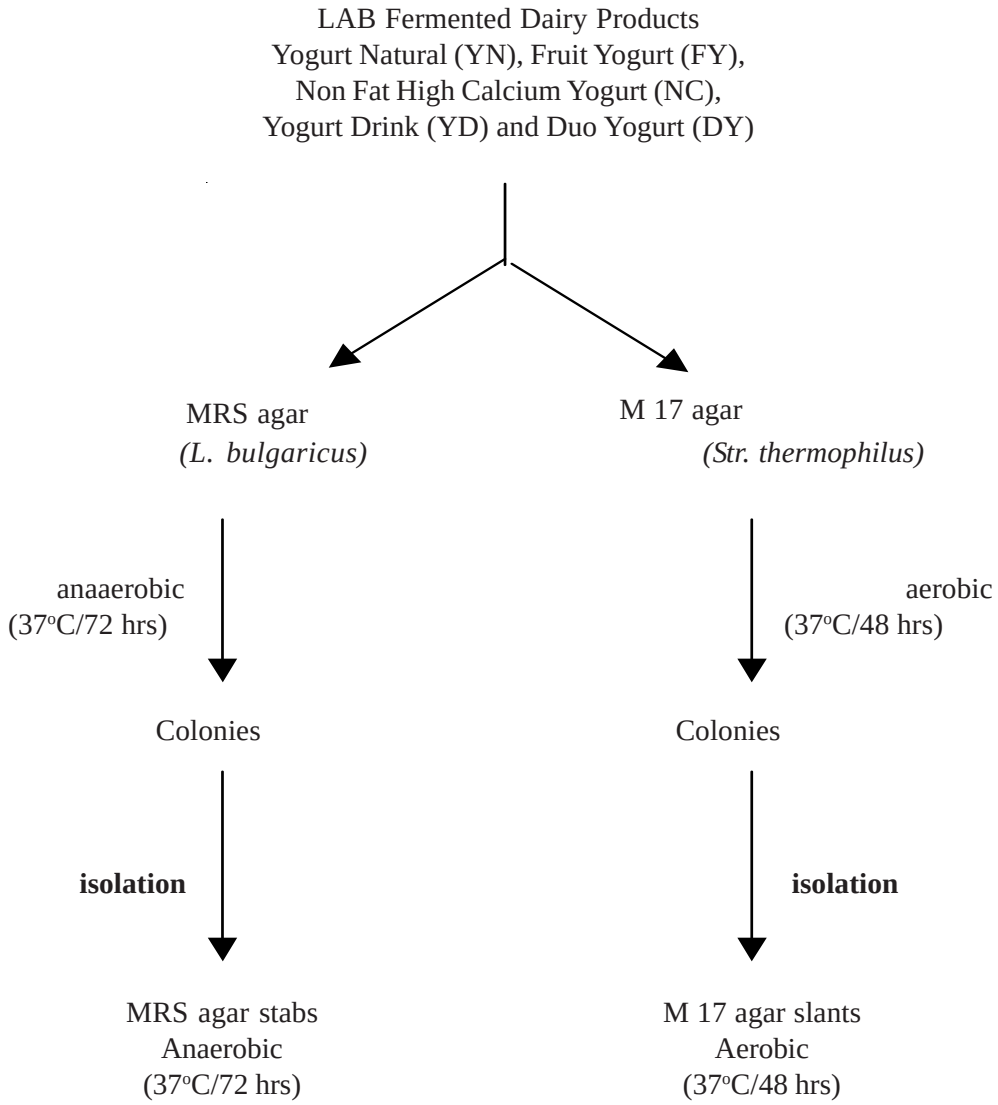

Figure 1. Plating and isolation of LAB (IDF, 1988).

examining colony growth, Gram reaction and cell size measurement (Benson, 1998). Catalase test was done according to Harrigan & McCance (1976). Biochemical characterization of the isolates was done following Holt et al. (1994) and using API 50 CH identification system (Biomeriux SA, 69280 Marcy l'Etoile, France). The scheme for phenotypic identification is presented in Figure 2.

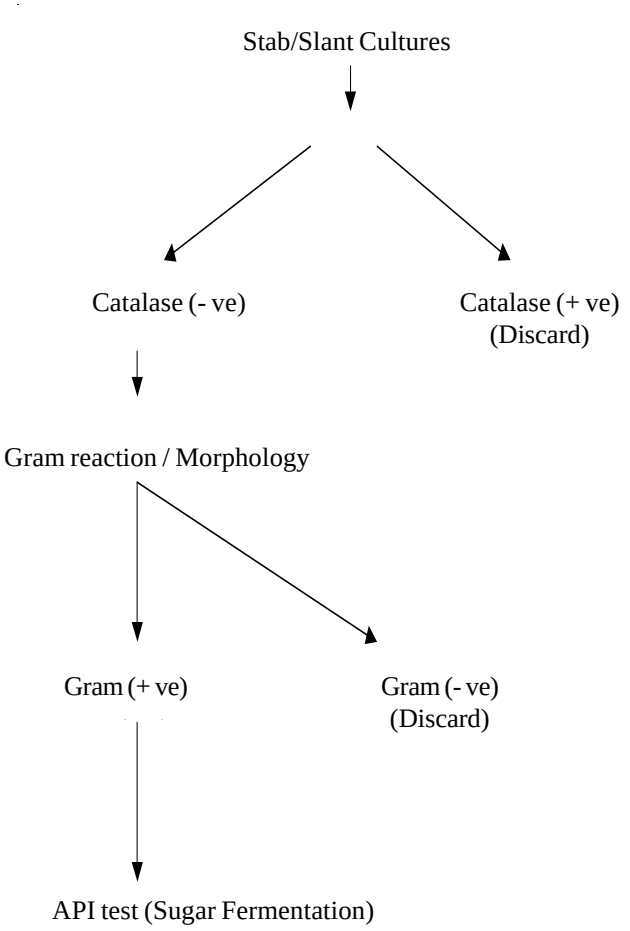

Figure 2. Phenotypic identification of LAB and probiotic bacteria

Probiotic Bacteria

Locally available probiotic dairy products from different supermarkets were used as the sources of probiotic bacteria. The probiotic dairy products and microorganisms claimed to be present in them are given in Table 1.Their manufacturing date, use by date and composition were carefully recorded. All samples were maintained at a temperature range of 4 to 6° C during transit to the laboratory for immediate microbiological examination. The scheme for plating and isolation of probiotic bacteria is presented in Figure 3. For Chamyto and Yakult, the diluents used were as recommended by IDF (1988). The diluents for Neslac and Nan were as specified in IDF (1999). Colonies were picked from the plate such that the number of isolates taken was equal to the square root of the total colony count.

Dairy Products	Probiotic Bacteria Claimed to be present	Manufacturer
Chamyto Plain (CP)	*Lactobacillus sp.*	NestlePhilippines, Cabuyao, Laguna, (Nestle,Switzerland)
Chamyto Orange (CO)	- do -	- do -
Neslac (Infant dried milk, Nes)	*Bifidobacterium bifidus*	- do -
Nan-2 (Infant dried milk, Nan	*Bifidobacterium bifidus*	- do -
Yakult Fermented milk, YK)	*L. casei strain Shirota*	Yakult Philippine Inc., Laguna (Collab. Yakult, Japan)

Table 1
Probiotic dairy product samples

Morphological, physiological and biochemical characterization were done similarly as for LAB, Figure 2. Identified LAB and probiotic bacteria were preserved as described by Kisworo & Barraquio (2003) and deposited at the Philippine Network of Microbial Culture Collections (PNMCC), Biotech, UPLB.

Statistical Analysis

Completely Randomized Design (CRD) with three replications per treatment was used. Fermented dairy products were the treatments to evaluate the presence of bacteria, their types, numbers and characteristics in the products (Gomez & Gomez, 1984). Analysis of Variance (ANOVA) in CRD using the SAS soft ware was done on the data on bacterial counts. The Least Significant Difference (LSD) test was used to determine differences among fermented dairy product means.

RESULTS AND DISCUSSION

LAB and Probiotic Counts of Dairy Products

The mean LAB and probiotic bacterial counts of different yogurt samples are given in Table 2. The data showed that the mean *Str. thermophilus* counts of FY was highest, 2.9 x 10^{20} CFU/g ($P \leq 0.01$) and YN was the lowest, 2.6 x 10^{11} CFU/g. The reason for the

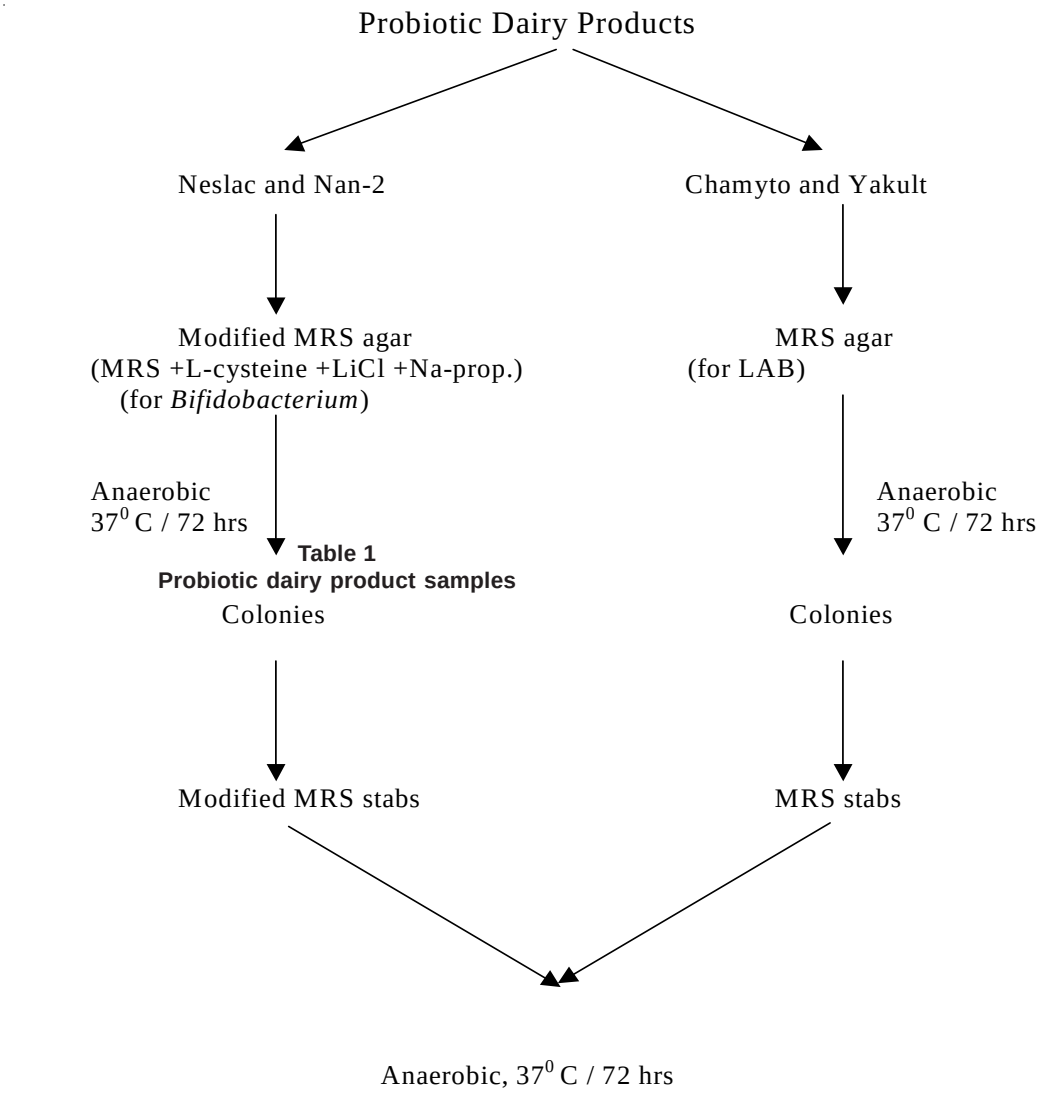

Figure 3. Plating and isolation of probiotic bacteria (IDF, 1999)

higher mean putative count of FY may be due to the differences in manufacturing dates of samples used.

The mean *Lactobacillus delbrueckii* ssp. *bulgaricus* counts of different yogurt samples are also given in Table 2. The data showed that the mean counts of DY, NC, FY and YN were statistically at par with each other but significantly higher than the YD.Highest *Lactobacillus delbrueckii* ssp. *bulgaricus* count was obtained in DY, 1.1 x 10^9 CFU/g, while YD was lowest, 8.0 x 10^7 CFU/g ($P \leq 0.05$), which may be due to the loss of nutrients in the yogurt and accumulation of waste with age (three weeks) of the sample (Kozaki et al., 1992).

The data also showed that the mean putative probiotic count of YK was significantly higher than the other samples. Probiotic bacterial count of YK was 2.30 x 10^8 CFU/g and Nes showed the lowest, 1.6 x 10^2 CFU/g. The reason for the differences in mean counts may be due to the nature of the product, Nes being a powder. The amount of water needed for growth of microorganisms varies. This water requirement is best expressed in terms of available water or water activity, aw. Most bacteria grow well in a medium with a_w approaching 1.00. Liquid milk has an a_w of about 0.98 while dried or powdered milk's aw is below 0.60 (Frazier and Westhoff, 1988). The mean probiotic count of Nan (1 year-old) was significantly higher than the Nes (2

years old), which may be due to the age of the sample, Nes having earlier manufacturing date.

Shah (2000) recommended a minimum LAB count of not less than 10^6 CFU/g in fermented dairy product. According to Robinson (1987), for the consumers to obtain the claimed health benefit, the minimum number of LAB and probiotic bacteria should be maintained at 10^5 CFU/g of cultured product. CP, Nes and Nan did not meet the prescribed minimum viable counts mentioned.

Colony Characteristics of LAB and Probiotic Bacterial Isolates

Colony characteristics of LAB and probiotic isolates were studied by picking up the typical, well isolated and representative colony that appeared on the plate. A single colony was aseptically picked-up and transferred to stab/slant for study of the growth pattern of isolates on the solid media. Growth of isolates was observed and cultural characteristics were described (Table 3).

SAMPLE	MEAN COUNTS (n=3, CFU/g)	
For LAB	*Str. thermophilus*[1]	*Lacto. bulgaricus*[1]
Fruit Yogurt (FY)	$2.9 \times 10^{20\ a}$	$2.6 \times 10^{8\ a}$
Yogurt Drink (YD)	$1.4 \times 10^{19\ b}$	$8.0 \times 10^{7\ b}$
Non fat high Ca Yogurt (NC)	$2.6 \times 10^{14\ c}$	$3.1 \times 10^{8\ a}$
Duo Yogurt (DY)	$1.8 \times 10^{13\ d}$	$1.1 \times 10^{9\ a}$
Yogurt Natural (YN)	$2.6 \times 10^{11\ e}$	$2.3 \times 10^{8\ a}$
For Probiotic bacteria[2]		
Yakult (YK)	$2.3 \times 10^{8\ a}$	
Chamyto, Orange (CO)	$3.6 \times 10^{6\ b}$	
Chamyto, Plain (CP)	$3.4 \times 10^{4\ c}$	
Nan - 2 (Nan)	$1.6 \times 10^{3\ d}$	
Neslac (Nes)	$1.6 \times 10^{2\ e}$	

[1] Means with the same superscript are not significantly different at $P \leq 0.01$ *(S.thermophilus)* and $P \leq 0.05$ *(L.bulgaricus)*.
[2] Means with the same superscript are not significantly different at $P \leq 0.01$
n = number of samples examined

Table 2
Mean LAB and probiotic bacterial counts

In this study, five isolates namely, YN-6, FY- 4, FY-5, CO-5 and CO-6 have colony characteristics that resembled the genus, Pediococcus as described by Sneath et al (1986). The colonies appeared grayish white in color, round, smooth entire and filiform, smooth and regular growth on stab. Fifteen isolates namely, NC- 4, NC-6, DY-1, DY-2, DY-3, DY-6, YD-1, YD-2, YK-1, YK-2, YK-3, YK-7, CP-4, CP-5 and CP-6 were found to be typical of genus *Lactobacillus* colonies of which generally appeared white to yellowish in color, round, spindle, triangular, star-like structure with effuse, filiform, irregular and arborescent on slant streak and stab.

Regarding probiotic bacteria, six (6) isolates namely, Nes-1, Nan-1, Nan-2, Nan-4, Nan-5 and Nan-6, showed colony characteristics that resembled *Bifidobacterium sp.* Holt et al. (1994) described the colonies as creamy white glistening with soft consistency, raised and convex elevation, round, triangular and spindle shaped. Isolates Nes-2 and Nan-3 resembled *Actinomyces*, which were creamy white in color, round, smooth, soft to mucoid which also resembled more or less *Bifidobacterium* (Sneath et al., 1986; Waksman,1967).

Morphological Characteristics of LAB and Probiotic Bacterial Isolates

The LAB and probiotic isolates were characterized morphologically by Gram staining. All 70 isolates were Gram positive. The morphological characteristics of LAB and probiotic isolates are presented in the Table 4. Cell measurements were based on 48 hr-old culture in case of *Str. thermophilus* and 72 hr-old culture in case of *L. delbrueckii* ssp. *bulgaricus* and probiotic bacterial isolates. Cell measurements of isolates were done under the high power objective (400 x). Cell measurements of LAB isolates ranged from 0.4 to 1.0 by 2 to 10 µm whereas the cell measurements of *Pediococcus* isolates ranged from 1.0 to 4.0 µm in diameter. The LAB belonging to the genus

Lactobacillus have rod shaped cells, usually regular but sometimes they are almost coccoid, commonly in short chains with cell size of 0.5 to 1.2 x 1.0 to 10 µm while the genus *Pediococcus* has spherical cells, 1.0 to 2.0 µm in diameter in pairs or tetrads (Holt et al., 1994).

Out of 28 isolates, fifteen (15) isolates namely: NC-4, NC-6, DY-1, DY-2, DY-3, DY-6, YD-1, YD-2, YK-1, YK-2, YK-3, YK-7, CP-4, CP-5 and CP-6 resembled the genus *Lactobacillus* as described by Holt et al., (1994). Five (5) isolates, namely YN-6, FL-4, FL-5, CO-5 and CO- 6, were found to be typical of the genus

NO.	ISOLATES	IDENTITY	COLONY CHARACTERISTICS
1	YN - 6	*Pedio.acidilactici*	Colonies round, smooth, white on solid media, surface growth on broth and arborescent growth on stab
2	NC - 4	*Lact.delb.delb*	Colonies round, smooth, raised and filiform and entire growth on solid media
3	NC - 6	*Lact. brevis*	Colonies round, raised, surrounded by transparent area on solid media and punctiform on slant.
4	F Y - 4	*Pedio.pentosaceus*	Colonies round, regular, flat and yellow color and effuse growth on solid media.
5	F L -5	*Pedio pentosaceus*	Colonies round, white, regular, raised and effuse growth on solid media.
6	DY -1	*Lact.delb delb*	Colonies round, smooth, raised and filiform entire growth on solid media
7	DY-2	*Lact. acidophilus*	Colonies round, white raised& smaller size effuse and entire growth on slant
8	DY- 3	*Lact. acidophillus*	Colonies round, smooth, flat, entire and white filliform, raised and entire growth on slant
9	DY -6	*Lact. acidophillus*	Colonies round, white raised and non-transparent filiform, raised and entire growth on slant
10	YD-1	*Lact. acidophilus*	Colonies round, white, convex & non transparent, regular & entire and slant growth is filiform
11	YD-2	*Lact.delb.delb..*	Colonies round, smooth white and flat on plate the growth is arborescent, white and rose
12	CO-5	*Pedio. damnosus*	Colonies round, yellow flat and filiform, smooth & regular growth on stab
13	CO-6	*Pedio pentosaceus*	Colonies small dot structure, white, raised, punctiform & beaded type growth on stab
14	YK-1	*Lact. paracasei*	Colonies round, light yellow, raised regular & punctiform growth on plates & filiform and irregular growth on stab
15	YK-2	*Lact.paracasei*	Colonies triangular, light yellow, raised, and punctiform on plate & filiform growth on stab
16	YK-3	*Lact. paracasei*	Colonies yellow, spindle, raised, regular and dot like on plate, arborescent and irregular on stab
17	YK-7	*Lact.paracasei*	Colonies triangular, white, raised, regular,star like on plate and arborescent and irregular on stab
18	CP-4	*Lact.delb bulg.*	Colonies small, raised, regular, non-transparent, arborescent and irregular growth on stab
19	CP-5	*Lact acidophilus*	Colonies triangular,irregular,white raised on plate and arborescent and irregular growth on stab
20	CP-6	*Lact. delb.delb.*	Colonies round, transparent, small, flat on plate arborescent and irregular growth on stab
21	Nes-1	*Bifidobacterium sp.*	Colonies round, regular, white glistening convex soft on plate and arborescent growth on stab
22	Nes-2	*Actinomyces israelii*	Colonies spindle, entire, white glistening, convex, smooth and filiform growth on stab
23	Nan-1	*Bifidobacterium sp.*	Colonies round, transparent, convex, smooth & entire and arborescent growth on stab
24	Nan-2	*Bifidobacterium sp.*	Colonies spindle, non transparent, convex, entire, smooth and cream color & filiform growth on stab
25	Nan-3	*Actinomyces israelii*	Colonies round creamy, convex, entire and arborescent growth on stab
26	Nan-4	*Bifidobacterium sp.*	Colonies triangular, cream color, convex, entire and arborescent growth on stab.
27	Nan-5	*Bifidobacterium sp*.	Colonies spindle, cream color, convex, entire and filiform growth on stab.
28	Nan-6	*Bifidobacterium sp.*	Colonies spindle, cream color, very small size, convex, entire and filiform growth on stab.

( ) not a LAB nor probiotic bacteria

Table 3. Colony characteristics of LAB and probiotic bacteria

Table 3. Colony characteristics of LAB and probiotic bacteria

Pediococcus in Gram reaction, cell shape, cell size and occurrence.

Six (6) of the probiotic isolates, namely, Nes-1, Nan-1, Nan-2, Nan-4, Nan-5 and Nan-6 appeared as thin, Gram positive rods, branched, arranged singly and pairs in V and Y arrangements, in chains, typical of *Bifidobacterium* as described by Holt et al. (1994). *Actinomyces israelii* was also isolated from Nes-2 and Nan-3 which might have come from the processing plant environment. Waksman (1967) explained that *Actinomyces* occurs virtually in every natural substrate such as fresh water basins, foodstuffs and the atmosphere and multiply most abundantly in various depths of soil and compost in temperate and tropical regions.

Sugar Fermentation of LAB and Probiotic Bacterial Isolates

Out of 70 isolates, only 28 isolates were selected and subjected to sugar fermentation test due to budgetary constraints. The sugar fermentation patterns of LAB and probiotic bacterial isolates are presented in Table 5. The summary of API output of LAB and probiotic bacterial isolates is presented in Tables 6 and 7, respectively, while the sources of LAB and probiotic isolates are shown in Table 8.

In general, in case of LAB, majority of the isolates were *Lactobacillus* with 5 different species identified followed by *Pediococcus* with 3 different species. Damelin et al. (1995), in his study on the biodiversity of LAB from food related ecosystem also reported that the *Lactobacillus* strain dominated all ecosystems and consisted 65% of *Lactobacillus* isolates. Kisworo and Barraquio (2003), also found in their study of raw milk and white soft cheese that the most predominant genus isolated was *Lactobacillus*, which comprised fourteen (14) out of twenty-three (23) isolates. Another work conducted by Tzanetakis and Litopoulou-Tzanetaki (1992), showed that Lactobacillus was the predominant genus over *Enterococcus* and *Pediococcus* in cheese. Rodrigues et al. (1995), reported that majority of species found in raw cow milk cheese were *Lactobacillus casei* ssp. *casei* followed by *Lactococcus lactis* ssp. *lactis*.

IDENTITY	SHAPE	AVERAGE CELL SIZE
Pediococcus acidilactici	Cells spherical, in tetrads, also in pairs	1.0 - 2.0 µm
Pediococcus pentosaceus	Cells spherical, in tetrads, also pairs	1.0 - 2.0 µm
Pediococcus damnosus	Cells spherical, tetrads, some are in pairs	1.0 - 4.0 µm
Lact. brevis	Cells rod shaped, regular rounded ends, occur singly/chains	0.6-1.0 x 4 - 6 µm
Lact. delb. delbrueckii	Cells rod shaped, regular, occurring in short chains	0.4-1.0 x 4-8 µm
Lact. acidophilus	Cells rod shaped, regular, occurring in short chains.	0.6-1.0 x 2-8 µm
Lact. delb.bulgaricus	Cells rod shaped, occur in pairs or chains with square ends	0.5-1.0 x 4 -10 µm
Lact. para. paracasei	Cells thin rods, with square ends and in chains	0.4 - 1.0 x 2-4 µm
Bifidobacterium sp.	Cells thin rods, branched, v and y arrangements in chains.	0.5-1.3 x 1.5-8 µm
(Actinomyces israelii)	Cells short rods with clubbed ends occurring in v and y arrangements in chains	0.5-1.x 2- 4µm

( ) not a LAB nor probiotic bacteria

Table 4
Morphological characteristics of LAB and probiotic bacterial isolates

	ISOLATES									
SUGAR	1	2	3	4	5	6	7	8	9	10
Glycerol	-	-	-	-	-	-	-	-	+	+
L-arabinose	-	-	-	-	+	+	+	+	-	-
D-arabinose	-	-	-	-	-	-	-	-	+	+
Ribose	-	+	-	-	+	+	-	+		
D-xylose	-	-	-	-	+	+	-	+	+	+
D-glucose	+	+	+	+	+	+	+	+	+	+
D-fructose	+	+	+	+	+	+	+	+	-	-
D-mannose	+	+	-	+	+	+	+	+	+	+
L-sorbose	-	+	-	-	-	-	-	-		
Sorbitol	-	+	-	-	-	-	-	-	-	-
Rhamnose	-	-	-	-	-	+	+	+	-	-
N-A.glucosamine	+	+	-	-	+	-	+	+		
Amygdalin	-	+	-	-	+	+	+	-		
Arbutine	-	+	-	-	+	+	+	+		
Esculin	+	+	-	+	+	+	+	+	+	+
Salicine	+	+	-	-	+	+	+	+	+	+
Manitol	-	+	-	-	+	-	-	-	+	-
Maltose	+	+	-	+	+	-	-	-	+	+
Lactose	-	+	+	+	+	-	-	-	+	+
α-M.D-glucoside	-	-	-	-	+	-	-	-		
Galactose	+	+	-	-	+	+	+	+	-	-
Gelatin	-	-	-	-	-	-	-	-	-	-
Trehalose	+	+	-	-	+	+	+	+	-	-
Cellobiose	+	+	-	-	+	+	+	+	+	+
Saccharose	+	+	-	+	+	-	-	-	+	+
Melezitose	-	+	-	-	-	-	-	-	+	-
Inulin	-	-	-	-	+	-	-	-		
D-raffinose	-	-	-	-	+	-	-	-	+	-
B-gentiobiose	+	+	-	-	+	+	+	+		
D-turanose	-	+	-	-	+	-	-	-		
D-tagatose	+	+	-	-	+	+	-	+		
D- arbitol	-	-	-	-	-	-	-	-		
Gluconate	-	+	-	-	-	-	-	-		
Adonitol	-	+	-	-	-	-	-	-		
Indol	-	-	-	-	-	-	-	-	-	-
Urease	-	-	-	-	-	-	-	-	-	-

1- *Lactobacillus acidophilus*
2- *Lactobacillus para.paracasei*
3 -*Lactobacillus delb. bulgaricus*
4 -*Lactobacillus delb. delbrueckii*
5- *Lactobacillus brevis*
6- *Pediococcus pentosaceus*
7 -*Pediococcus damnosus*
8 -*Pediococcus acidilactici*
9 -*Bifidobacterium spp.*
10- *Actinomyces israelii*

Table 5. Sugar fermentation patterns of LAB and probiotic bacterial isolates

No.	Isolates	IDENTITY	% Identification
01	Y N-6	*Pediococcus acidilactici*	99.9
02	NC -4	*Lact. delb delb rueckii*	96.8
03	NC-6	*Lact. brevis*	99.7
04	FY -4	*Pedio.pentosaceus*	85.1
05	FY -5	*Pedio. pentosaceus*	99.9
06	DY -1	*Lact. delb. delbrueckii*	90.7
07	DY - 2	*Lact. acidophilus*	99.1
08	DY - 3	*Lact. acidophilus*	99.9
09	DY - 6	*Lact. acidophilus*	98.2
10	YD - 1	*Lact. acidophilus*	95.8
11	YD - 2	*Lact. delb.delbrueckii*	99.9
12	CO-5	*Pedio. damnosus*	99.8
13	CO-6	*Pedio. pentosaceus*	99.9
14	YK - 1	*Lact. para. paracasei*	99.9
15	YK - 2	*Lact. para. paracasei*	99.9
16	YK - 3	*Lact. para. paracasei*	99.5
17	YK - 7	*Lact.para. paracasei*	92.5
18	CP - 4	*Lact. delb. bulgaricus*	99.7
19	CP - 5	*Lact. acidophilus*	99.7
20	CP - 6	*Lact. delb.delbrueckii*	98.2

Table 6
Summary of API identification output for LAB

No.	Isolates	IDENTITY	% Identification
1	Nes-1	*Bifidobacterium sp.*	98.2 %
2	Nes-2	*(Actinomyces israelii)*	95.2 %
3	Nan-1	*Bifidobacterium sp.*	90.2 %
4	Nan-2	*Bifidobacterium sp.*	94.5 %
5	Nan-3	*(Actinomyces israelii)*	95.2 %
6	Nan-4	*Bifidobacterium sp.*	96.5 %
7	Nan-5	*Bifidobacterium sp.*	95.7 %
8	Nan-6	*Bifidobacterium sp.*	96.5 %

( ) not a LAB nor probiotic bacteria

Table 7
Summary of API identification output for probiotic bacterial isolates

Sources	LAB and Probiotic Bacteria	No. of Isolates
Yogurt Natural	*Pedio. acidilactici*	1
Non Fat High Ca yogurt	*Lact.delb.delbrueckii*	1
	Lact. brevis	1
Fruit yogurt	*Pedio. pentosaceus*	2
Duo Yogurt	*Lact.delb.delbrueckii*	1
	Lact.acidophilus	3
Yogurt drink	*Lact. acidophilus*	1
	Lact.delb.delbrueckii	1
Chamyto (orange)	*Pedio. damnosus*	1
	Pedio. pentosaceus	1
Chamyto (plain)	*Lact.delb. bulgaricus*	1
	Lact. acidophilus	1
	Lact. delb. delbrueckii	1
Yakult (plain)	*Lact. para. paracasei*	4
Neslac (powder milk)	*Bifidobacterium sp.*	1
	(Actinomyces israelii)	1
Nan-2 (powder milk)	*Bifidobacterium sp.*	5
	(Actinomyces israelii)	1
	Total isolates	28

( ) not a LAB nor probiotic bacteria

Table 8
Sources of LAB and probiotic bacterial isolates

SUMMARY AND CONCLUSIONS

Cultural, morphological, physiological and biochemical characteristics showed that the following genera and species of LAB and probiotic bacteria were present in the dairy products examined: *Pediococcus acidilactici* (YN), *Pediococcus pentosaceus* (FY), *Lactobacillus delbrueckii delbrueckii* and *Lactobacillus brevis* (NC), *Lactobacillus acidophilus* and *Lactobacillus delbrueckii delbrueckii* (DY), *Lactobacillus delbrueckii delbrueckii* and *Lactobacillus acidophilus* (YD), *Pediococcus damnosus* and *Pediococcus pentosaceus* (CO), *Lactobacillus delbrueckii bulgaricus*, *Lactobacillus acidophilus* and *Lactobacillus delbrueckii delbrueckii* (CP), *Lactobacillus para. paracasei* (YK), and *Bifidobacterium sp.* (Nes and Nan).

Of the twenty-eight (28) isolates, five (5) were *Lactobacillus acidophilus*, four (4) each of *L.*

para.paracasei and *L. delbrueckii delbrueckii*, one (I) each of *L. brevis* and *L. delbrueckii bulgaricus.* There were three (3) *Pediococcus pentosaceus* and one each of *P. damnosus* and *P. acidilactici.* Of the probiotic bacterial isolates there were six (6) *Bifidobacterium sp.* found and two (2) *Actinomyces israelii.* The viable counts of all the products met the prescribed minimum viable count of 10^5 to 10^6 CFU/g for the claimed health benefits for the consumer except for Chamyto Plain, Neslac and Nan-2.

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