Tropical Agricultural Research Vol. 26 (3): 428 – 440 (2015)

Study on Isolation of Potentially Probiotic *Lactobacillus species* from Fermented Rice

N. Jeygowri, N. Parahitiyawa[1], S. Jeyatilake[1], S. Ranadheera[2] and T. Madhujith[3*]

Postgraduate Institute of Agriculture
University of Peradeniya
Sri Lanka

ABSTRACT: *Traditional fermented food products such as fermented rice are known to possess probiotic potential. Probiotics are live microorganisms that provide a myriad of health benefits. Despite the associated health benefits, fermented rice has not received due attention in the countries where rice is the staple diet. Many strains of probiotic bacteria have been isolated from different sources, however, not much work has been carried out on isolation of probiotic strains from fermented rice. In this backdrop, the present study was carried out to isolate and identify potentially probiotic microorganisms from fermented rice. Cooked and uncooked white and red rice were separately fermented and subsequently used to isolate potentially probiotic strains. The Man Rogosa Sharpe (MRS), MRS sorbitol (0.2%) and MRS L-cysteine (0.5%) culture media were used for the isolation of potentially probiotic bacteria. The samples of fermented rice were serially diluted, plated and incubated at 37 °C for 2-3 days under anaerobic conditions. The resulting colonies were purified and tested for catalase production and Gram-staining. Distinct cluster like cocci, diplo cocci and rods were observed with gram-positive and catalase-negative reactions. Most of the isolated cluster like cocci morphologically resembled Aercococcus or Peptococcus* species. *The rods were selected for motility and endospore test and sugar fermentation was studied using API 50CH kits. The biochemical characteristics (gram positivity and catalase-negativity) non-motile and non-endospore forming and colony and cell morphology of seven rod shaped bacteria resembled the genus Lactobacillus and were identified to species level by API 50CH kits. The subsequent physiological and molecular methods for species identification and probiotic characterization of these seven Lactobacillus spp will further confirm the application of them as potential probiotic starter cultures in the food industry.*

Keywords*: Catalase, fermented rice, gram-staining., Lactobacillus, probiotics*

INTRODUCTION

Probiotics are live microorganisms used as food supplements, which provide health benefits when consumed, through improving the intestinal microbial balance of the host (Fuller, 1989).They are live microorganisms which when administered in adequate amounts confer health benefits on the host (FAO-WHO, 2001). Microorganisms commonly used as

[1] Department of Oral Medicine and Periodontology, Faculty of Dental Sciences, University of Peradeniya, Sri Lanka
[2] Department of Animal and Food Sciences, Faculty of Agriculture, Rajarata University of Sri Lanka
[3] Department of Food Science and Technology, Faculty of Agriculture, University of Peradeniya, Sri Lanka
[*] Corresponding author: madujith@yahoo.com

probiotics include *Bifidobacteria, Lactobacilli* and certain yeasts. Research has shown that addition of probiotics to food provides many a health benefit. They are able to increase the frequency of bowel movements (Bekkali *et al.,* 2007) and stimulate cell-mediated immunity (Wold, 2001). Moreover, *Lactobacillus acidophilus* L1 has shown the ability to reduce serum cholesterol level indicating the potential of reducing the risk for coronary heart disease by 6% to 10% in hypercholesterolemic human (Anderson *et al*., 1999). *L. Rhamnosus* GG has shown anti-carcinogenic effects through decreasing the activity of β-glucuronidase (Nicole and Martjjn, 2000). Fermentation is one of the primary methods of adding value to food including improving palatability, safety, shelf life and nutritional value. This process could also be applied in the production of functional foods such as probiotic food (Salovaara and Simson, 2004).

The fermentation process is widely used for preservation of food, particularly in developing countries (Lei, 2006)**.** Yoghurt rice, "*dosa", "idli*" and hoppers are some examples of fermented rice products that constitute traditional Indian and Sri Lankan food (Wickramanayake, 2002). In the past, a vast majority of the farming community in Asian regions used to consume fermented rice for breakfast. This type of diet was known to provide adequate nutrition. The soothing effects of such meals were also well known. Fermented rice-milk beverage products show unique flavour, good taste and milky colour where *L.acidophilus* and *Streptococcus thermophillus* are used as starter cultures with rice steep liquor-milk (Yong-Jin *et al*., 2010). Production of primary metabolite, lactic acid and resultant decrease in pH is identified as the main preserving factor in fermentation of food (Jagadeeswari *et al.*, 2010).

Cereal constituents such as wheat bran-based ingredients fermented with probiotic, enhance the consumer health with the benefits of probiotics, bran fibre, and healthful bioactive components (Lamsal and Faubion, 2009). Poor eating habits, consumption of chlorinated drinking water, stress and certain disease conditions, consumption of alcohol and the use of antibiotics are known to alter the composition and activities of gut flora. These effects are akin to what occurs following systemic antimicrobial treatment on such occasions, the tilt of balance of the bacterial population dynamics results in overgrowth of opportunistic and undesirable bacteria in the gut. The reduction in the proportion of beneficial bacteria leads to development of excessive gas, bloating, constipation, intestinal toxicity and poor absorption of nutrients. Hence, it is essential to enrich the human gut with probiotics in order to maintain the probiotic balance through regular intake of probiotic foods. Many lactobacilli species possess probiotic determinants including resistance to low pH, bile tolerance, adhesive properties, antibacterial activity, and antibiotic susceptibility. There is evidence to suggest that organisms from different origins vary in their probiotic properties (Sandholm *et al.*, 2002)**.**

The possibilities of horizontal gene transfer and recombination to create harmful pathogens in genetically modified (GM) probiotics (Cummins and Wan Ho, 2006) limit the production and application of the GM probiotics**.** Therefore, many researchers explore novel strains from different sources with improved probiotic potential.

Fermented rice is one of the neglected foods in countries where rice is a staple. Furthermore, despite the numerous strains of probiotic bacteria isolated from different sources including traditional fermented food, there is a paucity of information on fermented rice and their constituent probiotic bacteria. In this backdrop, the objective of the current study was to isolate and identify potentially probiotic microorganisms from fermented rice.

METHODOLOGY

Materials

Red and white raw rice samples belonging to BG group were purchased from local market. All microbiological media were obtained from Oxoid, UK. Anaerogen sachets were purchased from Oxoid, UK.

Culture media

deMan Rogosa and Sharpe (MRS) agar (Oxoid, UK) was used as reported previously for isolation of lactic acid producing bacteria (De Man *et al*., 1960). MRS agar with 0.1-0.2% sorbitol interfere with the growth of *Staphylococcus* spp., *Bacillus* spp. and number of yeasts (Janet *et al.,* 2012) and more importantly is selective for *Lactobacillus* spp. (Tharmaraj and Shah, 2003). MRS media supplemented with L-cysteine (0.2-5%) is a selective medium for isolation of selected Lactobacilli and Bifidobacteria (Haddadin *et al*., 2004). MRS, MRS-sorbitol (0.2%) and MRS-L-cysteine (0.25%) media were used in order to improve the specificity of the medium for the isolation of different species of *Lactobacillus.* The pH of the MRS, MRS sorbitol (0.2%) and MRS L cysteine (0.25%) media were 6.2, 6 and, 5.8 respectively.

Preparation of rice samples

The red raw and white rice (75 g) were cooked with water (rice: water 1:3) for 30 min to obtain a soft consistency. Fermentation was carried out by soaking raw or cooked rice (50 g) in sterile distilled water (rice: water 1:3) overnight (12-16 hours) in clay pots (250 ml) at ambient temperature (27 ^{o}C).

Isolation of Lactic Acid Bacteria (LAB) from fermented rice

Fermented rice steep (10 ml) from both white and red cooked and uncooked rice were collected separately after shaking well in the Vortex and diluted in sterile saline (0.85%) solution to obtain concentrations 10^{0} , 10^{-1} , 10^{-2} and 10^{-3} dilutions. Aliquots (100 µl) of the prepared dilutions and undiluted sample were inoculated on to petri plates in duplicate using spread plate method. The inoculated plates were incubated at 37 ^{o}C for 3 days under anaerobic conditions created by Anaerogen gas packs (Oxoid) (Table 1). The colony of gram-positive, and catalase negative bacilli were sub-cultured and purified by streaking on plates containing relevant MRS media and subsequently purification was carried out 4 to 5 times for the confirmation of the purity of the culture.

Table 1. Experimental plan of the study

Culture medium	Source of bacteria	Serial dilutions
MRS	White Raw	$W_1 10^{0,} W_2 10^{0}, W_1 10^{-1,} W_2 10^{-1,} W_1 10^{-3,} W_2 10^{-3}$
	Red Raw	$R_1 10^{0,} R_2 10^{0}, R_1 10^{-1,} R_2 10^{-1,} R_1 10^{-3,} R_2 10^{-3}$
	White cooked	$W_1C10^{0,} W_2C10^{0}, W_1C10^{-2,} W_2C10^{-2,} W_1C10^{-3}, W_2C10^{-3}$
	Red cooked	$R_1C10^{0,} R_2C10^{0}, R_1C10^{-2,} R_2C10^{-2} R_1C10^{-3} R_2C10^{-3}$
MRS-sorbitol 0.2%	White Raw	$SW_1 10^{0,} SW_2 10^{0}, SW_1 10^{-2,} SW_2 10^{-2}$
	Red Raw	$SR_1 10^{0,} SR_2 10^{0}, SR_1 10^{-2,} SR_2 10^{-2}$
	White cooked	$SW_1C10^{0,} SW_2C10^{0}, SW_1C10^{-2,} SW_2C10^{-2}$
	Red cooked	$SR_1C10^{0,} SR_2C10^{0}, SR_1C10^{-2,} SR_2C10^{-2}$
MRS-L cysteine 0.25%	White Raw	$LW_1 10^{0,L} W_2 10^{0}, LW_1 10^{-2,} LW_2 10^{-2}$
	Red Raw	$LR_1 10^{0,L} R_2 10^{0}, LR_1 10^{-2,} LR_2 10^{-2}$
	White cooked	$LW_1C10^{0,L} W_2C10^{0}, LW_1C10^{-2,} LW_2C10^{-2}$
	Red cooked	$LR_1C10^{0,L} R_2C10^{0}, LR_1C10^{-2}, LR_2C10^{-2}$

W-white raw rice, R-red raw rice, WC-White cooked rice, RC-red cooked rice, S-Sorbitol and L-L-cysteine

Identification of *Lactobacillus* spp.

Confirmation of the isolates belonging to the genus Lactobacillus was accomplished by observing their colony morphology, staining and selected biochemical tests: catalase, endospore and motility test, as reported previously (Barrow and Felthman, 1993). The isolates were identified to species level using API 50 CH kits and CHL media (BiomÈrieux, France). The API profiles were analyzed using API web software (BiomÈrieux, France). In the instances where two or more significant taxa were indicated, a reference was made to standard texts for identification by considering their physical properties such as temperature and salt tolerance.

Long term preservation of isolates

Gram-positive, catalase negative isolates were preserved in MRS broth medium containing 20% (v/v) glycerol at -80 °C as frozen cultures. The glycerol stocks of samples were prepared by mixing 0.5 ml of active culture and 0.5 ml of MRS broth with 40% sterile glycerol.

Gram staining

The Gram characteristics of the isolates were determined using light microscope following staining. Lactobacillus is known to be gram-positive. Cultures were grown in appropriate MRS media and modified MRS media at 37 °C for 24 hours under anaerobic conditions (9 %-13 % Carbon dioxide). Cells from fresh cultures were used for Gram staining. After incubation, the cultures were aseptically transferred into 1.5 ml microcentrifuge tubes and centrifuged for 5 min at 1957xg. The resulting supernatant was discarded and the pellet containing cells was immersed in sterile distilled water. The morphological characteristics of isolates were determined under the light microscope with the assistance of an oil immersion lens.

Catalase test

Overnight cultures of isolates were grown on MRS broth at 37 °C. Fresh liquid cultures were used for the catalase test (Bisen and Verma, 1998) by dropping 3% hydrogen peroxide solution into 1 ml of culture broth on microscopic slides. The isolates, which did not give off gas bubbles were chosen.

Spore staining

Spore staining was performed as explained by Barrow and Felthman (1993). Briefly, a thin smear of fresh culture was made on slide and flooded with 5% aqueous malachite green. 5% aqueous safranin was used as counter stain and examined under oil immersion (10X100 magnification). Isolates without green staining of spores were selected as non-spore formers.

Motility

Young broth cultures were incubated at 37 °C for 18 hours. A hang drop preparation was made (Barrow and Felthman, 1993) using a cavity slide and examined under high-power dry objective (X40) with reduced illumination.

RESULTS AND DISCUSSION

Isolation of potentially probiotic bacteria from fermented rice

The number of colonies isolated from fermented red, white cooked and uncooked rice on MRS media, MRS sorbitol and MRS- L- cysteine plates are shown in Tables 2, 3 and 4. There were 168 colonies observed from both cooked and uncooked red and white rice on the different MRS media used. A total of 122 colonies were studied for Gram stain reaction and catalase tests. Three different morpho types cluster: coccus (48), diplococcus (30) and rod (30) were observed with gram-positive and catalase negative reactions. The number of studied colonies showed that the majority of the isolated microorganisms from fermented rice was grape like clusters of coccus and isolated rods with similar number of diplococcus.
Karna and Barraquio (2007) found in their study of fermented milk product, That the predominant genus was *Lactobacillu* sp., which comprised fifteen of twenty eight isolates while others included *Pediococcus* (5), *Bifidobacterium* (6) and *Actinomycesis israelii (2)* .274 strains of *Lactobacilli* sp were identified among 563 total isolates from Thai traditional fermented food (Srikanjana *et al*., 2008) Another study (Silvia and Medana, 2011) reported that fermented Romanian vegetables were rich source of LAB including 139 isolates with rod- (95) coccoid (32), cocobacillai (11) and diplococcal (1) shapes.

Table 2. Colonies observed on MRS media plates

Isolation source	Observed	Identified	Morpho types	Gram test	Catalase test	No.
White raw	21	17	Cluster coccus	+	-	14
			Candida	+	-	2
			Bacillus	+	-	1
Red Raw	21	16	Cluster coccus	+	-	14
			Candida	+	-	1
			Diplococcus	+	-	1
White cooked	11	8	Cluster coccus	+	-	2
			Candida	+	-	2
			Bacillus	+	-	2
			Diplococcus	+	-	2
Red cooked	7	2	Bacillus	-	-	1
			Candida	-	-	**1**
Total	60	43				

In the current study, sixty different types of colonies were observed from fermented cooked, raw white, fermented cooked and raw red rice on inoculated MRS media plates (Table 2). Among the studied colonies (n=43), 33 colonies were gram-positive catalase negative cocci (Cluster coccus: 30, diplo: 3) and 3 colonies were gram-positive catalase negative bacillus.

Fifty seven different types of colonies were observed from fermented cooked and raw white and fermented cooked and raw red rice on the MRS sorbitol (0.2%) media plates (Table 3). The 44 isolates were studied for colony morphology, catalase test and Gram's test. Among the studied colonies, 27 colonies were gram-positive catalase negative cocci (Cluster: 7, diplo: 20) and 9 colonies were gram-positive catalase negative bacilli (Table 3).

Fifty one different types of colonies were observed in fermented cooked and raw white and fermented cooked and raw red rice on the MRS- L-cysteine 0.25% (Table 4). Thirty five isolates were studied for colony morphology, catalase test and Gram test. Among the studied colonies, 17 colonies were gram-positive catalase negative coccus (Cluster: 11, diplo: 6) where 18 colonies were Gram-positive catalase negative bacillus.

It was observed that the number of gram-positive and catalase negative bacillus were higher on MRSL-cysteine 0.25% media plates (n=18) than on MRS-sorbitol 0.2% (n=9) and MRS media (n=4) (Table 5). The contamination of candida, gram-negative bacillus are higher in MRS media and MRS sorbitol media plates than MRS L cysteine plates during isolation as well as purification process. Therefore, the MRS L cysteine 0.25% media were selected for sub culturing and purification of isolated gram-positive and catalase negative bacillus. The higher number gram-positive and catalase negative bacilli were noted for higher isolated from white rice (23) compared with red rice (7).

Table 3. Colonies observed on MRS- sorbitol 0.2 % media plates

Isolation source	Observed	Identified	Morpho types	Gram test	Catalase test	No
White raw	17	16	Cluster coccus	+	-	6
			Diplococcus	+	-	7
			Bacillus	**+**	**-**	**3**
Red Raw	16	9	Diplococcus	+	-	9
White cooked	10	10	Cluster coccus:	+	-	1
			Bacillus	**+**	**-**	**3**
			Bacillus	-	-	3
			Diplococcus	+	-	3
Red cooked	14	9	**Bacillus**	+	-	**3**
			Bacillus	-	-	1
			Diplococcus	+	-	1
			Candida	+	-	1
			Strepto	-	-	2
			Cocco bacillus	-	-	1
Total	57	44				

Table 4. Colonies observed on MRS- L-Cysteine 0.25% media plates

Isolation source	Observed	Identified	Morpho types	Gram test	Catalase test	No
White raw	14	14	Cluster coccus	+	-	4
			Diplococcus	+	-	2
			Bacillus	+	-	8
Red Raw	16	11	Cluster coccus	+	-	6
			Bacillus	+	-	4
			Diplococcus	+	-	1
White cooked	9	6	Bacillus	+	-	6
Red cooked	12	4	Cluster coccus	+	-	1
			Diplococcus	+	-	3
Total	51	35				

Table 5. Number of gram-positive, catalase negative bacilli isolated from fermented rice

Medium	White raw	Red raw	White cooked	Red cooked
MRS	1	0	2	0
MRS-sorbitol 0.2%	3	0	3	3
MRS-L cysteine 0.25%	8	4	6	0
Total	12	4	11	3

Colony characteristics of isolates

Colony characteristics of isolates were studied by picking up the typical, well isolated and representative colony that appeared on the plate and streaked plates and the most prominent colony characters of the gram-positive and catalase negative bacteria were described (Table 6).

Table 6. Colony morphology of bacterial isolates

Colony Character	Isolates code							Coccus	
	Lb-1	Lb-2	Lb-4	Lb-8	Lb-10	Lb-11	Lb-17	Cluster	Diplo
Form	C	C	C	C	C	C	C	C/IR	C
Size (dia-mm)	0.5-2	0.5-2	0.5-2	2-4	0.5-2	0.5-1	0.5-1	1-5	<0.5
Elevation	R	R	R	F	R	R	R	R/F	R
Margin	E	E	E	E	E	E	E	E	E
Surface	SM	SM	SM	SM	SM	SM	SM	SM	SM
Opacity	O	O	O	T	O	O	O	O	O
Color	DW	DW	DW	GW	DW	DW	DW	MW/DW	DW
Visibility	D	D	D	D	D	D	D	D	D
Formation	S	S	S	S	S	S	S	S	S

C-Circular, IR-Iregular,R-Raised.F-Flat.E-entire,SM-smooth,O-Opaque,T-Translucent,Dw-Dull White,MW-Milky White, GW-Grey White, D-Descriptive ,S-Single.

Colonies of most of the gram-positive and catalase negative grapes cluster like cocci, diplococci and rods appeared in circular form, raised, opaque, with entire margin and smooth surface. Cluster cocci appeared as medium or large milky white colonies while the diplococcus and bacillus formed dull white punctiform (<1mm diameter) or medium size colonies (Table 6). Different colony morphological characters were observed for the morphologically identical microorganism. Grape cluster like coccus showed the circular or irregular form colonies. Identical colony morphological characters were observed for morphologically distinguished microorganism. Diplococci and bacillus appeared as punctiform (<1mm) dull white colour colonies.

In this study, all the isolates (Lb-1, 2, 4, 8, 10, 11 and 17) were descriptive single isolated colonies, circular with smooth surface and entire margin. Colonies of Isolate-11 and 17 were 0.5-1 mm diameter in size. Isolate - 1, 2,4,10 form 0.5-2 mm size colonies and colonies from Isolate 8 are larger (2-4mm diameter) than other. Isolate-8 shows morphologically distinct colony surface characters (flat, translucent and grey white in colour) and other isolate-1, 2, 4, 10, 11 and 17 show nearly morphologically identical raised and opaque colony surface characters with dull white.

Different species or the same species of lactic acid bacteria can show identical or distinct morphological colony characters. The isolated colonies of pediococcus from fermented milk product were generally grayish white in color, round, smooth, entire and colonies of genus *Lactobacillus* generally appeared white to yellowish in color, round, spindle, triangular, star-like structure (Karna and Barraquio, 2007). In another study, the colony characters of isolated *Lactobacillus* sp from the milk of domestic animals appeared to have smooth, round, concave and translucent anaerobic organisms (Priya *et al.,*2011). Colonies of *Bifidobacterium* sp. appears as creamy white glistening with soft consistency, raised and convex elevation, round, triangular and spindle shaped (Holt *et al*., 1994).In the current study, though, all isolated gram-positive and catalase negative microorganism have the lactic acid bacterial colony characteristics, further biochemical or molecular confirmation are needed to identify those colonies.

Morphological characteristics of isolates

The cell morphology of all isolates was evaluated by microscopic observations using the immersion objective (100) after Gram staining. The morphological characteristics of isolates are presented in Table 7. Four morphologically distinct types of *Lactobacillus* sp. Are in the current study. The isolates Lb- 1, 2, 10 and 11 were medium size regular rods (Figure1a) and the isolates Lb- 4, 17 and 8 appeared as very long (Figure 1b), long regular rod (Figure 2d) and short round ended (Figure 2c) rods, respectively. The bacterial cell of all the isolates appeared as single or in the V shaped arrangement as pairs or as a V shaped short chain.

Probiotic *Lactobacillus* strains isolated from milk and milk product has been described as translucent anaerobic organisms and observed in the culture plate in the form of long to very short rods often in chains (Priya *et al.,* 2010). *Lactobacillus sp.* have rod shaped cells, usually regular but sometimes coccoid, commonly in short chains while the genus *Pediococcu s*p. appeared as spherical cells in pairs or tetrads, *Bifidobacterium* sp. are gram-positive rods, branched, singly arranged and pairs in V and Y arrangements, in chains (Holt *et al*., 1994). Isolated all cocci shaped bacteria were spherical and arranged in grape like clusters or pairs. Among the cluster like coccus, majority of cells were arranged in cluster while few of them showed the tetrads or diploid nature along with cluster appearance. These were also included in grape like cluster coccus. Generally, *Pediococcus* sp appear in the tetrad arrangement while *Leconostoc* sp. arrange in pairs or chains, *Streptococcus sp* arrange in chains and *Aercococcus* sp. *or Peptococcus* arrange as clusters similar to coccus (Barrow and Felthman, 1993). The morphological arrangement of isolated coccus more or less resemble the small cluster appearance of *Aercococcus* sp. or *Peptococcus* sp. Some of the cluster like coccus show the tetrad or diploid nature which may be *Pediococcus* sp. or *Leconostoc* sp. The seven isolates morphologically resembled the genus Lactobacillus as described by Holt *et al*. (1994) and all isolates were identified up to species level using API Kits.

Table 7. Cell morphology of the isolates

Isolate	Isolated plate	Morphological characters
Lb-1	SW1100	Medium regular rod, occur single or V arrangement pairs or chain
Lb-2	SW1C100	Medium regular rod, occur single or V arrangement pairs or chain
Lb-4	SW210-2	Very long or medium, regular rod occur single or V arrangement pairs or chain
Lb-8	LW2C10-2	short rod regular rounded end, occur single or V arrangement pairs or chain
Lb-10	LW2100	Medium, regular rod occur single or V arrangement pairs or chain
Lb-11	SW2100	Medium ,regular, rod occur single or V arrangement pairs or chain

Lb-17	SW210-2	Long or medium, occur single or V arrangement pairs or chain

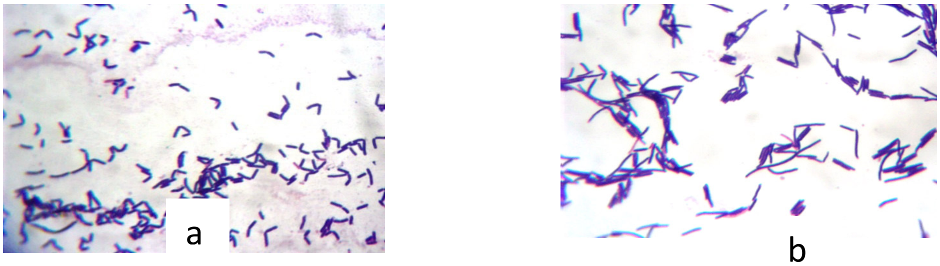

Fig. 1. Medium rod (a) and very long rod of the isolated rod shaped bacteria

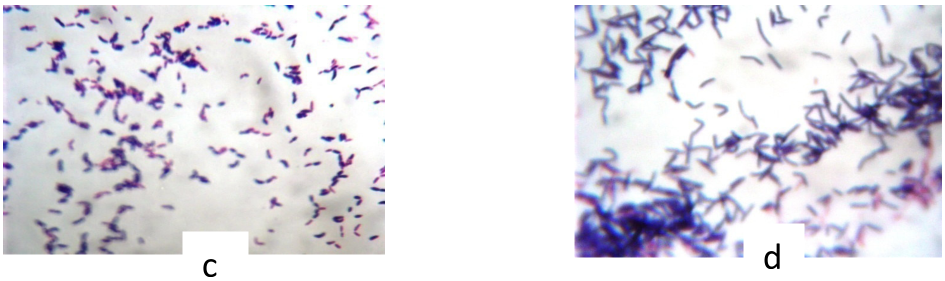

Fig. 2. Short rod (c) and long rod (d) of the isolated rod shaped bacteria

Identification of *Lactobacillus* spp.

Members of the genus *Lactobacillus* can be selected on solid culture media that have an acidic pH (MRS). Many identified *Lactobacillus* strains proliferate best under anaerobic conditions. *Lactobacilli* spp. are gram-positive, catalase negative, non-spore-forming and non motile rods (ranging from coccobacilli to long, slender bacilli) (Barrow and Felthman, 1993; Hoque *et al.,* 2010). Among gram-positive and catalase negative bacterial isolates (30), about 12 isolates survived after 3-4 subsequent purification process. The survived gram-positive and catalase negative rod shaped bacteria (n=12) were selected for motility and endospore test. Seven rods were identified as *Lactobacillus* sp. as they were gram-positive, catalase negative, non motile and non endospore forming. According to classical and biochemical tests (using API 50 CHl, BioMérieux, France) Isolated Lactobacillus were identified up to species level (Table 8).

Table 8. Identification of the related species of the *Lactobacilli* using API 50 kits

Isolate code	Species of Lactobacillus	Identity
Lb-1	*Lactobacillus curvatus ssp.curvatus*	98.9%
Lb-2	*Lactobacillus curvatus ssp.curvatus*	99.3%
Lb-4	*Lactobacillus helveticus*	86.3 %
Lb-8	*Lactobacillus delbrueckii ssp. delbrueckii*	95.5%
Lb-10	*Lactobacillus pentosus*	63.3%

Lb-11	*Lactobacillus curvatus ssp.curvatus*	99.3%
Lb-17	*Lactobacillus plantarum*	91.3%

Lactobacillus plantarum KUS and *Pediococcus pentosaceus* KUR isolated from rice milk and mixed culture of *L. plantarum* KUS and *P. pentosaceus* KUR were reported as the best starter of rice milk yoghurt (Boongird and Leelawatcharamas, 2006). Twenty five strains of lactic acid bacteria (LAB) isolated from South Indian traditional fermented foods 'Kallappam' batter, 'Koozh' and 'MorKuzhambu' and further 6 strains including *Lactobacillus plantarum* and *Lactobacillus fermentum. Lactobacillus plantarum* AS1 possesses antimicrobial activity (Kumar *et al.,* 2010) in the probiotic characterization. Therefore, Identification of these isolated species of *Lactobacillus* by molecular identification methods and probiotic characterization of them will further confirm the application as starter culture in the food industry.

CONCLUSIONS

Among 122 microorganism studied, the grape cluster like coccus (48), diplococcus (30) and bacillus (30) were observed as gram-positive and catalase negative. Among the isolated bacilli, seven were identified as *Lactobacillus* spp using API kits and showed that potentially probiotic *Lactobacillus* spp were present in fermented rice. Molecular identification and further probiotic characterization are required for confirmation.

REFERENCES

Anderson, J.W., Stanley, E. and Gilliland, S.E. (1999). Effect of Fermented Milk (Yogurt) Containing *Lactobacillus Acidophilus* L1 on Serum Cholesterol in hypercholesterolemic Humans. Journal of the American College of Nutrition*, 18,* 43 - 50.

Barrow, H.L. and Felthman, R.K.A., (1993). Cowman and Steels manual for the identification of medical bacteria. 3 rd ed. Cambridge, UK.

Bekkali, N.L.H., Bongers, M.E.J., Van den Berg, M.M., Liem, O. and Benninga, M.A. (2007). The role of a probiotics mixture in the treatment of childhood constipation: a pilot study. Nutrition Journal, *6*, 17. Available at http://www.nutritionj.com/content/6/1/17. doi:10.1186/1475-2891-6-17.

Bisen, P. S. and Verma, K. (1998). Hand Book of Microbiology.1st ed. CBS Publisher, New Delhi, India.

Boongird, J. and Leelawatcharamas, V. (2006). Screening and identification of lactic acid bacteria from rice milk for yoghurt production. The Proceedings of the 44th Kasetsart University Annual Conference*, 44,* 480 - 488.

Cummins, J. and Wan Ho, M. (2006). GM Probiotic Bacteria in Gene Therapy. ISIS (Institute of science and society) Report 03/07/06.

De Man, J.C., Rogosa, M. and Sharpe, M.E. (1960). A medium for the cultivation of lactobacilli. Journal of Applied Bacteriology, *23,* 130 - 135.

FAO-WHO, (2001). FAO and WHO Joint Expert Committee Report on Evaluation of Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO).

Fuller, R. (1989). Probiotics in man and animals. Journal *of Applied Bacteriology 66,* 365 - 378.

Haddadin, M.S.Y., Awaisheh, S.S. and Robinson, R.K. (2004). The Production of Yoghurt with Probiotic Bacteria Isolated from Infants in Jordan. Pakistan Journal of Nutrition*, 3,* 290 - 293.

Holt, J.G., Krieg, N.R., Sneath, P.H.A., Staley, J.T. and Williams. S.T. (1994). *Bergey's manual of determinative bacteriology*. 9th ed. Baltimore, Williams and Wilkins, USA.

Hoque, M.Z., Akter, F., Hossain, K.M., Rahman, M.S.M., Billah, M.M. and Islam, K.M.D. (2010). Isolation, Identification and Analysis of Probiotic Properties of *Lactobacillus Spp.* From Selective Regional Yoghurts. World Journal of Dairy and Food Sciences, *5,* 39 - 46.

Jagadeeswari1, S., Vidya, P., MukeshKumar, D.J. and Balakumaran, M.D. (2010). Isolation and Characterization of Bacteriocin producing *Lactobacillus* sp from Traditional Fermented Foods. Journal of Environmental Agriculture and Food*, 9,* 575 - 581.

Janet, E.L.C., Gordon, D.W.C. and Rosamund, M.B. (2012). *Handbook of Culture Media for Food and Water Microbiology*. Burlington House, Piccadilly, London, UK.

Karna, O.C.E. and Barraquio, V.L. (2007). Lactic Acid and Probiotic Bacteria from Fermented and Probiotic Dairy Products. B.K.L. Science Diliman*, 19*, 23 - 34.

Kumar, R.S., Ragu Varman,R., Kanmani, P., N. Yuvaraj, N., Paari, K. A., Pattukumar, V. and Arul V. (2010). Isolation, Characterization and Identification of a Potential Probiotics from South Indian Fermented Foods (*Kallappam*, *Koozh* and *MorKuzhambu*) and its use as Biopreservative. Probiotics and Antimicrobial Proteins, *2*, 145 - 151.

Lamsal, B.P. and Faubion, J.M. (2009). The Beneficial Use of Cereal and Cereal Components in Probiotic Foods. Food Reviews International*, 25,*103 - 114.

Lei, V. (2006). Probiotic potential of African fermented millet. Department of Food Science, University of Copenhagen.

Nicole, M.D. and Martijn, B.K. (2000). Effects of probiotic bacteria on diarrhea, lipid metabolism, and carcinogenesis: a review of papers published between 1988 and 1998. African Journal of clinical Nutrition*, 71,* 405 - 11.

Priya, R., Bharathi, B., Jayanthi. A. and Vidhyalakshmi, A. (2011). Studies on Isolation, Characterization and Entrapment of Probiotic *Lactobacillus* from Milk and Milk Products. Advanced Biotechology, *10*, 30 - 33.

Salovaara, H. and Simson, L. (2004). Fermented Cereal Based Functional Foods. *Hand Book of Food and Beverage Fermentation Technology.* CRC press. Marcel Dekker, New York.

Sandholm, T.M., Myllärinen, P., Crittenden, R., Mogensen, G., Fondén, R. and Saarela, M. (2002).Technological challenges for future probiotic foods. International Dairy Journal*, 12*, 173 - 182.

Silvia, S. G.T. and Medana. Z. (2011). Isolation and characterization of lactic acid bacteria from Romanian fermented vegetables. Romanian Biotechnological Letter*, 16,* 148 - 154.

Srikanjana, K., Helmut, V., Jakkapan, S. and Siripon, O. (2008). Probiotic Properties of lactobacilli Isolated from Thai Traditional Food. Scientia Pharmaceutica, *76,* 485 - 503.

Tharmaraj, N. and Shah, N.P. (2003). Selective enumeration of *Lactobacillus delbrueckii* spp. *bulgaricus, Streptococcus thermophilus, Lactobacillus acidophilus,* bifidobacteria*, Lactobacillus casei, Lactobacillus rhamnosus,* and propionibacteria. Dairy Science, *86,* 2288 - 96.

Wickramanayake, T.W. (2002). *Food and Nutrition*. reprinted HARTI, Sri Lanka.

Wold, A. E. (2001). Immune effects of probiotics. Food and Nutrition Research*, 145*, 76 - 75.

Yong-jin, H., Ren-jun,Z. and Yue ,W. (2010). Processing Technology of a Fermented Rice-Milk Beverage. Chinese Journal of Modern Food Science and Technology*, 4.* http://en.cnki.com.cn/Article_en/CJFDTotal-GZSP201004017.htm.