Diversity and antibiotic resistance patterns of *Lactobacillus* species in traditional curd in Sri Lanka

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Introduction

Lactobacillus is a genus which comprises of more than 50 species as described in the Bergey's Manual of Systematic Bacteriology (2009) *Lactobacilli* are Gram-positive, non-spore-forming rods or coccobacilli. They are generally considered as apathogenic, fastidious bacteria and one of their main habitats is fermented dairy products.

In their fermentative pathways, main metabolic end product is Lactic acid. The fermentation process of *Lactobacillus* increases the shelf-life of the fermented dairy product, as well as adds the taste and improves the digestibility of milk. There are adequate evidences to prove that traditionally fermented milk products have been produced for a long time in different countries. Curd (Sri Lanka and India), Kefer (Russia), Yakult, Yogurt, Kumisss (Russia), Tarhana (Turkey) are some examples.

There are *Lactobacillus* species that have achieved GRAS (Generally Recognized as Safe) or QPS (Qualified Presumption of Safety) status. However, during the past few decades there has been an emerging concern on spreading the antibiotic resistance in the environment. Due to the increasing use of antibiotics and disposing them in to the environment in a considerably inappropriate manner, concerns have arisen on the possibility and probability of spreading the antibiotic resistance genes to *Lactobacillus* in fermented food (Farthing, 2004). The main threat associated with these bacteria is that they can transfer resistance genes to pathogenic bacteria as well as to the commensal flora in the intestinal tract. A number of initiatives have been recently launched across the globe to address the biosafety concerns of starter cultures and probiotic microorganisms. The European Food safety Authority (2007) considers transferable antibiotic resistances as a safety concern. Many studies have detected the acquired antibiotic resistance in fermented food products. Nevertheless, the tetracycline resistance was shown to be able to transferred *in vitro* to *Enterococcous faecalis* from *Lactobacillus* species isolated from fermented sausages (Gevers *et al.*, 2003).

The objective of this study was to find out the diversity of *Lactobacillus* species in curd prepared by small and medium scale producers in Sri Lanka and to detect their antibiotic resistances.

Methodology

Total number of 32 curd samples representing different areas of the country were collected from small and medium scale produces (SMS) who do not use starter cultures directly for the process. Samples were enriched in modified Man Rogosa Sharpe broth at 37 C at room^{0} temperature for 24 h, under anaerobic conditions and plated on modified MRS agar medium using agar overlay technique and incubated at 37 ^{0}C, for 24-36 h under anaerobic conditions. *Lactobacillus delbrueckii subsp. bulgaricus* was used as the positive control. Morphologically different colonies were isolated. Identification of the different strains were carried out using morphological and biochemical tests according to the Bergey's Manual of Systematic Bacteriology. For the detection of antibiotic resistances, agar overlay disc diffusion method (Charteris *et al.*, 1998) was followed. The bacterial cultures used for this test were grown overnight in modified MRS broth and the densities were adjusted to OD $_{590nm}$ = 0.1 using spectrophotometer in order to obtain consistent growth. Penicillin G(P10),Tetracycline (TE30), Erythromycin (E15), Bacitracin (B10), Rifampicin (RD30) Nalidixic Acid (NA30), Vancomycin (VA30) and Polymixine B (PB300) were selected as the test antibiotics.

Results

For morphological characteristics, shape and colour of the colonies, Gram's reaction, shape and arrangement of cells, motility and endospore formation were observed. For the biochemical characteristics, reaction in the Gibson's medium, oxidase test, catalase test, Arginine utilization, sugar fermentation tests (Arabinose, Cellobioose , Esculin, Galactose, Maltose, Mannose, Melibiose, Raffinose, Ribose, Sucrose, Trehalose, Xylose, Salicin, Sorbitol, Mannitol, Rhamnose, Lactose) were detected.

A total Number of 51 *Lactobacillus* isolates were obtained in this study and all the 51 isolates were comprised with 16 different bacterial species. *Lactobacillus fermentum* (35%) *Lactobacillus acidophilus* (11.11%), *Lactobacillus plantarum* (5.55%) and *Lactobacillus amylolyticus* (8.33%) represented majority. When compare the antibiotic resistance patterns (Table 01), Polymixine B was reported to have 94.44% overall resistance. All the 51 isolates were reported to score 100% of overall resistance for both Vancomycin and Nalidixic Acid. Minimum percentages of antibiotic resistances were observed for Tetracycline (22.22%) and Erythromycin (27.78%).

Discussion

Considering the prevalence of different *Lactobacillus* species, the results obtained in this study correlate to a significant extent to some research carried out on traditional fermented dairy products (Yu, 2011). Conversely, several research studies have been *disclosed that L. delbrueckii subsp. bulgaricus is the most abundant Lactobacilli in* traditionally fermented milk products (Tendakyi *et al.*, 2001) and this species was not identified in this study.

Though the present study reveals less abundance of Tetracycline and Erythromycin resistance compared to that of most other countries it can be considered as an important finding. Correlate with the Tet- resistant species identified in this study, R-plasmids encoding Tet-resistance have been reported in *L. fermentum L. planatarum* and *L. amylolyticus.* Because the Tet-resistance has been transferred from *Lactobacillus* to *E. faecalis* in vitro it shows the possibility of in vivo process. Further studies can be aimed at determining the transferability of the Tet-resistance from *Lactobacillus* to commensal flora *in vitro*.

Table 01: Antibiotic resistance patterns of some selected *Lactobacillus* isolates.

Isolate No	Species	Tet	Ery	Bac	Rif	Pen	Poly	Nali	Van
L43	*L. planatarum*	R	S	S	R	R	R	R	R
L3	*L. planatarum*	S	S	R	MS	R	R	R	R
K1	*L. amylolyticus*	R	R	R	R	R	R	R	R
K2	*L. amylolyticus*	R	R	R	R	R	R	R	R
L25	*L. fermentum*	R	R	R	R	R	R	R	R
M2	*L. fermentum*	R	R	R	R	R	R	R	R
L11	*L. fermentum*	R	R	S	R	R	R	R	R
L8	*L.frintoshensis*	R	R	R	R	R	R	R	R
L29	*L. gastricus*	S	R	R	R	R	R	R	R
Tcb	*L. acidophilus*	S	S	R	R	R	R	R	R

S; Sensitive, R; Resistant, MS; Moderatly sensitive
Tet; Tetracycline, Ery; Erythromycin, Bac; Bacitracin, Rif; Rifampicin,
Pen; Penicillin G, Poly; Polymixine B, Nal; Nalidixic acid, Van; Vancomycin

Conclusions

16 different Lactobacillus species were isolated. *Lactobacillus fermentum* was the most abundant organism. Minimum percentages of antibiotic resistances were observed for Tetracycline and Erythromycin while maximum percentages of resistances were observed for Nalidixic Acid and Vancomycin. The relatively high percentage of vancomycin resistance (85%) is due to the fact that the majority of the lactobacilli are intrinsically resistant to this glycopeptide.

References

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