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CHARACTERISATION OF INDIGENOUS LACTIC ACID BACTERIA AND SURVIVAL STUDIES IN RESPONSE TO ACID AND BILE STRESS

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ABSTRACT

The claimed health benefits of probiotic bacteria have led to the active investigation of a large variety of sources for obtaining these "healthy bacteria". Several studies have now proven that many lactic acid bacteria exhibit probiotic potential. Tolerance to acid and bile conditions is considered a very important requirement to be a probiotic organism. In this study, putative lactobacilli isolated from dairy viz. curd and raw milk was subjected to acid (pH 2.5 and 3.5) and bile stress (0.3% and 1.5% bile). While there was no significant difference in resistance to low pH between isolates from milk and curd, there was a significant difference in their tolerance to bile. Overall, raw milk could be suggested as a better source, of potentially probiotic microorganisms (in terms of acid and bile tolerance), than curd.

Key words: Lactobacilli, Probiotics, Acid tolerance, Bile tolerance.

INTRODUCTION

Lactic acid bacteria belong to a group of microorganisms that are related by common physiological traits and metabolic functionality. They are Gram-positive, mostly nonspore forming rods or cocci, generally nonmotile, catalase negative and obligate fermenters. They convert glucose either almost quantitatively to lactic acid or may produce an equimolar mixture of lactic acid, ethanol or carbon dioxide. Microbes belonging to lactic acid bacteria include *Streptococcus*, *Lactococcus*, *Leuconostoc*, *Lactobacillus* and *Pediococcus*.

Lactic acid bacteria have been used for centuries in fermented and non-fermented foods mainly because of their ability to transform foods in terms of better taste and nutritive properties. Many of these microbes have been found to inhibit other microorganisms that cause food spoilage or even food borne illnesses (Mercenieret al.,2002; Senok et al., 2005; Gregor et al., 2003; Erika 2001). Hence, these microbes are now widely being used as what are known as probiotics. Probiotics seem to exert their beneficial effect on the host by influencing the competition and/or metabolic activity of the flora of the GI tract.The FAO/WHO report defines probiotics as live microorganisms which when administered in adequate amounts, confer a health benefit on the host (FAO/WHO 2001).Therefore, there is a growing need for obtaining these healthy bacteria for use in nutraceuticals and/or pharmaceuticals.

Lactic acid bacteria used as probiotics have been isolated from diverse sources including sourdough bread, vegetables, dairy products, meat products, animal/human digestive tract and even faeces (Patil *et al.,* 2010; Sieladie *et al.,* 2011; Sirilun *et al.,* 2010; Ahrne *et al.,* 1998; Haddadin *et al.,* 2004).Lactic acid bacteria from various sources show variations in their probiotic characteristics (Mercenier *et al.,* 2002). Milk and dairy products are ready sources of lactic acid bacteria. Curd or 'dahi' is a traditional dairy fermented product used throughout the various states of India. Being easily available and popularly consumed, milk and curds are both good candidates for source of lactic acid bacteria and may be a delivery vehicle for those lactic acid bacteria that have probiotic potential. Among the many characteristics that qualify a given microbial species/strain as probiotic is the ability to tolerate low pH levels prevalent in the stomach and presence of bile in the intestine (FAO/WHO 2001; ICMR-DBT 2011).

MATERIALS AND METHODS

Fresh curd and milk samples were obtained from different dairy farms in the city. These were streaked directly onto deMann Rogosa Sharpe (MRS, Hi Media) plates and incubated at 37°C for 24-48 hours. Well isolated colonies were further checked for purity following few more isolations and gram staining. Colonies that represented putative lactobacilli according to Bergey's manual of systematic bacteriology (2009) were chosen for the different tests. Six isolates were obtained from milk and four from curd, which could be maintained on repeated sub-culturing on MRS media.

PRELIMINARY TESTS

Catalase test was performed using H_2O_2, motility was tested using 0.4% agar in MRS broth and gelatin liquefaction test was performed using 12% gelatin in MRS broth.The isolates were also further categorized for their type of fermentation pathway using Gibson's semisolid media (Collins *et al.*, 2004; Harrigan, 2013).

RESISTANCE TO GASTRIC ACIDITY

Overnight grown cultures were inoculated (10^9cfu/ml) into MRS broth of pH 2.5 & 3.5 (adjusted using 1N HCl) to simulate acidic conditions of gut. Viable counts of the lactobacilli, present initially and after exposure for 2.5 hours, were carried out. Serial dilutions (10-fold) were done using saline and the cultures were plated onto MRS agar. The plates were incubated at 37°C for 24-48 hours and numbers of live organisms expressed as cfu/ml.

RESISTANCE TO BILE

The isolates showing good tolerance to acidic conditions were tested for their bile tolerance. Overnight grown cultures were inoculated (10^9cfu/ml) into MRS broth containing 0.3% and 1.5% respectively of Oxgall (HiMedia). After two hours of exposure, viable counts were determined as in the earlier method and numbers of live organisms expressed in cfu/ml.

RESULTS

All 10 isolates obtained were gram positive non-sporing, nonmotile rods and negative for catalase activity as well as gelatin liquefaction. Their fermentation pattern is presented in table 1. The response of the isolates to low pH and bile acid is shown in tables 2and3 respectively. A comparison of the survival rates between isolates from different sources viz. curd and milk are represented in figures 1 and 2. An overall survival response of the isolates belonging to different sources to acid and bile is represented in figure 3.

Table 1: Fermentation pattern of isolates from curd (Lc1-Lc4) and milk (Lm1-Lm6)

Isolate	Lc1	Lc2	Lc3	Lc4	Lm1	Lm2	Lm3	Lm4	Lm5	Lm6
Fermentation	2	1	1	1	1	1	1	2	1	1

1-Homofermentation; 2-Heterofermentation

Table 2: Effect of exposure to low pH on viability of the isolates

Isolate	Viability (cfu/ml)		
	Initial	pH 3.5	pH 2.5
Lc1	9.46	7.86	6.76
Lc2	9.72	7.86	6.76
Lc3	9.25	6.74	6.02
Lc4	9.1	6.64	6.69
Lm1	8.74	6.9	4.55
Lm2	9.51	7.16	6.52
Lm3	9.16	6.97	6.17
Lm4	9.2	6.87	6.58
Lm5	9.21	7.07	6.24
Lm6	9.18	7.07	5.54

Table 3: Effect of exposure to bile on viability of the isolates

Isolate	Viability (cfu/ml)		
	Initial	0.3% Bile	1.5% Bile
Lc1	10.67	4.85	3.41
Lc2	9.87	4.56	2
Lc3	9.18	4.2	4.17
Lc4	7.62	4.7	0
Lm1	9.75	4	3.08
Lm2	9.64	5.2	5.04
Lm3	8.36	6.54	5.24
Lm4	7.99	5.16	5.17
Lm5	9.66	6.18	4.56
Lm6	10.51	6.29	4.81

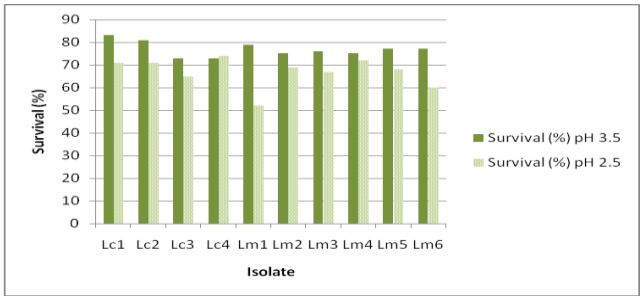

Figure 1: Comparative survival response of the isolates to low pH

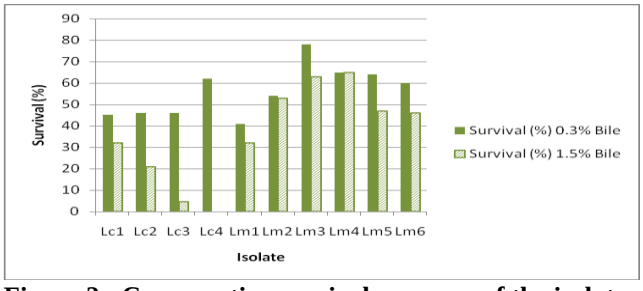

Figure 2: Comparative survival response of the isolates to bile

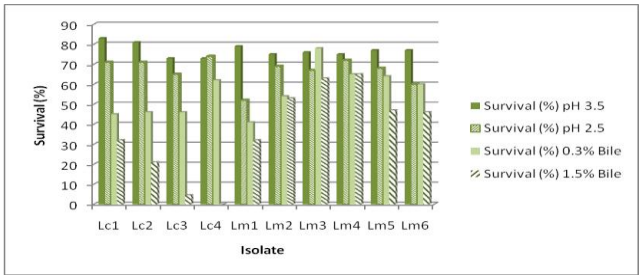

Figure 3: Overall survival response of the isolates to low pH and bile

DISCUSSION

The use of microorganisms such as lactic acid bacteria as probiotics has led researchers to explore various sources for their isolation. Among the lactic acid bacteria, lactobacilli have been now found to be very useful as a probiotic (Sieladie et al., 2011; Sirilun et al., 2010). Dairy is an easy to obtain and naturally considered GRAS source. In this paper, we would like to present the fact that dairy needs to be actively explored as a likely indigenous source of probiotic bacteria. Preliminary characterisation tests suggest the isolated organisms to be lactobacilli.

Two of the main characteristics required to be a probiotic microorganism, is tolerance to gastric acid and bile during gastrointestinal transit. The pH found in the stomach ranges from 1.4-2.1 (fasting) to 3.0-7.0 (during feeding), (Paulo 2014) while the bile acid concentration in the intestine is approximately 0.3% (Hill, 2002; Hood et al., 1988). Although the isolates from curd showed a greater tolerance to acid at both pH 3.5 and 2.5 as compared to the isolates from milk, there was no significant difference between their overall tolerance to acid ($P>0.05$). On the other hand, the isolates from milk exhibited a significant difference in their bile tolerance as compared to the isolates from curd ($P<0.05$).

CONCLUSIONS

While sources for probiotic bacteria are as varied as meat, sourdough, vegetables, livestock, the use of dairy as an indigenous source is strongly suggested by the authors. This is mainly because dairy is an easily available and also highly probable source for obtaining probiotic organisms. However, since all the isolates did not have a similar response to acid and bile stress, it shows that not all dairy inevitably contains organisms with high tolerance to acid and bile. Therefore, obtaining potential candidates for use in nutraceuticals or pharmaceuticals, would involve an extensive screening of these sources. Of this, raw milk seems to be a better source compared to curd when testing for key factors such as resistance to acid and bile environments.

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