

## Effect of potential Prebiotics on the survival of Probiotic *Lactobacillus acidophilus*

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**ABSTRACT.** Four locally available sources of Arrowroot (*Maranta arundinacea* L.), Hingurala (*Dioscorea alata*) Innala (*Plectranthus rotundifolius*), rice bran and commercially available raftiline<sup>®</sup> were tested for their prebiotic quality in obtaining increased biomass of probiotic *Lactobacillus acidophilus*. These sources were incorporated at two levels (1 and 3%) into sterile skim milk containing 12% (w/v) solids at two stages of the incubation process, the two stages being at the time of inoculation and 1 h after incubation. The inoculum used was one g of prepared culture containing  $10^9$  -  $10^{10}$  colony forming units /g of *Lactobacillus acidophilus*. All the samples were incubated at 37 °C anaerobically for 12 h followed by refrigeration at 4 °C. Plate counts and pH of each sample were measured throughout the storage period from 0, 3, 7, 14, 21, and 28 days. All the sources showed significant increase in the colony forming units compared to the control while arrow root and hingurala showed best results with 7.71 and 7.55  $\log_{10}$  /g respectively at the end of 28 days of storage. All the sources showed significant counts when added one hour after incubation. The control showed highest acidity at the end of storage (pH 4.11) while Innala showed the lowest (pH 4.32). The survival of *Lactobacillus acidophilus* was generally enhanced by all the potential prebiotics while maintaining the standards required for a probiotic.

### INTRODUCTION

Lifestyles and food habits of individuals contribute towards their overall health status. It is believed that the modern fast moving world with increased stress and foods of poor nutritional quality leads to growing incidences of intestinal infections and disorders, cancer and atherosclerosis to name a few. Increased awareness and consciousness of healthy and nutritive food among the consumers have created a huge market potential for superior nutritive qualities.

Probiotics are described as a live microbial feed supplement which beneficially affects the host by improving its intestinal microbial balance (Fuller, 1995). Species of genera *Lactobacillus*, *Streptococcus* and *Bifidobacterium* are recognized as probiotics on account of their various therapeutic health benefits (Talwalker *et al.*, 2003). The human colon is one of the body's most metabolically active organs where intestinal bacteria predominantly ferment undigested food

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material (Desai *et al.*, 2004). Intestinal microflora play a significant role in effective utilization of food consumed (Siddeshwar *et al.*, 2008) and being responsible for the resistance to intestinal

infections and disorders thus ensuring good health status of the host. The composition of intestinal microflora can change due to antibiotic therapy, foods of poor nutritional quality and various other reasons making the host susceptible to the said infections (Fuller, 1995). This highlights the importance of probiotics whereby via oral administration the intestinal microbial balance can be maintained.

Probiotics are found to enhance the population of beneficial bacteria in the human intestine, through suppressing pathogens, building up resistance against intestinal diseases, in treatment of rota viral diarrhea, lactose maldigestion, colonic diseases and reduction of serum cholesterol levels (Talwalker *et al.*, 2004). Probiotics have also shown to suppress the formation of carcinogens in relation to colonic and breast cancer (LeBlanc *et al.*, 2007).

Incorporation of probiotics into diets can easily be accomplished through fermented foods or in the form of powders and tablets. In order to ensure the efficacy of probiotic foods, standards require a minimum of  $10^7$  colony forming units (CFU) /mL of *L. acidophilus* and  $10^6$  CFU/mL of *Bifidobacteria* in fermented milk at the time of sale (Talwalker *et al.*, 2003).

Several studies have shown that the survival of probiotic bacteria in the market samples is very low (Shah, 2000). The use of probiotics, non-digestible ingredients that beneficially affects the host by selective stimulation of the growth or activity of one or a limited number of bacteria in the colon, along with probiotics has shown synergistic effect in improving the survival rates of probiotics (Gibson *et al.*, 1995). The oligosaccharides that are substantially available in prebiotics have the ability to stimulate the growth of probiotics. Cereals, yams, fruits and vegetables are considered to contain high levels of oligosaccharides while a recent research has revealed carrot, tomato, yams, garlic and banana to be good sources of prebiotics (Siddeshwar *et al.*, 2008). Another study showed that commercially available prebiotics could improve the survival rates of Lactobacilli in skim milk (Desai *et al.*, 2004). It is quite possible that a wide range of locally available sources can also be used as prebiotics in order to stimulate the growth of probiotics. Therefore the objective of this study was to investigate the effect of locally available sources expected to have prebiotic quality on the survival of the probiotic *Lactobacillus acidophilus*.

## MATERIALS AND METHODS

### Preparation of culture

Freeze dried culture of *L. acidophilus* was obtained from New Zealand Dairies® and inoculated into sterile skim milk of 12% (w/v) solids to obtain  $10^9$  -  $10^{10}$  CFU/g.

### Preparation of prebiotics

Arrowroot (*Maranta arundinacea L.*) and *hingurala* (*Dioscorea alata*) were obtained from small scale growers in the village Divulapitiya while *Innala* (*Plectranthus rotundifolius*) was obtained from sellers at the market of Minuwangoda. Rice bran from raw red rice was obtained from rice millers at Marandagahamula. Raftiline® (Orafti- Belgium) was used as a commercially available prebiotic. All the yam varieties were cleaned, washed and peeled followed by oven drying at 65 °C overnight and ground into fine powders.

### Sample preparation and growth of Lactobacilli

One gram of prepared culture containing  $10^9$  -  $10^{10}$  CFU /g of *L. acidophilus* was inoculated into sterile skim milk containing 12% (w/v) solids. The sources prepared as explained above were added at 1% and 3% (w/v) levels at two different stages of the incubation process. In One set of skim milk, sources were incorporated at the time of inoculation followed by incubation while in the other set, skim milk was allowed to incubate for a period of one hour after which the sources were incorporated and continued incubation. A control was maintained. All the samples were incubated at 37 °C for 12 hours and transferred into refrigerated storage at 4 °C.

### **Determination of colony counts and pH**

Aliquots (1ml) of each fermented milk was subjected to a series of dilutions and plated in MRS agar followed by incubation under anaerobic conditions at 37 °C for three days (Oxoid UK 2001). The colony forming units were obtained using a colony counter and the pH was measured using a digital pH meter . Testing was done throughout the storage period on day 0, 3, 7, 14, 21 and 28.

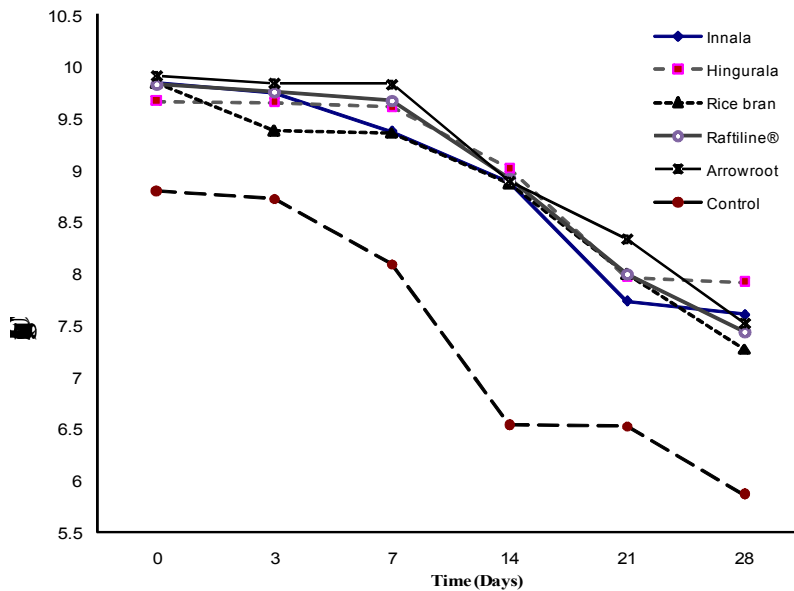
### **Statistical analysis**

Statistical analysis carried out in triplicate in a completely randomized design (CRD) and analyzed using SAS software package, version 8, 1999.

## **RESULTS AND DISCUSSION**

The counts for *L. acidophilus* grown in all the sources maintained higher values up to seven days of storage ranging from 9.91 - 9.36 CFU/g (Figure 1), however started declining drastically afterwards. This is a common fact observed in lactic acid fermentation where destruction of colonies can occur during storage due to hydrogen peroxide production, permeation of oxygen and post acidification. Statistical analysis revealed a significant difference ( $p < 0.05$ ) in the counts of innala and bran at 3% level while no significant difference was seen in other sources. Higher levels of sources may have hindered the survival of the bacterium due to lumps as observed in the product limiting their biochemical reactions. However results reveal that arrowroot has high performance compared to the rest of prebiotics (7.71 CFU/g) at the end of the storage period.

All the potential prebiotics have maintained standard counts as explained by Talwalker *et al.*, (2004) which should be greater than  $10^6$  CFU/mL for *L. acidophilus*, while rice bran has shown the lowest performance. All the three yam varieties, arrowroot, *hingurala* and *innala* indicated higher performance along with the commercially available prebiotic Raftiline®. This shows the possibility of using locally available sources to obtain higher growth rates and viability of probiotics.



**Figure 1. Change of *L. acidophilus* in media enriched with different prebiotics during 4 °C storage period**

The performance of all the sources was high when added one hour after incubation (Table 1). The counts of *L. acidophilus* grown as the control shows significant low numbers (Figure 1) while pH of the skim milk was at its lowest in the control as compared to the treatments at the end of storage life (Figure 2). This explains the destruction of colonies due to high levels of acidity during post-acidification.

Starch and rice bran have shown higher performance when added one hour following incubation than addition at the time of inoculation (Table 1). Addition of both microbial culture and the potential prebiotics together might have caused stress condition to the microorganisms where the sources are not utilized effectively. At the initial stage of fermentation supported by skim milk allowed the growth of colonies and acclimatize to the environment where sources added at this stage may have shown higher effects.

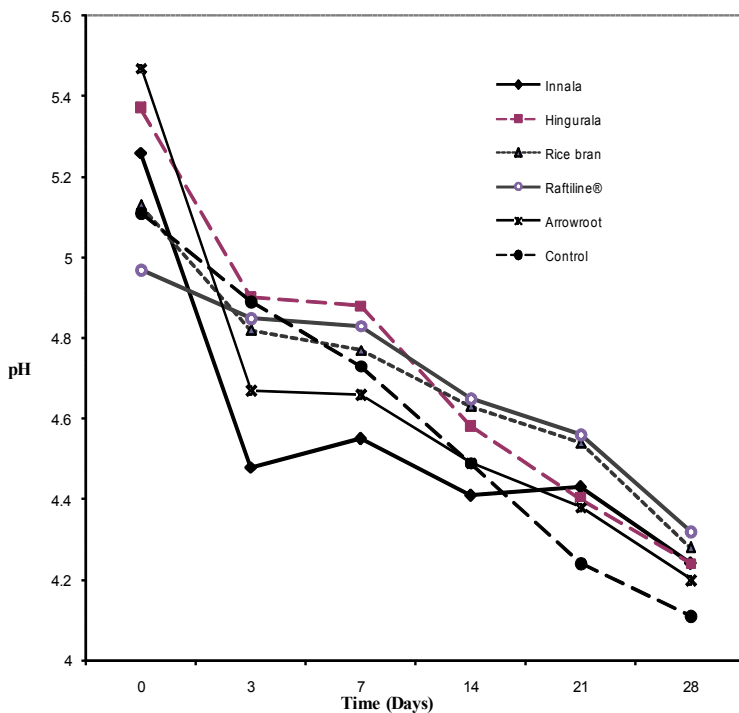
## CONCLUSIONS

Arrowroot and *hingurala* showed the highest performance in maintaining higher counts until the end of the 28<sup>th</sup> day. It can be concluded that these sources possess the properties of prebiotics which can be useful to obtain higher biomass of probiotics while maintaining their viability substantially throughout the expected storage life of a fermented product well above the standard levels.

**Table 1. Population of *L. acidophilus* on the third week of storage**

Stage of incorporation	Sources				
	Hingurala	Innala	Rice bran	Raftiline	Arrowroot
Sources added at time of inoculation	8.42 <sup>a</sup> ± 0.4	8.76 <sup>a</sup> ± 0.01	8.56 <sup>a</sup> ± 0.2	8.85 <sup>a</sup> ± 0.04	8.81 <sup>a</sup> ± 0.1
Sources added one hour after incubation	8.88 <sup>b</sup> ± 0.2	8.78 <sup>a</sup> ± 0.1	8.88 <sup>b</sup> ± 0.1	8.92 <sup>b</sup> ± 0.03	8.87 <sup>b</sup> ± 0.1

Values represented by different superscript letters in each column are significantly different at  $p < 0.05$ .



**Figure 2. Change of pH in skim milk preparation during storage period at 4 °C**

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## REFERENCES

- Gibson, G.R. and Roberfroid, M.B. (1995). Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J. Nutr.* 125: 1401-1412.
- Shah, N.P. (2000). Probiotic Bacteria. Selective enumeration and Survival in Dairy Foods. *J. Dairy Sci.* 83: 894-907.
- Talwalker, A. and Kailasapathy, K. (2004). A review of oxygen toxicity in probiotic yoghurts: influence on the survival of probiotic bacteria and protective techniques. *Food Tech.* 3: 117-124.
- Desai, A.R., Powell, I.B. and Shah, N.P. (2004). Survival and activity of probiotic Lactobacilli in skim milk containing prebiotics. *J. of Food Sci.* 69: 57-60.
- Fuller, R. (1995). Probiotics: their development and use. Old Hebron university, Reading UK.
- Siddeshwar, S., Reddy, K.K., Rao, T.M., Arun, K. and Reddy, P.V.M. (2008). Screening and estimation of prebiotic oligosaccharides in fruits and vegetables. *Curr. trends Biotechnol. Pharm.* 2 (1): 183-191.
- LeBlanc, A.M., Matar, C. and Perdigon, G. (2007). The application of probiotics in cancer. *Br. J. Nutr.* 98: 105- 110.