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Comparitive study of natural and commercial pectin on the growth of probiotic

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1. INTRODUCTION

The search for functional foods or functional food ingredients that can enhance health, is one of the leading trends in today's food industry **(Saarela *et al.*,2002).** In this context, probiotics and prebiotics receive much attention. Functional foods or functional food ingredients exert a beneficial effect on host health and/or reduce the risk of chronic disease beyond their nutritive value. A food can be made functional by addition of a potential health promoting entity, reducing or removing concentrations of harmful components and/or modifying the nature or the bioavailability of one or more components. The first generation of functional foods was based on enrichment/ fortification with vitamins and minerals (mainly calcium). However, the concept has recently moved towards food ingredients exerting a positive effect on the gut microbiota, introducing probiotics and prebiotics **(Christina 2007).**

Probiotics are defined as '*living microbial supplements that beneficially affect the host animals by improving its intestinalmicrobial balances*'**(Lay-Gaik Ooi & Min-Tze Liong,2010).**The most common probiotics currently used belong to the genera *Bifidobacterium* and *Lactobacillus***(Christina 2007)**. Prebiotics are '*indigestible fermented food substrates that*

selectively stimulate the growth, composition and activity of microflora in gastrointestinal tract and thus improve hosts' health and well-being'. When probiotics and prebiotics are used in combination, they are known as 'synbiotics'. The use of probiotics and prebiotics has only acquired scientific recognition in recent years although their applications as functional foods have been well-established throughout generations. In the interest of their promising effects on health and well being, probiotics and prebiotics have become increasingly recognized as supplements for human consumption. In addition to improving gut health, probiotics have also been documented to exert other health promoting effects such as strengthening of the immune system, antihypertensive effects, prevention of cancer, antioxidative effects, reduction of dermatitis symptoms, facilitation of mineral absorption, amelioration of arthritis, reduction of allergic symptoms and improvement of vulvovaginal candidiasis in women. Probiotics have been reported to lower cholesterol (**Lay-Gaik Ooi & Min-Tze Liong,2010).).**

Fructo - oligosaccharides, inulin, oligofructose, lactulose, and galacto- oligosaccharides have been identified as prebiotics due to characteristics such as resistance to gastric acidity, hydrolysis by mammalian enzymes and are fermented by gastrointestinal microflora to selectively stimulate the growth and/or activity of beneficial intestinal bacteria **(Lay-Gaik Ooi & Min-Tze Liong,2010).**Generally, prebiotics offer promising health benefits such as improving gastrointestinal microflora by selectively promoting the growth of probiotics and/or inhibition of pathogenic microorganisms , stimulation of the immune system , cancer prevention, stimulation of mineral absorption and bone stability and treatment of irritable bowel-associated diarrhoeas. Prebiotics are utilized by the intestinal microbial population to produce short-chain fatty acids

which may lead to the reduced incidence of gastrointestinal disease, cancers and cardiovascular diseases; and improvement of lipid profiles **(Lay-Gaik Ooi & Min-Tze Liong,2010).**

Probiotics must survive in the acidic gastric environment if they are to reach the small intestine and colonize the host, thereby imparting their benefits **(Lay-Gaik Ooi & Min-Tze Liong,2010).** Probiotic bacteria cannot thrive well in digestive tract without prebiotics **(Govind*et al.*, 2011**). Acidity is an environmental condition commonly encountered by lactic acid bacteria and bifidobacteria in the gastrointestinal tract and fermented foods (**Noritoshi *et a.,l* 2004).** The probiotic strains must be resistant to acidic conditions (gastric pH 1-4), alkaline conditions (bile salts present in the small bowel), enzymes present in the intestine (lysozyme) and toxic metabolites produced during digestion **(Aziz *et al.*, 2012)**. Since there are not many researches on the effect of pH on the growth of probiotics .

The current study aims at determining the variation in growth of probiotics (*Lactobacillus acidophilus, Lactobacillus casei* and *Bifidobacterium bifidum*) at different pH range (3.0- 6.0) . The study also looks into the effect of natural and commercial pectin on the growth of probiotic strains at pH 6.0 and pH 3.0

2. MATERIALS AND METHODS

2.1. Extraction of Pectin from Fruit Wastes:

The putrefied fruits of *Solanum lycopersicum* (tomato) were washed and blenderized with distilled water in the ratio of 1:1.5, acidified with lime juice to pH 2 and autoclaved. The mixture was cooled and filtered through cheese cloth. The filtrate was mixed with iso-propyl alcohol in

the ratio 1:1 and refrigerated overnight. This mixture was centrifuged at 2000 rpm for 10 minutes. The residue (pectin) was dried at 48ºC.

2.2. Culture maintenance:

Starter culture of *Lactobacillus acidophilus*, *Lactobacillus casei and Bifidobacterium bifidum* were procured from the Department of Dairy Microbiology, Dairy Science College UAS, Bangalore. Probiotic strains were maintained individually in sterile skim milk at 37ºC for 4hours, and then refrigerated at 4ºC. Subcuturing was done every 7 days.

2.3. Determination of growth parameters of probiotic bacteria at different pH:

Probiotic bacteria were individually inoculated in acidified (with 0.1N hydrochloric acid) sterilized MRS (Himedia) (De Man, Rogosa and Sharpe) broth adjusted to pH 3.0 and pH 6.0. They were incubated at 37ºC for 2hours. After incubation the cultures were serially diluted in sterile distilled water and pour plated on MRS agar. They were incubated at 37ºCfor 48hours. After 48 hours the colonies were enumerated by placing the plates on colony counter. The experiment was carried out in triplicates.

2.4. Determination of growth parameters of probiotic bacteria with addition of commercially available pectin at different pH:

The prebiotic pectin (Himedia) was added to the acidified broth (pH 3.0 - pH 6.0) with culture, and incubated at 37ºC for 2hours. After the incubation period, the cultures were serially diluted in sterile distilled water and pour plated on MRS agar in triplicates and then incubated for

growth at 37ºCfor 48hours. After 48 hours the colonies were enumerated by placing the plates on colony counter.

2.5. Comparison of commercially available prebiotics with natural pectin from *Solanum lycopersicum* (Tomato)

The probiotics (*Lactobacillus acidophilus, Lactobacillus casei* and *Bifidobacterium bifidum)* were added to the natural pectin, commercially available pectin individually in milk samples and also in combination with all the 3 cultures and the natural pectin, commercially available pectin to see the combined effect of the probiotics on the prebiotics. They were incubated at 37ºCfor 2 hours . After the incubation period, the cultures were serially diluted in sterile distilled water and pour plated on MRS agar in triplicates and then incubated for growth at 37ºCfor 48hours. The experiment was carried out in triplicates.

3. RESULTS AND DISCUSSION

3.1.Extraction of pectin:

Pectin is a prebiotic which is selectively metabolized by probiotic bacteria thereby acting as a growth enhancer. Pectin was isolated from putrefied fractions of *Solanum lycopersicum* (tomato)) and it was seen that the yield of pectin was obtained from *Solanum lycopersicum* is 7.14%.

3.2.Growth of probiotics at various pH (3.0-6.0)

Acidity is an environmental condition commonly encountered by lactic acid bacteria and bifidobacteria in the gastrointestinal tract and fermented foods **(Noritoshi *et al.*, 2004**). There fore the lactic acid bacteria and bifidobacteria were subjected to different pH of pH 3.0 and pH 6.0.

The MRS broth culture was maintained at pH 6.0 (normal pH of the broth ranges between 5.7-6.3) and the colony forming units / mL was counted (Table 1). It was seen that at pH 6.0 the *Lactobacillus casei* had higher number of colonies as compared to *Lactobacillus acidophilus and Bifidobacterium bifidum (*Figure 1). The probiotics must be resistant to acidic conditions (gastric pH 1.0-4.0), therefore the probiotics were subjected to the acidic environment that could mimic the gastrointestinal pH (pH 3.0). The MRS broth culture was maintained at pH 3.0 and the colony forming units / mL (Table 1) showed that *Lactobacillus casei* had higher number of colonies as compared to *Lactobacillus acidophilus, Bifidobacterium bifidum* .The number of colonies were compared at pH 6.0 and pH 3.0, which showed less variation in *Lactobacillus casei* and *Lactobacillus acidophilus. Bifidobacterium bifidum* showed a decrease in number of colonies at pH 3.0 when compared to pH 6 .0.

3.3. Growth of probiotics in presence of potential prebiotics at various pH (3.0-6.0).

Possibilities of synbiotic production was reported by **Kneifel *et al,*. (2000**) who observed growth of *Lactobacillus* and *Bifidobacterium* in media with the addition of prebiotics.

New compounds with gut resistant properties and selective fermentability by intestinal microorganisms are continuingly being identified and developed as prebiotics. These include

oligosaccharides (isomaltooligosaccharides, lactosucrose, xylooligosaccharides and glucooligosaccharides), sugar alcohols and polysaccharides and and pectin.When the bacteria were grown with commercial pectin at the concentration of 0.25%, *Lactobacillus casei* had the highest number of colonies as compared to *Lactobacillus acidophilus* and *Bifidobacterium bifidum* at pH 3.0 (0.25%) (Table 2). At 0.5% concentration of commercial pectin the number of colonies were approximately equal in all three species at pH 6.0 (Table 3) .However there was a minimal increase in number of colonies at pH 6.0 when compared to pH 3**. 0.**

3.4. Comparison of commercial pectin to natural pectin from fruit source *Solanum lycopersicum* on the probiotic bacteria.

The number of colonies with combination of all the probiotic bacteria with commercially available pectin and natural pectin individually showed that the natural pectin had highest number of colonies when compared to the commercially available pectin **(Figure 3).**An attempt was also made to combine the commercially available pectin and natural pectin with the probiotic bacteria (*Lactobacillus acidophilus, Lactobacillus casei and Bifidobacterium bifidum)* which showed decrease in bacterial counts than the prebiotics when included individually in the probiotic culture.

The commercially available probiotic drink and low fat probiotic curd were serially diluted and plated to count the number of colonies were found to 2688 and 1632 colonies respectively which is lesser .

4. Conclusion:

Prebiotics, natural or synthetic, can enhance thegrowth and activities of probiotics, and gut microflora, which are beneficial to the health and well being of humans and animals **(Paiboon *et al.*, 2009)**. Prebiotics that can withstand acidic and enzymatic digestion in the small intestine and can be utilized by probiotics in the large intestine for their growth. An attempt was made to study the impact of gastrointestinal pH on the growth of selected probiotic bacteria. The effect of commercially available prebiotic agents such as pectin was compared with naturally extracted fruit pectin (tomato) on the growth of probiotics.

The findings clearly indicates that there was minimal variation in the number of colony forming units of the probiotic bacteria, except *Lactobacillus casei* when subjected to various pH. *Lactobacillus casei* showed the maximum growth when subjected to prebiotic pectin. Comparative study between the commercially available prebiotics and naturally extracted pectin reveal that natural pectin was efficient in increasing the number of bacteria. The results of the current study suggests that natural pectin from fruit sources can be used as an efficient prebiotic agent.

This study could pave the way to test the effect of prebiotic agents on probiotics in animal models. The study would lead to product or process development methodologies, including the addition of pectin to enhance the survival of probiotic bacteria in milk product. The study benefits consumers by determining product development strategies that could enhance the survival of probiotic in food product, thereby delivering the desired levels of probiotic at the point of consumption.

REFERENCES:

Aziz ,Homayouni., Aslan, Azizi., Mina, Javadi., Solmaz, Mahdipour., & Hanie, Ejtahed.(2012). Factors Influencing Probiotic Survival in Ice Cream: A Review. *International Journal of Dairy Science*, 7, 1-10.

Christina, S., Venter. (2007). Prebiotics: an update. *Journal of Family Ecology and Consumer Sciences* ,35, 17-25.

Govind, Babu., & Nithyalakshmi, V. (2011) Influence of Prebiotic Composition on Probiotic Survivability in Calcium Alginate Coated Synbiotic Microcapsules at Thermal Incubation. *Agricultural Journal* , 6 , 231-236.

Kneifel, W., Rajal, A., & Kulbe, KD. (2000) In vitro growth behavior of probiotic bacteria in culture media with carbohydrates of prebiotic importance. *Micr. Ecol. Health Dis*, 12, 27-34.

Lay-Gaik, Ooi., Min-Tze Liong. (2010) Cholesterol-Lowering Effects of Probiotics and Prebiotics:A Review of *in Vivo* and *in Vitro* Findings. *Int. J. Mol. Sci, 11*, 2499-2522.

Noritoshi ,Takahashi., Jin-Zhong, Xiao ., Kazuhiro Miyaji , Tomoko Yaeshiima ., Akinori, Hiramatsu ., Keiji, Iwatsuki., Sadayuki, Kokubo., & Akiyoshi ,Hosono. (2004). Selection of acid tolerant Bifidobacteria and evidence for a low-pH-inducible acid tolerance response in *Bifidobacterium longum. Journal of Dairy Research*, 71, 340-345.

Paiboon, Thammarutwasik., Tipparat, Hongpattarakere., Suphitchaya, Chantachum., Kongkarn, Kijroongrojana., Arunporn, Itharat., Wantana, Reanmongkol., Supinya, Tewtrakul., & Buncha Ooraikul. (2009). Prebiotics – A Review**.** *J. Sci. Technol*, 31, 401-408.

Saarela, M., lähteenmäki, L., Crittenden, R., Salminen, S., Mattila-Sandholm ,T.(2002). Gut bacteria and health foods – the European perspective. *International Journal of Food Microbiology*,78, 99-117.

Table 1 : Growth of Probiotics at pH 6.0 (control) and pH 3.0

Growth of *L. acidophilus*			
	Colony forming unit / mL(10^{-4}) Mean ± SD	**Colony forming unit / mL(10^{-5}) Mean ± SD**	**Colony forming unit / mL(10^{-7}) Mean ± SD**
pH 6.0	226 ± 8	34 ± 6.24	2 ± 1
pH 3.0	234 ± 19.28	30.66 ± 6.80	26.6 ± 8

Growth of *L. casei*			
	Colony forming unit / mL(10^{-4}) Mean ± SD	**Colony forming unit / mL(10^{-5}) Mean ± SD**	**Colony forming unit / mL(10^{-7}) Mean ± SD**
pH 6.0	429.33±20.33	74.66± 3.78	4.33 ± 2.08
pH 3.0	420 ± 49.78	281.33±20.59	72 ± 13.06
Growth of *B.bifidum*			
	Colony forming unit / mL(10^{-4}) Mean ± SD	**Colony forming unit / mL(10^{-5}) Mean ± SD**	**Colony forming unit / mL(10^{-7}) Mean ± SD**
pH 6.0	281.66±8.08	20.33±8.50	2 ± 1
pH 3.0	118.3 ± 8.08	47.33 ± 7.63	2 ± 1

Table 2: Growth of Probiotics at pH 3.0 and 6.0 with 0.25% Commercial Pectin

Growth of *L. acidophilus* with 0.25% commercial pectin			
	Colony forming unit / mL(10^{-4}) Mean ± SD	**Colony forming unit / mL(10^{-5}) Mean ± SD**	**Colony forming unit / mL(10^{-7}) Mean ± SD**
pH 3.0	306±14.10	99±15.52	28±3.06
pH 6.0	608.66±24	93.66±11.15	35±12.76

Growth of *L. casei* with 0.25% commercial pectin			
	Colony forming unit / mL(10^{-4}) Mean ± SD	**Colony forming unit / mL(10^{-5}) Mean ± SD**	**Colony forming unit / mL(10^{-7}) Mean ± SD**
pH 3.0	506.3±44.00	102.3±9.07	2.33±0.57
pH 6.0	255±4	39±15.71	3±1
Growth of *B.bifidum* with 0.25% commercial pectin			
	Colony forming unit / mL(10^{-4}) Mean ± SD	**Colony forming unit / mL(10^{-5}) Mean ± SD**	**Colony forming unit / mL(10^{-7}) Mean ± SD**
pH 3.0	174±37.02	21±6.08	11.33±2.08
pH 6.0	283±17.34	41±9.16	8.23±3.51

Table 3: Growth of probiotics at pH 3.0 and pH 6.0with commercial pectin (0.5%)

Growth of *L. acidophilus* with 0.5% commercial pectin			
	Colony forming unit / mL(10^{-4}) Mean ± SD	**Colony forming unit / mL(10^{-5}) Mean ± SD**	**Colony forming unit / mL(10^{-7}) Mean ± SD**

pH 3.0	557±43.57	237.33±24.37	4±2
pH 6.0	658±18.08	146.33±19.39	12.33±1.52
Growth of *L. casei* with 0.5% commercial pectin			
	Colony forming unit / mL(10^{-4}) Mean ± SD	**Colony forming unit / mL(10^{-5}) Mean ± SD**	**Colony forming unit / mL(10^{-7}) Mean ± SD**
pH 3.0	475.33±24.21	41.33±23.43	12.66±2.08
pH 6.0	651.33±36.07	168±20.42	27.33±2.08
Growth of *B.bifidum* with 0.5% commercial pectin			
	Colony forming unit / mL(10^{-4}) Mean ± SD	**Colony forming unit / mL(10^{-5}) Mean ± SD**	**Colony forming unit / mL(10^{-7}) Mean ± SD**
pH 3.0	488.66±29.90	77.33±4.04	5±3
pH 6.0	671.66±23.28	67±4.35	8.66±2.51

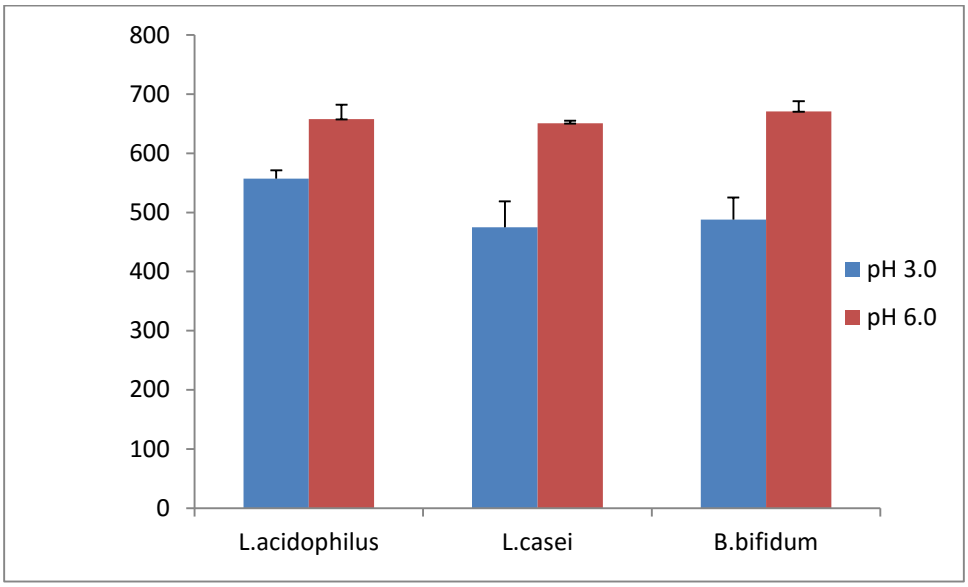

Figure 1 : Effect of 0.25% commercial pectin on Probiotic growth

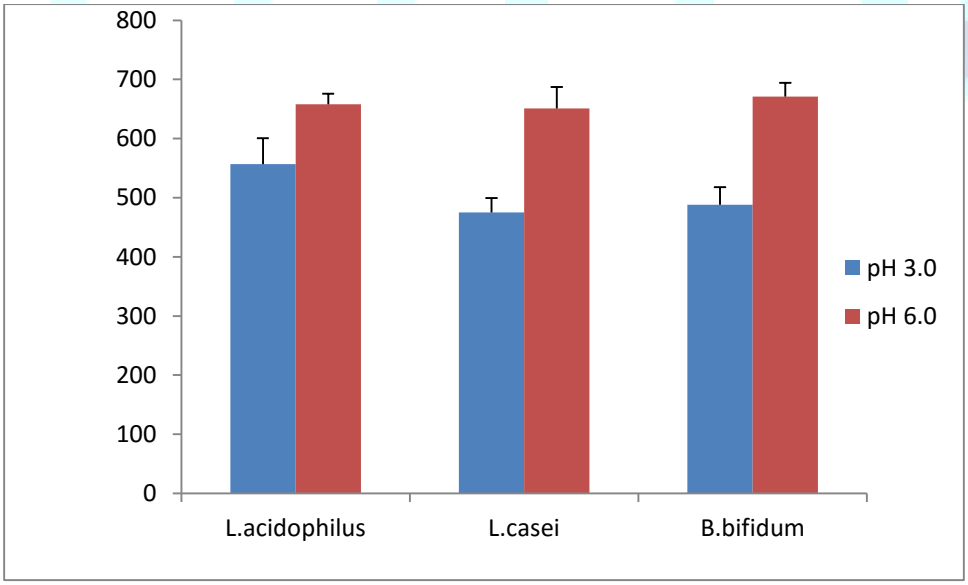

Figure 2: Effect of 0.5% commercial pectin on Probiotic growth

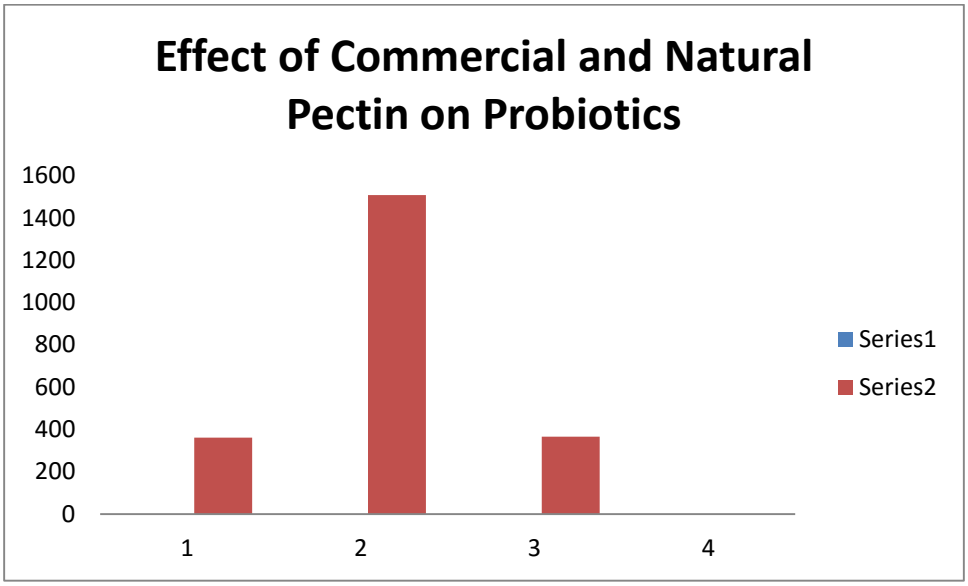

Figure 4: Effect of Commercial and Natural Pectin on Probiotics