

Fruit waste pectin in enhancing the establishment of probiotic bacteria

Abstract

The influence of pectin derived from the peels of discarded fruits in enhancing the growth of the health promoting probiotic organisms like *Bifidobacterium bifidum* and *Lactobacillus acidophilus* was studied by incorporating the derived pectin in the growth media. It was found that the pectins extracted from the different fruit wastes (apple- *Malus domestica*, lemon- *Citrus limon*, orange- *Citrus sinensis*) enhanced the CFU counts of the tested organisms at different pH ranging from pH 2 to 8. This clearly indicates that the presence of pectin as prebiotic supports the growth and survival of the probiotics in the gastrointestinal tract at extreme acidic to alkaline conditions. This suggests that waste fruits and vegetable peels can be used as a source of pectin to serve as prebiotics. This serves as an effective means of waste management.

Keywords: probiotic bacteria, *Bifidobacterium bifidum*, *Lactobacillus acidophilus*, *Malus domestica*, *Citrus limon*, *Citrus sinensis*

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Abbreviations: GI, gastrointestinal tract; FOS, fructo-oligosaccharides; GOS, galacto-oligosaccharides; FAO, food & agriculture organization

Introduction

The human gastrointestinal (GI) tract contains a variety of microflora including both pathogenic and beneficial bacteria.¹ Harmful bacteria in the GI tract may cause health problems in the host such as diarrhoea, infections, or liver damage. These bacteria may produce substances that increase the risk of selective growth and activity of certain beneficial bacteria in the colon.² Probiotics and their suitable prebiotics can be designed into food products to improve metabolic activities and stimulate their growth through synergistic effects.³

Probiotics should be able to recover and compete with microflora in the colon for nutrients and colonization sites. The beneficial bacteria such as *Bifidobacteria* and *Lactobacilli* can promote health of host by inhibiting the growth of these harmful bacteria, stimulating the immune system, and enhancing the digestion and absorption of essential nutrients such as short chain fatty acids.² Certain species of *Lactobacillus* and *Bifidobacteria* have been used as probiotic supplements in food products to provide better microbial balances in the GI tract and consequently improve the health.^{4,5} However, probiotic applications are limited because these bacteria are likely to be in a stressed state when they reach the colon due to exposure to diverse barriers in the host such as gastric acid and bile acids.^{2,6}

Prebiotics are normally commercially extracted from fruits and vegetables through methods such as enzymatic hydrolysis of polysaccharides from dietary fibers or starch. Potential prebiotic carbohydrates include fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), lactulose, lactosucrose, soybean oligosaccharides, iso-maltooligosaccharides, palatinose, xylo-oligosaccharides, and gluco-oligosaccharides.^{1,7} Hernandez-Hernandez et al.,⁸ have shown that prebiotic carbohydrates can enhance the survival of beneficial probiotic bacteria during exposure to gastric conditions. Prebiotics are food ingredients which are indigestible in the human upper GI tract and reach the colon to positively influence

the health of the host by selectively promoting the growth and activity of certain beneficial bacteria in the colon.² Probiotics and their suitable prebiotics can be designed into food products to improve metabolic activities and stimulate the growth of the probiotic bacteria through their synergistic effects.³ However, pectins have been reported to be able to reduce serum cholesterol in both rats and humans⁹ and are thought to have anti-carcinogenic effects.¹⁰ Pectin and pectic oligosaccharides have been shown to induce apoptosis in human colonic adenocarcinoma cells.¹¹ Using prebiotics and probiotics in combination is often described as a symbiotic. The United Nations Food & Agriculture Organization (FAO) has recommended the term synbiotic to be used only when the net health benefit is synergistic. Alternatively the prebiotic must support the increase in the population and functioning of the associated probiotic organism.¹²

The partially purified pectins extracted from the fruit peels of apple (*Malus domestica*), lemon (*Citrus limon*) and orange (*Citrus sinensis*), were considered in the present study to find out their effect on the growth and survival of probiotic bacteria at different pH (2, 6, 7 and 8). To represent the gastric acidity, control conditions, and the alkaline conditions in the intestine.

Methods and materials

Culture acquisition

Starter culture of *Lactobacillus acidophilus* was procured from Microbial Type Culture Collection and Gene Bank Institute of Microbial Technology, Chandigarh (MTCC number 10307) and the culture of *Bifidobacterium bifidum* from the Department of Dairy Microbiology, Dairy Science College UAS, Bangalore. Probiotic strains were maintained individually in sterile MRS (Himedia) (De Man, Rogosa and Sharpe) broth at 37°C for 4 hours, and then refrigerated at 4°C. Subculturing was carried out every 7 days.

Determination of growth parameters of probiotic bacteria at different pH

Probiotic bacteria were individually inoculated into MRS broth,

adjusted to pH 2 with 0.1N hydrochloric acid and for pH 7 and 8 with 0.1N Sodium hydroxide. pH 6 served as control. They were incubated at 37°C for 2 hours. After incubation the cultures were serially diluted 10^{-4} , 10^{-5} , and 10^{-7} were pour plated on MRS agar. They were incubated at 37°C for 48 hours. After 48 hours the colonies were enumerated by using colony counter. The experiment was replicated five times for each parameter.

Extraction of crude pectin from the peels of apple, carrot, lemon and orange

The peels were washed and blended using 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 48°C.

Determination of growth parameters of probiotic bacteria with different prebiotic samples at different pH

The samples of pectin from apple, lemon, orange, and commercial pectin were added to the broth at pH 2, 6, 7 and 8 along with the bacterial culture, and incubated at 37°C for 2 hours. After the incubation period, the cultures were serially diluted and the dilution 10^{-7} (for *L. acidophilus*) and 10^{-4} (for *B. bifidum*) were pour plated on MRS agar and then incubated at 37°C for 48 hours. After 48 hours the colonies were enumerated using colony counter.

Results and discussion

L. acidophilus showed a higher number of colonies as compared to *B. bifidum* at all the pH levels indicating that *L. acidophilus* exhibited better increase in CFU compared to *B. bifidum*. *L. acidophilus* at different pH where in maximum growth of colonies was at pH 6 and least at pH 2 (Table 1). This indicates that although these organisms do not survive at pH 6 under acidic conditions, the growth was still substantial. The colony count at pH 8 indicated that they remained unaffected by alkaline situations. One way ANOVA showed that there was clear indication of effect of pH on the growth of these organisms. The findings with *L. acidophilus* corresponded to that of Gomes and Malcata¹³ who in their study showed that acid tolerance of *L. acidophilus* varied from 0.3% to 1.9% titratable acidity, with an optimum pH lying at 5.5±6.0. This indicates that *L. acidophilus* has evolved some mechanisms for tolerance to wide ranges of pH in the gut as a result of which it does not require any additional protective mechanisms.

B. bifidum also showed the highest CFU at pH 6 which is in accordance to the growth characteristics of MRS media (optimum pH being 6.5±0.2). The growth at pH 2 was nil indicating that in order to survive the acidic pH these organisms require protective mechanisms. The growth at pH 7 and pH 8 was higher compared to pH 2 and this was expected as these organisms are typical flora of the alkaline intestine (Table 2). Furthermore, one way ANOVA showed that pH had a significant effect on the growth of these organisms. The growth characteristics of *B. bifidum* were in accordance with the results obtained by Gomes and Malcata¹³ and O'May et al.,¹⁴ stating that the optimum pH for growth of *B. bifidum* is 6, with virtually no growth at pH 4.5 or below or at pH 8.0 or above.

The pectin samples were found to be potential prebiotic agent

that increased the growth of *B. bifidum* (Table 3) as compared to *L. acidophilus* (Table 4). The growth in *B. bifidum* and *L. acidophilus* was highest at pH 6 in all the experimental conditions. It was observed that the growth of *B. bifidum* was highest in presence of apple pectin at pH 2 and that of orange pectin at pH 6, 7 and 8 at 10^{-4} dilution (Table 3). The growth of *L. acidophilus* was highest with pectin from apple at pH 2, with orange pectin at pH 6, apple pectin at pH 7 and apple pectin at pH 8 at 10^{-7} dilution (Table 4). Two way ANOVA with interaction was also calculated and the results were found to be statistically significant.

Table 1 Growth of *L. acidophilus* at different pH at various dilutions (n=5)

pH	Dilution		
	10^{-4}	10^{-5}	10^{-7}
2	631.2±30.38	142.8±19.06	3.6±0.89
6	2265.6±78.26	829.6±36.07	14.2±1.92
7	2258.4±52.05	535.2±68.67	7.8±1.30
8	1420.8±83.88	622.4±74.65	15±1.58

*The results are statistically significant

Table 2 Growth of *B. bifidum* at different pH at various dilutions (n=5)

pH	Dilution		
	10^{-4}	10^{-5}	10^{-7}
2	Nil	Nil	Nil
6	416±29.52	69.8±2.68	2.8±1.3
7	118.6±41.87	10.2±1.78	Nil
8	183.2±21.79	25.8±6.76	10.8±3.7

*The results are statistically significant

Thus in the case of *B. bifidum*, it was seen that each pectin sample enhanced the number of colony forming units to a very great extent. At pH 2 the growth enhancement was approximately 350 fold indicating that pectin might provide protection against the acidic pH in the stomach. Furthermore, at pH 6 the growth of two fold increase, at pH 7 it was seven folds and at pH 8 it was of five folds (Table 3). This indicates that pectin is readily metabolized by *B. bifidum* and as a result their growth rate is greatly enhanced. Various growth responses of the bacteria to the pectin samples at various pH was also observed by Palframan et al.¹⁵ The fermentation carried out at different pH will have direct effect on the substrate metabolism because of the change in enzyme activity at different pH. However, they found that in general, the pectin samples increased the growth of Bifidobacteria. Similar results were also reported by Olano-Martin et al.,¹¹ who investigated the effect of various pectin and pectin-oligosaccharides on Bifidobacteria.

However, pectin was able to increase the growth of *L. acidophilus* only to a certain extent in comparison to high growth in *B. bifidum*. At pH 2 there was very little or no response to enhancement of growth indicating that pectin might not provide any protection against the acidic pH in the stomach. This result was different from the findings of Nazzaro et al.¹⁶ They had found that the initial growth of *Lactobacillus* at acidic pH in the presence of pectin significantly improved the growth of the bacteria. However, at pH 6 the growth enhancement was four folds which were higher than in the case of *B. bifidum*. At pH 7 and pH 8 there was hardly any increase in growth which indicates

that these organisms do not require pectin as growth enhancing molecule. Pectin is already being used in microencapsulation of probiotics¹⁷ in order to create more effective delivery systems for the probiotic bacteria. However, it has not been as well characterized as other prebiotics such as inulin whose prebiotic mechanisms have been effectively studied. Further understanding of the protective mechanisms conferred by pectin will further enhance the numerous

beneficial effects of probiotics. This will be useful because fruit and vegetable peels are rich sources of pectin and can thus be used to produce pectin in an efficient manner reducing the amount of vegetable waste. The potential of fruit and vegetable waste is not only limited to the production of value added products but could also be utilized in enhancing the growth of the probiotic bacteria and can eventually bring down the level of contamination in the environment.

Table 3 Growth of *B.bifidum* at different pH with different pectin samples at 10⁻⁷ dilution (n=5)

Sample	pH 2	pH 6	pH 7	pH 8
Apple	523±5.73	586.4±45.13	683.2±7.69	852±16.49
Lemon	350.4±39.04	743.2±44.13	881.6±33.66	1152±21.73
Orange	360.6±52.15	752±54.04	953.6±67.76	1276.4±55.72
Commercial Pectin	764.8±20.08	2964±22.45	1072.8±16.83	1056.8±36.81

*The results are statistically significant

Table 4 Growth of *L. acidophilus* at different pH with different pectin samples at 10⁻⁴ dilution (n=5)

Sample	pH 2	pH 6	pH 7	pH 8
Apple	5.4±0.55	48.8±12.48	9.6±0.55	22.2±3.03
Lemon	2.4±0.57	55.4±6.19	8.4±0.55	18.4±1.82
Orange	1.6±0.55	77.4±1.67	9.2±0.45	16.2±2.17
Commercial Pectin	3.8±0.45	60.4±3.65	10.2±0.45	17.8±1.10

*The results are statistically significant

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None.

Conflict of interest

Author declares that there is no conflict of interest.

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