Phytase Activity of Lactic Acid Bacteria Isolated from Dairy and Pharmaceutical Probiotic Products

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ABSTRACT
Phytate, the major storage form of phosphorus in plant seeds, can form insoluble complexes with minerals such as iron, zinc and calcium thus reducing their bioavailability. Phytase enzymes are often used to upgrade the nutritional quality of phytate-rich foods and feeds such as grains. The phytate-degrading activity of 43 lactic acid bacteria including isolates from commercial probiotic preparations, dairy products and type strains were measured. The phytate-degrading activity of bifidobacteria and lactobacillus isolates from pharmaceutical probiotics, dairy products and type strains were determined. The enzyme activity of probiotic bacteria ranged between 1.1-5.4 mU and was strain not species specific. Phytase activity may thus be a useful additional attribute of probiotics to be used as food supplements.

Keywords: Dairy Products; Probiotics; Lactic Acid

1. Background
Phytate, myo-inositol (1, 2, 3, 4, 5, 6) hexakisphosphate, the major storage form of phosphorus in the plant seeds, is an important anti-nutritional factor in all kinds of grains, seeds, nuts, vegetables and fruits. Phytate may form insoluble complexes with minerals such as iron, zinc and calcium thus reduce their bioavailability. The bioavailability of dietary minerals can be improved by reducing of the phytate content in plant foods and feeds. Phytase enzymatic activity produces available phosphate and a compound, which is not a metal chelator. Phytases are then considered to be enzymes of great value in upgrading the nutritional quality of phytate-rich foods and feeds.

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Sources of phytases include plants, animals and microorganisms. For example, the phytase activity was observed in Bacillus subtilis and Klebsiella pneumoniae (4, 5). Some lactic acid fermentations of dairy products or vegetables decrease the content of phytate. Extracellular phytase activity was suggested to be responsible for the observed reduction in phytate content during lactic acid fermentation (1, 6).

2. Objectives

Since lactic acid bacteria have been used traditionally in food fermentation, they may be a very useful source of microbial phytase. Thus, phytase producers could be used as starter cultures for preparing sourdough bread, pancakes, idli, dosa, dhokla, porridges, alcoholic and non-alcoholic beverages, beans or dairy products (7). Also, if the lactic acid bacteria be probiotic, and food is not subsequently cooked, they may be able to produce this enzyme in gut providing a double benefit. The aim of this work was to evaluate the phytase activity of 38 probiotic bacteria and 5 lactobacilli and bifidobacteria type strains.

3. Materials and Methods

Table 1 shows the list and source of lactobacilli and bifidobacteria in the present study. All bacteria were maintained on de Man Rogosa and Sharp (MRS) broth (Oxoid CM359)/anaerobic/37°C. Lactobacillus and bifidobacterium (38 strains) were isolated and identified from dairy products or Pharmaceutical probiotics according to Chen et al. (8). Bacterial type strains included L. acidophilus NCIMB 1748, L. casei NCIMB 11970, L. rhamnosus NCIMB 8010, B. bifidum NCIMB 702715 and B. longum NCIMB 702259.

Table 1. The List and Sources of Probiotic Isolates

| Name of product                     | Organisms stated on label                  | Species isolated/codes of isolates                                      |
|-------------------------------------|-------------------------------------------|-----------------------------------------------------------------------|
| Solgar Advanced Acidophilus         | L. acidophilus, B. lactis                 | L. acidophilus/1C2, 1C3, L. pl. arabinosis/1C, 1C5                     |
| Quest digestive Aids                | Acidophilus, L. casei, L. casei-rhamnosus | L. acidophilus/2C1, 2C3, L. casei-rhamnosus/2C4, L. casei-rhamnosus/2C2|
| Holland & Barret Acidophilus        | Lactobacillus                             | L. plantarum/3C12, 3C14, L. rhamnosus/3C15                           |
| Holland & Barret Non-Dairy Acidophilus | L. acidophilus, L. rhamnosus, L. bifidum  | L. rhamnosus/3C23, L. casei/3C21, 3C22                               |
| Seven Seas Multibionta              | L. acidophilus PA 16/8, B. bifidum MF, B. longum | L. acidophilus/4C1, 4C2, L. rhamnosus/4C3                          |
| Pharmadas Heath Aid Acidophilus     | L. acidophilus Acidophilus bifidus        | L. acidophilus/5C1, 5C2                                              |
| American Health Chewy Bears         | L. acidophilus, L. rhamnosus, L. plantarum, L. sporogenes, B. longum | L. acidophilus/6C6, L. rhamnosus/6C2, L. plantarum/6C1, 3, 4 & 5   |
| DanonActiMEL                       | L. casei-Munitass                         | L. casei (Munitass)/4D1, 4D2                                         |
| Yakult milk                         | L. casei-shirota                          | L. casei (shirota)/6D1, 6D2                                          |
| DanoneActiva                        | Bifidobacterium                          | Bifidobacterium sp./7D1                                             |
| Muller Vitality yogurt              | Bifidobacterium Bb-12                     | Bifidobacterium sp./8D1                                             |
| Tesco Probiotic-yogurt              | Bifidobacterium Bb-12                     | Bifidobacterium sp./9D1                                             |

Abbreviations: ND; Not determined, Now B. bifidum; No phenotypic profile available in the literature

4. Results

Phytase activity of probiotic isolates: phytase activity of all the isolated strains was between 1 to 5.4 mU (Table 2 A, B, C). Phytase activity of isolated strains and type strains were divided into three groups of low, medium and high activity. Phytase activity was categorized as low (<3 mU), moderate (3 mU < 4 mU) and high (> 4 mU). Among 43 strains tested, phytase activity of 6, 6 and 3 dairy isolates was high, moderate and low, respectively. Similarly, phytase activity of 6, 12 and 5 pharmaceutical isolates was high, moderate and low, respectively. Phytase activity of

E. coli isolated from the faecal flora of a healthy donor was used as a non-lactic acid bacteria negative control. Phytase activity: All isolates were grown overnight in modified MRS broth where the only source of phosphate was sodium phytate (Sigma P8810) 0.1%, centrifuged at 400 rpm and bacteria supernatants (bfs) were screened for phytase activity (9). Phytase activity of the probiotic isolates were tested using the "phytic acid ColorKit-complete phytase assay system" (Innova Biosciences) according to the manufacturer’s instructions.
2, 2 and 1 type strains was high, moderate and low, respectively. Most of bifidobacteria strains (4 out of 5) were in high activity group. No correlation between phytase-activity of species was observed. Phytase activity was not species related but appeared to be a strain characteristic.

Table 2 A. Phytase Activity of Bacterial Supernatant from 36 Hours Cultures

| Strains                  | Phytase activity (mU) |
|--------------------------|-----------------------|
| Low activity             |                       |
| 4D2 (L. casei Immunitass)| 1.05                  |
| 4D1 (L. casei Immunitass)| 1.09                  |
| 3C12 (L. plantarum)      | 1.7                   |
| 2C1 (L. acidophilus)     | 1.8                   |
| L. casei T               | 2                     |
| 6D2 (L. casei Shirota)   | 2.6                   |
| 1C2 (L. acidophilus)     | 2.7                   |
| 2C4 (L. casei)           | 2.6                   |
| 6C6 (L. acidophilus)     | 2.8                   |

Table 2 B. Phytase activity of bacterial supernatant from 36 hours cultures

| Strains                  | Phytase activity (mU) |
|--------------------------|-----------------------|
| Medium activity          |                       |
| 6D1 (L. casei Shirota)   | 3                     |
| 3C22 (L. casei)          | 3                     |
| 3D3 (L. lactis)          | 3.01                  |
| 5D2 (no-identified LAB)  | 3.3                   |
| 2C3 (L. acidophilus)     | 3.6                   |
| 1D2 (L. plantarum)       | 3.6                   |
| 3C21 (L. casei)          | 3.6                   |
| 3D1 (L. lactis)          | 3.6                   |
| 3D2 (L. lactis)          | 3.6                   |
| 6C3 (L. plantarum)       | 3.6                   |
| 5C1 (L. acidophilus)     | 3.6                   |
| 4C2 (L. acidophilus)     | 3.6                   |
| 1C1 (L. plantarum)       | 3.7                   |
| 2C2 (L. rhamnosus)       | 3.7                   |
| 6C4 (L. plantarum)       | 3.7                   |
| 6C5 (L. plantarum)       | 3.7                   |
| B. longum T              | 3.7                   |
| L. rhamnosus T           | 3.7                   |
| 3C15 (L. rhamnosus)      | 3.8                   |
| 3C23 (L. rhamnosus)      | 3.8                   |

5. Discussion

Phytase enzymatic activity produces available phosphate and a compound, which is not a metal chelator(1). The phytase activity of lactic acid bacteria isolated from natural vegetable fermentations was shown by Zamudio et al. 2001 (10). Some bacteria that have phytase enzymes can decrease phytate content of food substrates (11). Fermentation of food with lactic acid bacteria can improve the nutrient content. For example the bioavailability of Fe is enhanced by fermentation of carrot juice (12) and maize (13). It has also been suggested that gut microbiota is responsible for degradation of phytic acid (9) and flavonoids (14,15). The presence of phytases in sourdough Lactobacillus has been investigated by several authors (6,16-18).

Our studies revealed that more dairy product isolates showed high phytase activity than those isolates from pharmaceutical products (40% vs 27%). These higher values are in agreement with the results of Zamudio et al. who found activity between 3.5-6.3 mU (19). Lopez et al. measured the phytase activity of strains of L. plantarum and L. acidophilus but did not observe any differences between strains in the level of phytic acid hydrolysis (20). Similar to our results, Phengnuman and Suntornsuk investigated the detoxifying toxic and anti-nutritional compounds in J. curcas seed cake by fermentation with Bacillus spp. They saw that after fermentation, phytate, phorbol esters, and trypsin inhibitor were reduced by 42%, 62% and 75%, respectively and suggested that the reduction of phytate, phorbol esters and trypsin inhibitor was related to phytase, esterase and protease activities,
respectively (21). All bifidobacteria isolates in present study were among the high phytase activity group. This was in agreement with Wongputtisin that showed B. subtilis MR10 and TK8 were suitable fermentative bacteria for FCSBM production according to their ability of phytase production (22).

The method used in the present study for measuring phytase activity was detection of free Pi in the medium in which the only source of Pi was phytate. In this method we were able to detect phytase activity and compare the activity of different bacteria. More evaluation such as enzyme extraction and purification using HPLC or gel purification is necessary to confirm the presence of phytase enzyme in these bacteria. Zamudio et al. reported phytase activity in L. plantarum similar to that observed in L. amylovorus by Sreeramulu et al. (1992) (10). They purified phytase from culture supernatant by gel filtration. The molecular weight of the enzyme was lower than phytase extracted from Bacillus subtilis and E. coli. They concluded that L. plantarum phytase activity was due to non-specific acid phosphatase. Haros et al. used sodium phytate and p-nitrophenol phosphate as substrates for evaluation of phytase activity (9). The ability of bifidobacterium strains to produce inorganic phosphorous from each substrate, was used as a measure of the activity due to phytase or phosphatase. They reported that B. pseudocatenulatum, frequently isolated from infant faeces, showed the highest level of phytase activity. This species has not been used as a probiotic so far and their other characteristics should be assessed before this is considered.

In conclusion, it still needs to be ascertained whether this phytase activity is due to non-specific or specific phytases, as phytase activity is an important property for the food industry. The main value of Phytase being its ability to totally degrade the anti-nutritional compound phytate, whereas phosphatase causes only partial degradation. Bearing in mind that isolates used here are safe for the food industry, the presence of phytase activity makes these isolates of greater value. Addition of probiotic bacteria with phytase activity to the foods of families, especially in areas with higher prevalence of iron deficiency, may improve nutrient bioavailability.

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Authors’ Contribution

Zohreh Khodaii and Mahboobeh Mehrabani were responsible for designing and performance of project. Mohammad H. Naseri, Mahdi Goudarzvand and Hillary Dodson were practical and scientific advisers of the project.

Financial Disclosure

There is no conflict of interest.

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References

1. Reale A, Konietzny U, Coppola R, Sorrentino E, Greiner R. The importance of lactic acid bacteria for phytate degradation during cereal dough fermentation. J Agric Food Chem. 2007;55(8):2993-5.
2. Tang AL, Wilcox G, Walker KZ, Shah NP, Ashton JF, Stojanovska L. Phytase activity from Lactobacillus spp. in calcium-fortified soy milk. J Food Sci. 2010;75(6):M373-6.
3. Turk M, Sandberg AS, Carlsson NG, Andlid T. Inositol hexaphosphate hydrolysis by Baker’s yeast. Capacity, kinetics, and degradation products. J Agric Food Chem. 2000;48(1):100-4.
4. Greiner R, Haller E, Konietzny U, Jany KD. Purification and characterization of a phytase from Klebsiella terrigena. Arch Biochem Biophys. 1997;341(2):201-6.
5. Powar VK, Jaganathan V. Purification and properties of phytase-specific phosphatase from Bacillus subtilis. J Bacteriol. 1982;155(3):1002-8.
6. Reale A, Mannina L, Tremonte P, Sobolev AP, Succi M, Sorrentino E, et al. Phytate degradation by lactic acid bacteria and yeasts during the wholemeal dough fermentation: a 31P NMR study. J Agric Food Chem. 2004;52(20):6300-5.
7. Sreeramulu G, Srinivasa DS, Nand K, Joseph R. Lactobacillus amylovorus as a phytase producer in submerged culture. Lett Appl Microbiol. 1992;15(3):385-8.
8. Chen YS, Yanagida F, Shinhohara T. Isolation and identification of lactic acid bacteria from soil using an enrichment procedure. Lett Appl Microbiol. 2005;40(3):195-200.
9. Haros M, Bielecka M, Sanz Y. Phytase activity as a novel metabolic feature in Bifidobacterium. FEMS Microbiol Lett. 2005;247(2):231-9.
10. Zamudio M, Gonzalez A, Medina JA. Lactobacillus plantarum phytase activity is due to non-specific acid phosphatase. Lett Appl Microbiol. 2001;32(3):381-4.
11. Hallberg L, Sandstrom B, Ralph A, Arthur J. Iron, zinc and other trace elements. in JS Garrow, WPT James & A Ralph (eds), Human nutrition and dietetics. 2000.
12. Bergqvist SW, Andlid T, Sandberg AS. Lactic acid fermentation products. FEMS Microbiol Lett. 1997;150(7):2749-54.
13. Marotti L, Bonetti A, Biasotti B, Catizone P, Dinelli G. Biotransformation of common bean (Phaseolus vulgaris L.) flavonoid glycosides by bifidobacterium species from human intestinal origin. J Agric Food Chem. 2007;55(7):2749-54.
14. Leenhardt F, Levrat-Verny MA, Chailanuda E, Remy C. Moderate decrease of pH by sourdough fermentation is sufficient to reduce phytate content of whole wheat flour through endogenous phytase activity. J Agric Food Chem. 2000;48(1):98-102.
15. De Angelis Maria, Gallo Giovanna, Corbo Maria Rosaria, McSweeney Paul LH, Faccia Michele, Giorno Marinella, et al. Phytase activity in sourdough lactic acid bacteria: purification and characterization of a phytase from Lactobacillus sanfranciscensis CBI. International Journal of Food Microbiology. 2003;87(1):259-270.
16. Lopez HW, Krespine V, Guy C, Messager A, Demigne C, Remy C. Prolonged fermentation of whole wheat sourdough reduces...
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phytase activity is due to non-specific acid phosphatase. Letters in Applied Microbiology. 2001;32(3):181-184.
21. Lopez HW, Ouvry A, Bervas E, Guy C, Messager A, Demigne C, et al. Strains of lactic acid bacteria isolated from sour doughs degrade phytic acid and improve calcium and magnesium solubility from whole wheat flour. J Agric Food Chem. 2000;48(6):2281-5.
22. Phengnuam T, Suntrornsuk W. Detoxification and anti-nutrients reduction of Jatropha curcas seed cake by Bacillus fermentation. J Biosci Bioeng. 2013;115(2):168-72.
23. Wongputtisin P, Khanongnuch C, Khonghantad W, Niamsup P, Lumyong S. Screening and selection of Bacillus spp. for fermented corticate soybean meal production. J Appl Microbiol. 2012;113(4):798-806.