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

Zohreh Khodaii [1,*], Mahboobeh Mehrabani Natanzi [1], Mohammad Hassan Naseri [2,3], Mahdi Goudarzvand [4], Hillary Dodson [5], Anna M. Snelling [5]

[1] Department of Biochemistry, Genetic and Nutrition, Faculty of Medicine, Alborz University of Medical Sciences , Karaj, IR Iran
[2] Department of heart surgery, Baqiyatollah University of Medical Sciences, Tehran, IR Iran
[3] Chancellor of Alborz University of Medical Sciences, Karaj, IR Iran
[4] Department of Physiology and Pharmacology, Faculty of Medicine, Alborz University of Medical Sciences, Karaj, IR Iran
[4] Department of Biomedical Sciences, University of Bradford, UK

Corresponding author: Zohreh Khodaii, Department of Biochemistry, Genetic and Nutrition, Faculty of Medicine, Alborz University of Medical Sciences , Karaj, IR Iran. Tel: +98-2634336007, Fax: +98-2634319188, E-mail: zkhodaii@yahoo.com

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 probotic 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 (1). Phytate may form insoluble complexes with minerals such as iron, zinc and calcium thus reduce their bioavailability (2). The bioavailability of dietary minerals can be improved by reducing of the phytate content in plant foods and feeds (3). Phytase enzymatic activity produces available phosphate and a compound, which is not a metal chelator(1). 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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Implication for health policy/practice/research/medical education:
Addition of probiotic bacteria with phytase activity to foods of families, especially in areas with higher prevalence of iron deficiency, may improve nutrient bioavailability.

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Sources of phytases include plants, animals and microorganisms. For example the phytase activity was observed in Bacillus subtillis and Klebsiellaterrigena(4, 5). Some lactic acid fermentations of dairy products or vegetables decrease the content of phytate. Eextracellular 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/ 37oC. 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. caseicasei* 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. arabinisis*/1C, 11C5
Quest digestive Aids	*Acidophilus, L. caseicasei,L. caseirhamnosus*	*L. acidophilus*/2C1, 2C3L. *caseicasei*/2C4L. *caseirhamnosus*/2C2
Holland & Barret Acidophilus	*L.acidophilus*	*L. plantarum*/3C12, 3C14 *L. rhamnosus* 3C15
Holland & Barret Non-Dairy Acidophilus	*L. acidophilus, L. rhamnosus, L. bifidum*	*L. rhamnosus*/3C23 *L. casei*/3C 21, 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
DanoneActimel	*L. caseiImunitass*	*L. casei* (Imunitass)/4D1, 4D2
Yakult milk	*L. caseishirota*	*L. casei* (shirota) 6D1, 6D2
DanoneActiva	*BifidusEssensis*	*Bifidobacterium sp.*/7D1
MüllerVitality yogurt	*Bifidobacterium Bb-12*	*Bifidobacterium sp.*/8D1
Tesco Probioticyogurt	*Bifidobacterium Bb-12*	Bifidobacterium sp./9D1

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

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 free 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.

4. Results

Phytase activity of probiotic isolatess: 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, 3 mU< moderate < 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

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 caseiImmunitass*)	1.05
4D1 (*L caseiImmunitass*)	1.09
3C12 (*L. plantarum*)	1.7
2C1(*L. acidophilus*)	1.8
L. casei T	2
6D2 (*L. caseiShirota*)	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. caseiShirota*)	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

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

Strains	Phytase activity (mU)
High activity	
4C3 (*L. rhamnosus*)	4
1C5 (*L. acidophilus*)	4.2
B.bifidum	4.2
5D1 (*L. delbrueckii*)	4.6
6C1(*L. plantarum*)	4.6
2D3 (*L. sanfrancisco*)	4.6
7D1 (*Bifidobacterium sp.*)	4.6
9D1 (*Bifidobacterium sp.*)	4.7
4C1(*L. acidophilus*)	4.7
1D1(*L. plantarum*)	4.7
E. coli sp.	4.74
1C3 (*L. acidophilus*)	4.8
8D1 (*Bifidobacterium sp.*)	5.4
5C2 (*L. acidophilus*)	5.4
L. acidophilus T	5.4

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. plantarumphytase 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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