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REVIEW ARTICLE

Phytases of Probiotic Bacteria: Characteristics and Beneficial Aspects

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Abstract Probiotics play a vital role in clinical applications for the treatment of diarrhea, obesity and urinary tract infections. Phytate, an anti-nutrient, chelates essential minerals that are vital for human health. In the past few decades, research reports emphasize extensively on phytate degradation in animals. There is a growing need for finding alternate strategies of phytate utilization in human, as they are unable to produce phytase. At this juncture, probiotics can be utilized for phytase production to combat mineral deficiency in humans. The main focus of this review is on improving phosphate bioavailability by employing two approaches: supplementation of (1) fermented food products that contain probiotics and (2) recombinant phytase producing bacteria. In addition, several factors influencing phytase activity such as bacterial viability, optimal pH, substrate concentration and specificity were also discussed.

Keywords Probiotic · Anti-nutrient · Phytate · Phytase · Fermented foods

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Introduction

The joint initiative of Food and Agricultural Organization (FAO) as well as the World Health Organization (WHO), defined probiotics as mono or mixed cultures of "live microorganisms which when administered in adequate amounts beneficially affect the host" [1]. The history of probiotics dates back to 1907 when Russian scientist Ellie Metchnikoff postulated the idea of using lactic acid bacteria for modulating intestinal flora. Since then intensive research was undertaken in the field of probiotics for improving human health and the timeline of these applications are presented in Fig. 1.

Phytate also known as *myo*-inositol hexaphosphate is the principal storage form of phosphorus in plant-based foods.

The chemical structure of phytate consists of six phosphate groups (five of them present in equatorial position with the last one placed in axial position) attached to its inositol ring. The overall negative charge contributed by phosphates (Fig. 2) help in chelating divalent and trivalent metal ions. Therefore, phytate acts as an anti-nutrient and limit mineral bioavailability in human. Despite causing a major nutritional deficiency, phytate can be regarded for its beneficial effects especially in treating colon cancer, AIDS, Alzheimer's disease, Arthritis and Parkinson's diseases [2].

Phytases of plants and microorganisms degrade phytate into inositol and free orthophosphates. These enzymes differ in their structure. Plant-based phytases predominantly exist in alpha/beta form whereas, bacterial phytases belong to the beta class of proteins [3].

The crystal structure of bacterial phytase consists of antiparallel β-sheets (Fig. 3). The active site of enzyme consists of "catalytic" (phosphate 1) and "affinity" (phosphate 2) binding sites. The two phosphates of the substrate (phytate) bind to these sites causing structural

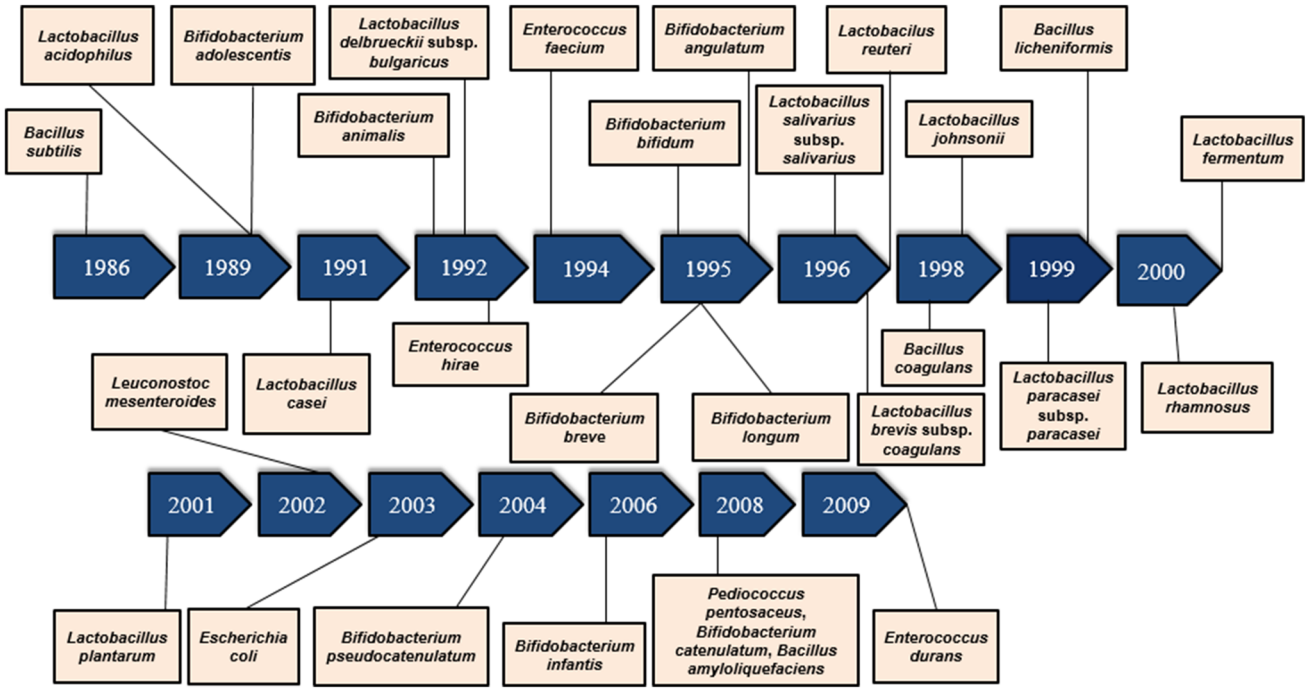

Fig. 1 Timeline of probiotics

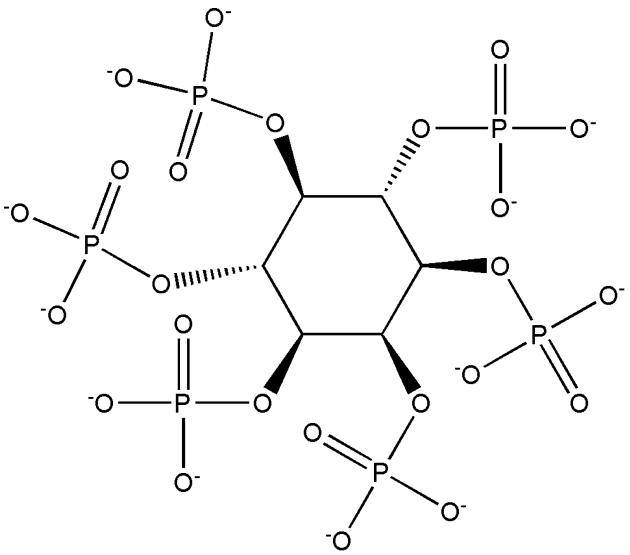

Fig. 2 Chemical structure of phytate (retrieved from PubChem)

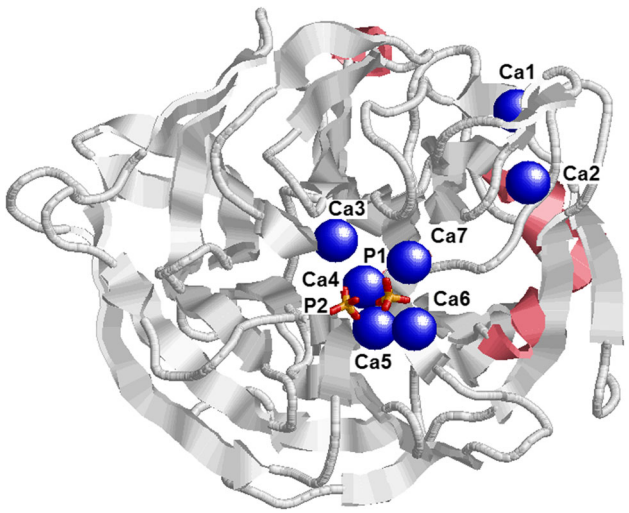

Fig. 3 Structural configuration of phytase (PDB id: 1H6L)

conformations via both ionic and hydrogen bonding. Then the catalytic site is activated by three Ca^{2+} ions, which are vital for substrate binding. Besides, three more Ca^{2+} bind to the affinity site that improves the substrate specificity and overall stability of the enzyme. Binding of phosphate ions also activates the presence of a seventh Ca^{2+} ion in the structure. Catalysis is initiated by an attack of hydroxide ion of water molecule on the first phosphate group, triggering its cleavage. Thereafter, every alternate phosphate is cleaved leading to the final product. Phytases cleave equatorial phosphate groups readily as compared to axial ones [4]. Probiotic bacteria can be efficiently utilized for phytate utilization as compared to their plant-based counterparts owing to the former's increased substrate specificity and catalytic efficiency [5].

Humans have a monogastric digestive system which lacks sufficient phytase producing bacteria in their intestine for phytate utilization. This leads to a serious nutritional deficiency in the host. The undigested phytate from food passes into the soil and lead to environmental phosphate pollution [2].

Hence, this review is focused on probiotic bacteria which are capable of degrading phytate in humans.

Probiotic Selection Criteria

Probiotics have been used for centuries due to their broad spectrum of biological activities in human. For bacteria to be used as probiotics, they must be non-pathogenic, non-invasive, non-carcinogenic, adhere to intestinal epithelium, resistant to gastric acidity, stable in food matrix during intestinal digestion, aid in immunomodulation, and colonize for a stable time period. Moreover, they must have verified strain properties, be effective against the specified target and exhibit bile salt hydrolase activity [6].

These probiotics can be administered through enteral route or by enema. Enteral route is widely preferred for consumption of fermented food products. Whereas, an enema is preferred for fecal microbiota transplantation (FMT) [7]. The review is focused only on fermented food products for administering probiotics via an enteral route as it is cost-effective and convenient when compared to FMT [8, 9]. The choice of fermented foods that contain viable bacteria, which are capable of degrading phytate are listed in Table 1.

Probiotic bacteria in fermented food products have an impact on human health. Probiotics isolated from a wide variety of functional foods ranging from sourdough to fermented bread, dairy products are capable of effective phytate degradation thereby aiding in improved mineral uptake in humans.

In general, probiotics competitively bind to the host intestinal epithelium and stimulate an immune response by activating specific signaling cascades and cell-based reactions. Each bacterial genera has a unique action mechanism for the corresponding biological functions.

Probiotics belonging to *Bacilli* group are gram-positive, facultative aerobes which produce non-pathogenic spores. The spores play an important role in increased shelf-life of the probiotic product as they are thermostable, recalcitrant to a wide range of pH fluctuations and viable under extreme intestinal conditions. The presence of an outer thick peptidoglycan layer protects bacterial spores from extreme heat, organic acids, lysosomal degradation and helps them in several beneficial activities on human health including treatment of rheumatoid arthritis and blood clotting.

Escherichia coli are gram-negative probiotic microbes which predominately participate in anti-inflammatory activity through modulation of the host immune system. They are also helpful in treatment of colitis and constipation.

Microbes belonging to genera *Lactobacilli, Enterococci*, *Bifidobacteria*, *Pediococcus* and *Leuconostoc* represents a group of non-pathogenic, facultative anaerobes which are commonly present in the human gut. In addition to their gastric viability, they efficiently colonize and have prolonged epithelial adherence. They also produce bacteriocins which competitively inhibit other pathogenic bacteria and maintain a healthy balance of beneficial microbes. These bacteria find applications ranging from preparation of fermented food products to clinical therapeutics such as inflammatory bowel disease (IBD), diarrhea, and cholesterol regulation.

Functional Foods for Phytase Production

Fermented foods like cheese, sausages and caper berry contain viable lactic acid producing bacteria that colonizes in the human intestine. In addition, it enables the sustenance of enzyme source and stability by continuous multiplication. Probiotic bacteria from fermented foods recorded high phytate degradation (Table S-1, see supplementary material). Fermented soybean containing *Bacillus subtilis* produced phytase activities ranging up to 1,354,906.6 U/mL.

Lower phytase, phosphatase activities were recorded for *Lactobacilli* sp. grown in sweet potato base medium (SPM) with sodium phytate, *p*-nitrophenyl phosphate as substrate. *Lactobacillus casei* 1 K produced highest phosphatase activity of 162,119.2 U/mL with 0.48% phytate hydrolysis.

The level of phytase activity in vivo depends on factors like; the concentration of phytate, bacterial viability, optimum pH, the accessibility of phytate, presence of inorganic phosphate and other organic acids. Human intestine normally maintains a constant temperature of 37 °C with pH ranging from 5 to 7. Probiotic bacteria isolated from humans showed efficient phytate degradation (Table S-2, see supplementary material).

Bifidobacterium sp. BIF *longum* 12R, *catenulatum* 31S and *breve* 211 were isolated from human infants and adults after consumption of whole wheat bread. They produced high specific phytase activities of 6.92, 6.59 and 6.57 U/mg at pH 7.2. *L. reuteri* CECT 9025 produced the highest phosphatase activity in modified MRS medium containing sodium phytate.

In addition to above-mentioned parameters, molecular mass of phytase, substrate specificity and the presence of minerals also influence phytate degradation in the human intestine.

Microbial phytases may be monomeric or dimeric depending on the conformational state of the enzyme. The characteristics of phytases and phosphatases from probiotic bacteria are tabulated (Table 2), where most of them contain monomeric chains. The exception being *L. brevis,* which has dimeric chains each with different molecular masses 73 and 34 kDa, respectively.

Table 1 Fermented food products as the source of probiotics

Microorganisms	Fermented foods	References
Bacillus subtilis (natto) N-77, *B. subtilis* CF92, *Bacillus coagulans* IDCC 1201	Commercial natto (traditional Japanese food), fermented soybean, pharmaceuticals	[10–12]
Lactobacillus strains *acidophilus* 16A, *brevis* 14G, *fermentum* 6E, *plantarum* DC400	Italian sourdough	[13]
L. acidophilus BS, *L. casei* 1 K, *L. casei* DSM20011, *L. fermentum* DSM 20052, *L. plantarum* 110	Commercial fermented milk, cheese, fermented beets, fermented plant food	[14]
L. acidophilus $1C_5$, $4C_1$ and $1C_3$ and $5C_2$, *L. casei*Immunitass $4D_2$, $4D_1$ and *Lactobacillus rhamnosus* $4C_3$, $2C_2$, T, $3C_{15}$ and $3C_{23}$, *L. casei*Shirota $6D_1$, $6D_2$, *Bifidobacterium longum* T, *Lactobacillus delbreuckii* $5D_1$, *Bifidobacterium bifidum* MF, *L. plantarum* $6C_1$, $1D_1$, $3C_{12}$, $1D_2$, $6C_3$, $1C_1$, $6C_4$, 6C5	Dairy and pharmaceutical products	[15]
L. acidophilus, *L. fermentum* and *L. plantarum*	Fermented sorghum–Irish potato gruel	[16]
L. casei, *L. fermentum*, *L. plantarum* and *Pediococcus pentosaceus*	(Eragostis tef) Atmit	[17]
L. casei NRRL B-1445, *L. delbrueckii* NRRL B-445, *L. fermentum* NRRL B-4524, *L. fermentum* NRRL B-4524, *Leuconostoc mesenteriodes* NRRL B-512F, *P. pentosaceus* NRRL B-14009 and *L. plantarum* NRRL B-4496	Natural vegetable fermentation	[18]
L. casei MF50, 54, *L. fermentum* MF25, *P. pentosaceus* MF32, 33 and 35 and *L. plantarum* MF79	Ethiopian injera (African soft pancake)	[19]
Enterococcus sp. *hirae*, *faecium* and *durans*	Indian fermented soybean foods	[20]
E. faecium RJ16	Goat cheese	[21]
E. faecium A86, *L. plantarum* H5, *L. plantarum* L3	Pizza dough, sourdough, sausages	[22]
L. brevis, *L. plantarum*	Southern Italian sourdough	[23]
L. brevis, P. pentosaceus, *E. durans* and *L. plantarum*	Fermented Himalayan vegetables	[24]
L. brevis	Hatay boiled cheese	[25]
Lactobacillus reuteri and *L. plantarum*	Iranian Sangak bread	[26]
Lactobacillus pentosus, *L. fermentum*, *L. brevis* and *L. plantarum* Lb 29, L10	Carper berry	[27]
L. plantarum strains 17bp30, 17bp31, 17bp48, Mb25, Mb26, 17bp29, Mb46, Mb50, Mb61, Mb67 and *Lactobacillus paracasei* strains C3-70, C3-89	Spanish farmhouse cheese	[28]
L. brevis G11, G25, *L. fermentum* N33, N25 and *L. plantarum* A6	Fermented corn	[29]
L. plantarum, *L. mesenteriodes*	Moroccan sourdough bread	[30]
L. plantarum	Greek dry fermented sausages	[31]
L. acidophilus, L. plantarum and *L. mesenteroides*	Iranian Sangak bread	[32]
L. plantarum	Fermented food (Shalgam)	[33]
L. plantarum, *L. mesenteroides*	Italian Cornetto di Matera sourdough	[34]
P. pentosaceus KTU05-8 and KTU05-9	Wholemeal wheat bread	[35]
L. acidophilus EF7, *L. plantarum* 299v, *L. rhamnosus* GG B103, *L. reuteri* M 14-C, CF2 7-F, DSM 20016, SD 2112, MM7 and MM2-3	Sweet potato	[36]
L. plantarum DPC2739, *L. plantarum* W723	Dairy products, Sorghum-Ogi	[37, 38]

Phosphorylated compounds are generated during digestion of food, mineral metabolism and their presence in the intestinal cavity may interfere with phytate degradation. Most of the bacterial phytases are highly specific towards sodium phytate.

Functional foods contain a good amount of trace elements and minerals important for human nutrition. These metal ions in different concentrations might affect probiotic phytate degradation. The effect of different minerals on phytase activity are tabulated (Table 3).

Ca^{2+} plays an important role in stimulating, stabilizing bacterial phytases at optimum concentrations. However, excess Ca^{2+} can lead to competitive inhibition of enzymatic activity by binding to the enzymatic active site. The presence of inorganic phosphate also inhibits phytate degradation.

Role of Recombinant Phytases and Their Expression

Oral consumption of fermented food products and FMT are effective strategies for probiotic delivery. However, these strategies are associated with certain disadvantages including lack of site specific action and short-term efficacy. This may be overcome by recombinant phytases (Table 4).

Table 2 Characteristics of probiotic phytases and phosphatases

Enzyme	Microorganisms	Substrate specificity	Molecular mass (kDa)	References
Phytase	*B. subtilis* (natto) N-77	Sodium phytate	38	[10]
	B. subtilis CF92	Sodium phytate (high), α-naphthyl phosphate and Adenosine triphophosphate (ATP) (low)	46	[11]
	B. coagulans IDCC 1201	Sodium phytate	–	[12]
	L. brevis	–	73, 34	[25]
	L. plantarum	–	46	[33]
Phosphatase	*L. plantarum* NRRL B-4496	Acetyl phosphate, sodium phytate, *p*-nitrophenyl phosphate and α-D-glucose-1 phosphate	–	[18]
		–	52	[29]
	L. plantarum DPC2739	–	27	[37]

Table 3 Effect of minerals on phytate degradation

Microorganisms	Activators	Inhibitors	References
B. subtilis (natto) N-77	Ca^{2+}	Zn^{2+}, Cd^{2+}, Ba^{2+}, Cu^{2+}, Fe^{3+} and Al^{3+}	[10]
B. subtilis CF92	–	Mn^{2+}, Zn^{2+}, Fe^{2+}, Cu^{2+}, Mg^{2+} and Co^{2+}	[11]
B. coagulans IDCC 1201	Co^{2+}	–	[12]
L. plantarum	–	Ca^{2+}, Hg^{2+}, Mg^{2+}, Mn^{2+}, Zn^{2+}, Ni^{2+}, Cu^{2+}, Co^{2+} and Fe^{2+}	[33]
L. plantarum DPC2739		Fluoride, hexametaphosphate at 0.5 mM and orthophosphate, tripolyphosphate and pyrophosphate at 5 mM (phosphatase)	[37]

Probiotics belonging to *Bacilli* genera are efficiently utilized for expression of cloned phytases. Phytase from *B. licheniformis*, when expressed in *E. coli* efficiently degraded phytate producing a phytase activity of 0.96 U/mL with a recombinant protein having a molecular mass of 66 kDa.

Lactic acid bacteria (LAB) can be effectively used for recombinant phytase expression [44]. They are safe, cost-effective and also yield enzyme with high purity and stability. On the contrary, high species diversity, obscurity in the route of administration, and stringent monitoring due to lack of clinical trial data pose critical challenges in employing these recombinant probiotic phytases [45].

Well-known probiotics belonging to genus *Bacilli* and *Lactobacilli* are capable of adherence and colonization in the human intestine. Most often they are inferred by their high cell counts. They produce bacteriocins, which competitively inhibit pathogens thereby aiding overall improvement of gut microbiome. Moreover, consumption of fermented foods containing viable bacteria provides a scope for phytate degradation.

Conclusion and Future Scope

Phytases from probiotics provide a solution for phosphate utilization in humans. This can be achieved by consuming fermented food products, an ideal vehicle for delivering probiotics. It was noted that *L. brevis* and *B. subtilis* in fermented food products are capable of producing higher amount of phytase. Most of the probiotic phytases are very specific towards phytate and are stimulated by Ca^{2+} ions for effective activity. Alternatively, recombinant phytases can also be used for intestinal phytate utilization. Despite the wide usage of probiotics, it is yet to be approved by US Food and Drug Administration (FDA). Therefore, more research needs

Table 4 Recombinant phytases

Source of phytase gene	Vector plasmid	Host microorganism	References
B. subtilis NCDC-070, NCIM-2712	Ins T/A	*E. coli* JM-109	[39]
B. longum subsp. *infantis* and *B. pseudocatenulatum*	pNGPHYpseudso, pNGPHYlongum	*L. casei*	[40]
B. licheniformis PB-13	pET32a(+)	*E. coli* BL21	[41]
E. coli	pET22b	*Pichia pastoris*	[42]
B. amyloliquefaciens DSM 1061		*B. amyloliquefaciens* DSM 1061	[43]

to be done on improvising probiotic strains for phytase production as well as optimizing probiotic dosage for phytate utilization.

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Compliance with Ethical Standards

Conflict of interest None.

References

1. FAO and WHO (2002) Joint working group report, "Guidelines for the evaluation probiotics in food"
2. Bohn L, Meyer AS, Rasmussen SK (2008) Phytate: impact on environment and human nutrition. A challenge for molecular breeding. J Zhejiang Univ Sci B 9:165–191. doi:10.1631/jzus.B0710640
3. Lei XG, Porres JM, Mullaney EJ, Brinch-Pedersen H (2007) Phytase: source, structure and application. In: Polaina J, MacCabe AP (eds) Industrial enzymes-structure, function and applications. Springer, Netherlands, pp 505–529
4. Shin S, Ha NC, Oh BC, Oh TK, Oh BH (2001) Enzyme mechanism and catalytic property of beta propeller phytase. Structure 9:851–858
5. Konietzny U, Greiner R (2004) Bacterial phytase: potential application, in vivo function and regulation of its synthesis. Braz J Microbiol 35:11–18. doi:10.1590/S1517-83822004000100002
6. Shewale RN, Sawale PD, Khedkar CD, Singh A (2014) Selection criteria for probiotics: a review. Int J Probiotics Prebiotics 9:17–22
7. Hudson LE, Anderson SE, Corbett AH, Lamb TJ (2017) Gleaning insights from fecal microbiota transplantation and probiotic studies for the rational design of combination microbial therapies. Clin Microbiol Rev 30:191–231. doi:10.1128/CMR.00049-16
8. Lee WJ, Lattimer LDN, Stephen S, Borum ML, Doman DB (2015) Fecal Microbiota Transplantation: a review of emerging indications beyond relapsing *Clostridium difficile* toxin colitis. Gastroenterol Hepatol 11:24–32
9. Baxter M, Colville A (2016) Adverse events in faecal microbiota transplant: a review of the literature. J Hosp Infect 92:112–127. doi:10.1016/j.jhin.2015.10.024
10. Shimizu M (1992) Purification and characterization of phytase from *Bacillus suhtilis* (natto) N–77. Biosci Biotechnol Biochem 56:1266–1269. doi:10.1271/bbb.56.1266
11. Hong SW, Chu IH, Chung KS (2011) Purification and biochemical characterization of thermostable phytase from newly isolated *Bacillus subtilis* CF92. J Korean Soc Appl Biol Chem 54:89–94. doi:10.3839/jksabc.2011.012
12. Lee SH, Kwon HS, Koo KT, Kang BH, Kim TY (2006) Characterization of phytase from *Bacillus coagulans* IDCC 1201. Korean J Microbiol Biotechnol 34:28–34
13. De Angelis M (2003) Phytase activity in sourdough lactic acid bacteria: purification and characterization of a phytase from *Lactobacillus sanfranciscensis* CB1. Int J Food Microbiol 87:259–270. doi:10.1016/S0168-1605(03)00072-2
14. Haros M, Bielecka M, Honke J, Sanz Y (2008) Phytate-degrading activity in lactic acid bacteria. Pol J Food Nutr Sci 58:33–40
15. Khodaii Z, Mehrabani Natanzi M, Naseri MH, Goudarzvand M, Dodson H, Snelling A (2013) Phytase activity of lactic acid bacteria isolated from dairy and pharmaceutical probiotic products. Int J Enterpathog 1:12–16. doi:10.17795/ijep9359
16. Adegbehingbe KT (2015) Effect of starter cultures on the anti-nutrient contents, minerals and viscosity of Ogwo, a fermented sorghum–Irish potato gruel. Int Food Res J 22:1247–1252
17. Urga K, Keshava N, Narasimha HV (1997) Effects of natural and mixed culture of lactobacilli fermentation on in vitro iron and zinc bioavailability in tef (*Eragrostis tef*) atmit. Bull Chem Soc Ethiop 11:101–109. doi:10.4314/bcse.v11i2.21018
18. Zamudio M, Gonzalez A, Medina J (2001) *Lactobacillus plantarum* phytase activity is due to non-specific acid phosphatase. Lett Appl Microbiol 32:181–184
19. Fischer MM, Egli IM, Aeberli I, Hurrell RF, Meile L (2014) Phytic acid degrading lactic acid bacteria in tef-injera fermentation. Int J Food Microbiol 190:54–60. doi:10.1016/j.ijfoodmicro.2014.08.018
20. Chettri R, Tamang JP (2014) Functional properties of Tungrymbai and Bekang, naturally fermented soybean foods of North East India. Int J Fermented Foods 3:87–103. doi:10.5958/2321-712X.2014.01311.8
21. Abriouel H, Lucas R, Ben Omar N, Valdivia E, Maqueda M, Martínez-Cañamero M, Gálvez A (2005) Enterocin AS-48RJ: a variant of enterocin AS-48 chromosomally encoded by *Enterococcus faecium* RJ16 isolated from food. Syst Appl Microbiol 28:383–397. doi:10.1016/j.syapm.2005.01.007
22. Anastasio M, Pepe O, Cirillo T, Palomba S, Blaiotta G, Villani F (2010) Selection and use of phytate-degrading LAB to improve cereal-based products by mineral solubilization during dough fermentation. J Food Sci 75:M28–M35. doi:10.1111/j.1750-3841.2009.01402.x
23. Reale A, Mannina L, Tremonte P, Sobolev AP, Succi M, Sorrentino E, Coppola R (2004) Phytate degradation by lactic acid bacteria and yeasts during the wholemeal dough fermentation: a 31 P NMR study. J Agric Food Chem 52:6300–6305. doi:10.1021/jf049551p
24. Tamang JP, Tamang B, Schillinger U, Guigas C, Holzapfel WH (2009) Functional properties of lactic acid bacteria isolated from

ethnic fermented vegetables of the Himalayas. Int J Food Microbiol 135:28–33. doi:10.1016/j.ijfoodmicro.2009.07.016
25. Sumengen M, Dincer S, Kaya A (2012) Phytase production from *Lactobacillus brevis*. Turk J Biol 36:533–541. doi:10.3906/biy-1111-2
26. Didar Z, Khodaparast MHH (2011) Effect of different lactic acid bacteria on phytic acid content and quality of whole wheat toast bread. JFBT 1:1–10
27. Pulido RP, Omar NB, Abriouel H, López RL, Cañamero MM, Guyot J, Gálvez A (2007) Characterization of lactobacilli isolated from caper berry fermentations. J Appl Microbiol 102:583–590. doi:10.1111/j.1365-2672.2006.03067.x
28. Lavilla-Lerma L, Pérez-Pulido R, Martínez-Bueno M, Maqueda M, Valdivia E (2013) Characterization of functional, safety, and gut survival related characteristics of Lactobacillus strains isolated from farmhouse goat's milk cheeses. Int J Food Microbiol 163:136–145. doi:10.1016/j.ijfoodmicro.2013.02.015
29. Roger T, Léopold TN, Funtong MCM (2015) Nutritional properties and antinutritional factors of corn paste (Kutukutu) fermented by different strains of lactic acid bacteria. Int J Food Sci. doi:10.1155/2015/502910
30. Chaoui A, Faid M, Belhcen R (2003) Effect of natural starters used for sourdough bread in Morocco on phytate biodegradation. East Mediterr Health J 9:141–147
31. Papamanoli E, Tzanetakis N, Litopoulou-Tzanetaki E, Kotzekidou P (2003) Characterization of lactic acid bacteria isolated from a Greek dry-fermented sausage in respect of their technological and probiotic properties. Meat Sci 65:859–867. doi:10.1016/S0309-1740(02)00292-9
32. Najafi MA, Rezaei K, Safari M, Razavi SH (2012) Use of sourdough to reduce phytic acid and improve zinc bioavailability of a traditional flat bread (Sangak) from Iran. Food Sci Biotechnol 21:51–57. doi:10.1007/s10068-012-0007-3
33. Sumengen M, Dincer S, Kaya A (2013) Production and characterization of phytase from *Lactobacillus plantarum*. Food Biotechnol 27:105–118. doi:10.1080/08905436.2013.781507
34. Zotta T, Ricciardi A, Parente E (2007) Enzymatic activities of lactic acid bacteria isolated from Cornetto di Matera sourdoughs. Int J Food Microbiol 115:165–172. doi:10.1016/j.ijfoodmicro.2006.10.026
35. Cizeikiene D, Juodeikiene G, Bartkiene E, Damasius J, Paskevicius A (2015) Phytase activity of lactic acid bacteria and their impact on the solubility of minerals from wholemeal wheat bread. Int J Food Sci Nutr 66:736–742. doi:10.3109/09637486.2015.1088939
36. Hayek SA, Shahbazi A, Worku M, Ibrahim SA (2014) Enzymatic activity of *Lactobacillus* grown in a sweet potato base medium. Br Microbiol Res J 4:509–522
37. Magboul AA, McSweeney PL (1999) Purification and characterization of an acid phosphatase from *Lactobacillus plantarum* DPC2739. Food Chem 65:15–22
38. Onipede GO, Banwo K, Ogunreni OR, Sanni A (2014) Influence of starter culture lactic acid bacteria on the phytic acid content of Sorghum-Ogi (an indigenous cereal gruel). Ann Food Sci Tech 15:121–134
39. Bawane R, Tantwai K, Rajput LPS, Kadam-Bedekar M, Kumar S, Gontia I, Tiwari S (2011) Molecular analysis of phytase gene cloned from *Bacillus subtilis*. Adv Stud Biol 3:103–110
40. García-Mantrana I, Monedero V, Haros M (2015) Reduction of phytate in soy drink by fermentation with *Lactobacillus casei* expressing phytases from Bifidobacteria. Plants Food Hum Nutr 70:269–274. doi:10.1007/s11130-015-0489-2
41. Kumar V, Sangwan P, Verma AK, Agrawal S (2014) Molecular and biochemical characteristics of recombinant β-Propeller phytase from *Bacillus licheniformis* strain PB-13 with potential application in Aquafeed. Appl Biochem Biotechnol 173:646–659. doi:10.1007/s12010-014-0871-9
42. Wua T, Chen C, Cheng Y, Ko T, Lin C, Lai H, Huang T, Liu J, Guo R (2014) Improving specific activity and thermostability of *Escherichia coli* phytase by structure-based rational design. J Biotechnol 175:1–6. doi:10.1016/j.jbiotec.2014.01.034
43. Xu W, Shao R, Wang Z, Yan X (2015) Improving the neutral phytase activity from *Bacillus amyloliquefaciens* DSM 1061 by site-directed mutagenesis. Appl Biochem Biotechnol 175:3184–3194. doi:10.1007/s12010-015-1495-4
44. Garcia-Fruitos E (2012) Lactic acid bacteria: a promising alternative for recombinant protein production. Microb Cell Fact 11:157. doi:10.1186/1475-2859-11-157
45. Wyszyńska A, Kobierecka P, Bardowski J, Jagusztyn-Krynicka EK (2015) Lactic acid bacteria—20 years exploring their potential as live vectors for mucosal vaccination. Appl Microbiol Biotechnol 99:2967–2977. doi:10.1007/s00253-015-6498-0