ELBA BIOFLUX
Extreme Life, Biospeology & Astrobiology
International Journal of the Bioflux Society

Potential application of microbial phytase in aquaculture

[1]Christopher M. A. Caipang, [2]Rande B. Dechavez, and [3]Mary Jane A. Amar

[1]Faculty of Biosciences and Aquaculture, University of Nordland, Bodø 8049, Norway. [2]College of Fisheries, Sultan Kudarat State University, Kalamansig, Sultan Kudarat 9808, Philippines. [3]National Institute of Molecular Biology and Biotechnology, University of the Philippines Visayas, Miag-ao 5023, Iloilo, Philippines.
Corresponding author: C. M. A. Caipang, cmacaipang@yahoo.com

Abstract. Phytases are histidine acid phosphatases that catalyze the hydrolysis of phytic acid to inorganic phosphorus and *myo*-inositol phosphates. The phosphorus that is being released is used in various metabolic processes. These enzymes are able to promote the growth of animals and plants as well as increase the nutritional value of feedstuffs through dephytinization. Phytases are widespread in nature because they are found in animals, plants and microorganisms. This paper discusses the different microbial phytases that have been characterized and their beneficial roles in aquaculture. In order to be incorporated in the feed, microbial phytases must have the ability to withstand high temperatures during processing, and to be stable in the gastrointestinal tract of the fish, the enzymes must be active at low pH levels. The various effects of microbial phytases on the bioavailability of phosphorus in fish, on growth performance, on the immune responses, on the excretion of phosphorus and on the environment are also reviewed.
Key Words: phytase, bacteria, bacterial enzyme, aquaculture.

Introduction. The hydrolysis of phytic acid to *myo*-inositol and inorganic phosphate by phytases (*myo*-inositol hexakisphosphate phosphohydrolase) is an important reaction in most biological systems. Phytic acid (*myo*-inositolhexakis phosphate) is an organic form of phosphorus (P) that is abundantly present in plants materials such as legumes, cereals, oilseeds and nuts (Vats & Banerjee 2004; Rao et al 2009). The presence of phytic acid in plant-derived food acts as an anti-nutritional factor because it causes mineral deficiency due to the chelation of metal ions such as Ca^{2+}, Mg^{2+}, Zn^{2+} and Fe^{2+} with proteins, thus affecting their digestion (Harland & Morris 1995). It also inhibits the activities of digestive enzymes such as α-amylase, trypsin, acid phosphatase and tyrosinase.

The ruminants digest phytic acid through the action of phytases produced by the anaerobic gut fungi and bacteria present in their intestinal microflora. On the other hand, monogastric animals such as pig, chicken and fish utilize phytate phosphorus poorly because they are deficient of phytases in their gastrointestinal tract. Due to the lack of adequate levels of phytases in monogastric animals, phytic acid is excreted in the feces. Therefore, inorganic phosphate is added to their feed to meet the phosphate requirement and to ensure good growth. The antinutritive effect of phytic acid is problematic in the feeding of fish (Richardson et al 1985), due to its short gastrointestinal tract and thus limiting the use of plant-derived protein in fish feed. These problems could be solved by the hydrolysis of phytate through the use of supplemental phytase (Simell et al 1989). Therefore, phytase has become an important industrial enzyme and is the subject of extensive research. By working efficiently on the substrate under optimum conditions, supplemental phytase could diminish the antinutritive effects of phytic acid and reduce the cost of diets by removing or at least reducing the need for supplemental inorganic phosphate. In addition, phytase could be an environmentally friendly product by reducing the amount of phosphorus that enters the environment (Kerovuo 2000).

During the last few years, phytases have attracted significant attention in the areas of nutrition, environmental protection, and biotechnology. Increased public concern regarding the environmental impact of high phosphorus levels in animal wastes and uneated feed has resulted in intensive biotechnological development of phytase and its application in animal nutrition. Feeding trials have shown the effectiveness of supplementing microbial phytases for the increased utilization of phytate-P as well as phytate-bound minerals by various animals including fish (Lei & Stahl 2001; Cao et al 2007; Selle & Ravindran 2007; Rao et al 2009). As a result, the phosphorus excretion of these animals can be significantly reduced (Lei et al 1993; Vohra et al 2006).

Recent reviews have mainly focused on the production, characteristics and the basic applications of phytases (Greiner & Konietzny 2006; Kaur et al 2007; Fu et al 2008; Rao et al 2009). However, no such reviews have been done on the application of microbial phytases in aquaculture. Hence, this mini-review documents the different studies on microbial phytases and their beneficial effects on fish.

Phytic acid, its sources and physiological functions. Phytic acid (*myo* – inositol hexakis dihydrogen phosphate) is the major storage form of phosphorus in cereals, pollen, legumes and oilseeds. The chemical description of phytic acid is *myo*-inositol 1, 2, 3, 4, 5, 6-hexakis dihydrogen phosphate (IUPAC-IUB 1977) and the salts of phytic acid are described as phytates. It is considered to be an antinutritional factor since it chelates minerals such as magnesium, zinc and calcium and may also react with proteins, therefore decreasing the bioavailability of protein and nutritionally important minerals.

Phytic acid occurs primarily as salts of mono- and divalent cations, *e.g.*, potassium magnesium salt in rice and calcium-magnesium-potassium salt in soybeans. It accumulates in seeds and grains during ripening and is incorporated with other storage substances such as starch and lipids. In cereals and legumes, phytic acid accumulates in the aleurone particles and globoid crystals, respectively (Reddy et al 1989). The endosperm of wheat and rice kernels is almost devoid of phytate, as it concentrates in the germ and aleurone layers of the cells of the kernel. Ferguson & Bollard (1976) found that 99% of the phytate in dry peas was in the cotyledons and 1% in the embryo taxis. The highest amount of phytate among cereals is found in maize, and among legumes in dolique beans (Reddy et al 1989).

The physiological roles of phytic acid in plant seeds are the following: 1) acts as storage of phosphorus, 2) source of energy, 3) source of cations, 4) source of *myo*-inositol, which is a cell wall precursor, and 5) acts as an initiator of dormancy (Reddy et al 1989). Phytic acid also acts as a natural antioxidant in seeds during dormancy (Graf et al 1987). This antioxidant property of phytic acid is based on the assumption that it effectively blocks iron-driven hydroxyl radical formation. Phytic acid has been shown to exert an antineoplastic effect in animal models of both colon and breast carcinomas (Dvorakova 1998). The presence of undigested phytic acid in the colon may protect against the development of colonic carcinoma.

Pallauf & Rimbach (1996) have demonstrated that phytic acid showed strong antinutritive effect due to its unusual structure. At complete dissociation, the six phosphate groups of phytic acid carry a total of twelve negative charges. Therefore, phytic acid effectively binds different mono-, di-, and trivalent cations and their mixtures, forming insoluble complexes (Reddy et al 1989). The formation of insoluble phytate mineral complexes in the intestinal tract prevents mineral absorption, thus reducing reducing their bioavailability (Davies 1982). Zinc appears to be the trace element of which its bioavailability is most influenced by phytic acid. Phytic acid interacts with proteins over a wide pH range, forming phytate-protein complexes. At a low acidic pH, phytic acid has a strong negative charge due to total dissociation of the phosphate groups. Under these conditions a negative influence of phytic acid on the solubility of proteins is observed because of the ionic binding between the basic phosphate groups of phytic acid and protonized amino acid (lysyl, histidyl and arginyl) residues (Fretzdorff et al 1995). Under acidic conditions, phytic acid is tightly bound to plant proteins, because the isoelectric point of plant proteins is generally around pH 4.0 - 5.0. In the intermediate pH range (6.0 to 8.0) both phytic acid and plant proteins have a net

negative charge. However, under these conditions complex formation occurs between phytic acid and proteins. Possible mechanisms include direct binding of phytic acid to the -NH2 terminal groups groups of lysine residues and multivalent cation-mediated interactions (Cheryan 1980). By binding to plant proteins, phytic acid has lower solubility and digestibility, thereby reducing the nutritive value.

In addition to complexing with minerals and proteins, phytic acid interacts with enzymes such as trypsin, pepsin, amylase and galactosidase, resulting in a decreased activity of these important digestive enzymes (Singh & Krikorian 1982; Inagawa et al 1987).

Phytase, its sources and biological activities. Phytase is an enzyme known as *myo*-inositol-hexaphosphate phosphohydrolase (Class 3: hydrolases) and is produced either by microorganisms or present in some plants (Simons et al 1990). It catalyzes the hydrolysis of *myo*-inositol hexakisphosphate (phytic acid) to inorganic monophosphate and lower *myo*-inositol phosphates, or in some cases to free *myo*-inositol. The Enzyme Nomenclature Committee of the International Union of Biochemistry distinguishes two types of phytase, the 3- phytase (EC 3.1.3.8) and 6-phytase (EC 3.1.3.26). 3-Phytase is typical for microorganisms and 6-phytase for plants. Microbial phytase is an enzyme that is used to hydrolyze phytate in plant protein meal-based animal feeds. Table 1 shows the different microbial phytases that have been characterized.

Table 1

Optimum culture conditions of different microorganisms for the production and/or activity of phytases

Species	Optimum		Reference
	pH	Tempt	
Yeasts			
Arxula adeninivorans	5.5	28	Sano et al (1999)
Pichia anomala	6.0	25	Vohra & Satyanarayana (2002)
P. rhodanensis	4.5	70	Nakamura et al (2000)
P. spartinae	4.5	75	Nakamura et al (2000)
Schwanniomyces castellii	4.4	77	Segueilha et al (1992)
Bacteria			
Bacillus subtilis	7.0	37	Kerovuo (2000)
B. amyloliquefaciens	6.8	37	Idriss et al (2002)
B. pumilus	6.0	35	Dechavez et al (2011)
B. megaterium	6.0	35	Dechavez et al (2011)
B. coagulans	6.0	35	Dechavez et al (2011)
B. licheniformis	6.0	35	Dechavez et al (2011)
Escherichia coli	7.0	37	Greiner et al (1993)
Klebsiella aerogenes	7.0	30	Tambe et al (1994)
Pseudomonas sp.	7.0	15	Lazado et al (2010)
Psychrobacter sp.	7.0	15	Lazado et al (2010)

Phytases are also broadly categorized into two major classes based on the pH for activity: acid phytases and alkaline phytases. Acidic phytases have been given more attention because of their applicability in animal feeds and broader substrate specificity than those of alkaline phytases. Recently, phytases have also been classified as HAP (Histidine acid phosphatase), BPP (β-Propeller phytase), CP (cysteine phosphatase) and PAP (purple acid phosphatase) based on their catalytic properties (Mullaney & Ullah 2003). Phytase is present in grains and oilseeds and in the digestive tract of animals, but its activity is usually not high enough to release phosphorus from phytate to any extent (Bitar & Reinhold 1972; Lall 1991).

Monogastric animals cannot produce this enzyme. Presence of phytase in some animals is of microbial origin. In the case of fish, phytase has been detected from gut-associated microbiota (Roy et al 2009; Lazado et al 2010). Microbial phytase either as a dry powder or as a liquid is available commercially. Commercialization requires not only a practical use and delivery of the enzyme but also the ability to produce the enzyme economically. So far, large scale production of cheap phytase for animal feed has been solved through fermentation of genetically modified organisms (Yao & Fan 2000). One key problem that has been solved is instability of the phytase upon heating. Phytase cannot withstand high temperature. Schwarz & Hoppe (1992) found that pelleting a diet with phytase supplementation at 70% reduces the activity by 15 – 25%.

Phytases are widely present in the plant kingdom. They have been isolated and characterized from cereals and beans. Phytase activity has also been detected in white mustard, potato, radish, lettuce, spinach, grass and lily pollen (Dvorakova 1998). To a certain extent, traces of of phytase activity has been detected in monogastric animals (Copper & Gowing 1983; Chi et al 1999). Generally, intestinal phytase does not play a significant role in food-derived phytate digestion in monogastric animals (Williams & Taylor 1985). A phytase-like enzyme was also described in the protozoan *Paramecium* (Freund et al 1992).

Microbial phytase activity is most frequently detected in fungi such *Aspergillus* spp. Shieh & Ware (1968) screened over 2000 microorganisms isolated from soil for phytase production. Most of the positive isolates produced only intracellular phytase, and extracellular phytase activity was observed only in 30 isolates. All extracellular phytase producers are filamentous fungi. Twenty-eight belong to the genus *Aspergillus*, one to *Penicillum* and one to *Mucor*. Of the 28 phytase-producing *Aspergillus* isolates 21 belong to the *A. niger* group. Other studies have also demostrated that *A. niger* strains were the best producers of extracellular phytase (Howson & Davis 1983; Volfova et al 1994).

There are various bacteria that have been detected for phytase production, *e.g*. *Aerobacter aerogenes* (Greaves et al 1967), *Pseudomonas* sp. (Irving & Cosgrove 1971; Lazado et al 2010), *Bacillus subtilis* (Powar & Jagannathan 1982), *Klebsiella* sp. (Shah & Parekh 1990), *Escherichia coli* (Greiner et al 1993), *Enterobacter* sp. (Yoon et al 1996) and *Psychrobacter* sp. (Lazado et al 2010). The only bacteria producing extracellular phytase are those of the genera *Bacillus* and *Enterobacter*. The phytase from *E. coli* is a periplasmic enzyme.

The optimum pH levels of phytases vary from 2.2 to 8. Most microbial phytases, particularly those of fungal origin, have optimum pH values between 4.5 and 5.6. Some bacterial phytases, especially those from *Bacillus*, optimum pH levels at 6.5 - 7.5. A study done by Dechavez et al (2011) showed that the optimum pH of phytase obtained from the different *Bacillus* strains ranged from 5.5 to 7. The optimum pH values of plant seed phytases range from 4.0 to 7.5, most having an optimum value between 4.0 and 5.6. Two alkaline plant phytases having an optimum pH value at about 8.0 have been described in legume seeds (Scott 1991) and lily pollen (Hara et al 1985).

The optimum temperature of phytases varies from 45 to 77^{o} C. Wyss and co-workers (1998) studied the thermostability of three acid phosphatases of fungal orgin (*A. fumigatus* and *A. niger* phytase, and *A. niger*) by circular dichroism (CD) spectroscopy and fluorescence, and by measuring the enzymatic activity. They concluded that *A. niger* phytase is not thermostable, neither it has the capacity to refold after heat denaturation. At temperatures between 50 and 55^{o}C it undergoes an irreversible conformational change that result in 70-80% loss of enzyme activity. Compared to two phytases, *A. niger* had higher intrinsic thermostability. At temperatures up to 80^{o}C, only minor changes in CD spectral characteristics and only slight, but irreversible enzyme inactivation were observed. However, exposure to 90^{o}C resulted in an irreversible conformational change and complete loss of activity. *Bacillus* sp. strain DS11 phytase (Kim et al 1998) has an optimum temperature at 70^{o} C, which is higher than the optimum temperature values of most of phytases. It is also termostable, *i.e.*, there was 100% residual activity after 10 min incubation at 70^{0} C, in the presence of $CaCl_2$. The enzyme stability of the phytase from *Bacillus* sp. strain DS11 was drastically reduced above 50^{o}C in the absence of $CaCl_2$, whereas it was rather stable up to 90^{o}C in the

presence of $CaCl_2$. The phytase of the different *Bacillus* strains that were studied showed that the enzyme had maximum activity at a 37°C and retained its activity even at 80°C (Dechavez et al 2011). In addition, 50% of the activity was retained at 70°C.

As to their activities, phytase from *Enterobacter sp* is inhibited by Zn^{2+}, Ba^{2+}, Cu^{2+} and Al^{3+} (Yoon et al 1996). Similarly, the phytase from *B. subtilis* strain N-77 was inhibited by metal ions including Zn^{2+}, Cd^{2+}, Ba^{2+}, Cu^{2+}, Fe^{2+}, and Al^{3+} (Shimizu 1992). Both of these enzymes, as well as two other phytases from *Bacillus* spp. (Kim et al 1998; Powar & Jagannathan 1982), were inhibited by EDTA, indicating that a metal ion (calcium) is needed for their activity. Wyss et al (1999) reported that Cu^{2+} considerably reduced the enzyme activities of phytases from *E. nidulans* and *A. terrus*, and that several metal ions inhibited *A. fumigatus* phytase. The activity of *A. fumigatus* phytase was stimulate by EDTA up to 50%, whereas EDTA had no major effects on the enzymatic activities of the other fungal phytases that were tested such as *E. nidulans*, *A. niger* and *A. terrus*.

In addition to the calcium-dependent *Bacillus* phytases, a phytase obtained from the pollen of *Typha latifolia* and phytases from some other plants require Ca^{2+} for optimal activity (Laboure et al 1993; Scott & Loewus 1986).

A structural analog of the substrate, *myo*-inositol hexasulfate, has been shown to be a potent competitive inhibitor of both PhyA and PhyB enzymes from *A. ficuum* (Ullah & Sethumadhavan (1998). Fluoride is a known inhibitor of different phytases and phosphatases (Nayini & Markakis 1986). The phytase from cotyledons of germinating soybean seeds was strongly inhibited by fluoride, vanadate and inorganic phosphate (Gibson & Ullah 1988). Inorganic phosphate was a competitive inhibitor of soybean seed phytase. Competitive product inhibiton of phytate hydrolysis caused by inorganic phosphate is recognized for bacterial, fungal and oat spelt phytases (Howson & Davis 1983; Greiner et al 1993; Konietzny et al 1995).

Molybdate and vanadate are known to inhibit phosphatase enzymes. It has been suggested that these transition metal oxoanions inhibit phosphomonoesterases by forming complexes that resemble the trigonal bipyramidal geometry of the transition state (Zhang et al 1997). Fungal phytase activity has been shown to be inhibited by substrate concentrations exceeding 1 mM (Ullah 1988), whereas maize root and soybean phytases were found to be inhibited at 300 μM and 20 mM substrate concentration, respectively (Hubel & Beck 1996; Sutardi & Buckle 1986).

Application of microbial phytase in aquaculture. Soybean meal is the most widely used plant-based meal in animal feed formulations. It is used as a primary plant protein source in animal feeds due to its relatively high crude protein level (>40%), high protein availability, good amino acid compound compared to other oilseed meals, and relatively low cost compared to fish meal. Hardy (1995) pointed out that soybean meal is the most promising alternative protein source for fish feeds in terms of future availability. It is widely studied in fish feed research and its use in commercial fish feeds is increasing (Pongmaneerat & Watanabe 1992; Oliva-Teles et al 1994; Vielma et al 2000). One disadvantage of using plant protein meals such as soybean meal in fish feeds is that approximately two-thirds of the total phosphorus in plant protein meals is present as phytate, hence, the bioavailability of phytate to fish is almost absent (NRC 1993).

Effects on the bioavailability of phosphorus. Addition of phytase to high phytate striped bass diets improved the absorption and utilization of phosphorus (Hughes & Soares (1998). According to Reddy et al (1982) microbial phytase is effective in improving bioavailability of phytate phosphorus, which could be expected since phytase hydrolyzes to orthophosphate. The treatment of fish feed with phytase resulted in the improvement of protein digestibility and retention in fishes (Storebakken et al 1998; Papatryphon et al 1999; Cheng & Hardy 2002; Usmani & Jafri 2002; Vielma et al 2004; Debnath et al 2005; Baruah et al 2005; Ai et al 2007; Hassan et al 2009). Studies showed that adding microbial phytase to diets have been found to increase phytate hydrolysis and the availability of phosphorus and other minerals that are chelated by phytic acid (Lei et al 1993; Adeola et al 1995). Dietary phytase also improved the

nutritive value of canola protein concentrate and decreased phosphorus output in rainbow trout (Forster et al 1999). Several authors reported similar results for different species including rainbow trout (Rodehutscord & Preffer 1995), channel catfish (Li & Robinson 1997), African Catfish (Van Weerd et al 1999), common carp (Schafer et al 1995) and *Pangasius pangasius* (Debnath 2003). Digestibility of phytate phosphorus increased by as much as 50% in common carp fed a diet containing 500 units of microbial phytase kg^{-1} (Schafer et al 1995). Microbial phytase is effective in enhancing the bioavailability of phosphorous considerably, thereby reducing the phosphorous output in the feces.

Effect on the growth performance of fish. The weight gain rates and specific growth rates of Indian major carp, *Labeo rohita* significantly decreased when phytic acid was included in diet at levels above 1 percent (Alvi 1994). Similar effects were evident on the growth performance and body composition of *Cirrhinus mrigala* fry (Usmani & Jafri 2002). It was reported that Chinook salmon, *Oncorhynchus tshawytscha*, fed semi-purified diets containing various levels of calcium, phosphorous, zinc, and sodium phosphate with a high dietary phytic acid (2.58%) exhibited depressed growth (Richardson et al 1985). In contrast, the growth performance increased when microbial phytase was incorporated in the diets. An increase in weight gain was reported in channel catfish fed phytase supplemented diets containing only plant protein or a combination of plant and animal protein sources (Jackson et al 1996). Weight gain and feed composition increased by 23.52 and 11.59%, respectively, compared to the control group. The same trend was also observed in *Pangasius pangasius* (Debnath 2003) and African catfish, *Clarias gariepinus* (Van Weerd et al 1999). The better performance of fish fed phytase-supplemented diets implied that either the phosphorous requirement was met along with other nutrients or that phytase has other positive effect on performance.

Phytase derived from *Aspergillus niger* has been shown to be effective in improving bioavailibity of phytate phosphorous in diets of several animals, including poultry (Simons et al 1990); rainbow trout, *Oncorhychus mykiss* (Cain & Garling 1995); and common carp, *Cyprinus carpio* (Schafer et al 1995). Laboratory studies have shown that a concentration of 250 to 500 FTU (phytase unit) microbial phytase kg^{-1} diet resulted in maximum weight gain and bone phosphorous deposition in channel catfish (Jackson et al 1996; Li & Robinson 1997), and 250 FTU kg^{-1} can effectively replace the dicalcium phosphate supplement in the diet (Li & Robinson 1997).

Results of the study conducted by Jackson et al (1996) in ponds using catfish showed that the level of phytase required for maximum weight gain and bone phosphorus deposition is 500 units phytase kg^{-1} diet. However, the effective level of microbial phytase according to Jackson et al (1996) was lower than the reported levels (500 to 1,000 units kg^{-1}) of microbial phytase that are effective forr other animals (Simons et al 1990; Rodehutscord & Preffer 1995; Schafer et al 1995).

Phytase supplementation increased the apparent absorption of phosphorus, nitrogen (protein), ash, calcium, magnesium, copper, iron, strontium and zinc in low–ash diets containing soybean meal, but had little effect in high–ash diets containing both soybean and fish meal.

Effects on the immune system. Supplementation of microbial phytase in the diets of fish to boost the immune system is an area that is least understood and needs intensive research. Lazado et al (2010) extracted crude phytase from *Pseudomonas* sp. and *Psychrobacter* sp. Using the shake flask method. When these crude phytases were added to primary cultures of Atlantic cod, *Gadus morhua* head kidney leukocytes, the enzymes stimulated cell proliferation, induced higher myeloperoxidase and alkaline phosphatase activites. However, extracellular responses such as respiratory burst activities and hydrogen peroxide production were not affected as well as the ability of the cells to inhibit the proliferatio of pathogenic bacteria, *Vibrio anguillarum* and *Aeromonas salmonicida*. The preliminary results indicated that the crude microbial phytases when used to incubate immune-related cells such as head kidney leukocytes, resulted in enhanced intracelular immune responses but not the extracellular responses.

Effects on fecal phosphorus. Li & Robinson (1997) reported that fecal phosphorus decreased an average of 73% in channel catfish using 250, 500 and 750 units of microbial phytase in comparison to fish fed the basal diet, which did not contain a phosphorus supplement. It appeared supplementation with microbial phytase improved the digestibility of phytate phosphorus.

Effects on the environment. The environmental impact assessment of the aquaculture industry is getting increased attention and rigorous restrictions are being set on this industry by governments and environmentalists. Farmers who are involved in freshwater aquaculture and coastal marine operations are facing increasing pressure from various organizations to control farm discharge into the surrounding waterways. This discharge, particularly high in phosphorous content, leads to eutrophication. The phosphorous in the feed ingredients occurs in number of forms, either in the inorganic form or as phosphate complexes of proteins, lipids and carbohydrates. These forms are available to the fish. Phosphorous that is present in most grain and seed by-products is generally unavailable to finfish and monogastric animals. Fish excrete phosphorous and the phosphates have direct Effect on the water quality. The particulate forms accumulate in the sludge and the phosphorous is released slowly to the water. Dissolved reactive phosphorous is usually regarded as the most important factor that affects water quality, because it is the most available nutrient that is needed for the growth of phytoplankton. Microbial phytase supplementation in the diet of fish can overcome this problem. It makes the chelated phosphorous available to fish and hence there is less fecal excretion, thereby reducing environmental pollution (Baruah et al 2004).

The use of phytase in feeds also reduces or sometimes eliminates the necessity of mineral supplementation, which would decrease the cost of feeds. Although phytase was first used for environmental reasons, it been recently demonstrated that there are other nutritional and health benefits from using these enzymes (Baruah et al 2004).

Conclusions. In summary, microbial phytases have different beneficial roles in aquaculture. When incorporated in the fish diets, these enzymes increased bioavailability of phosphorus, enhanced growth, stimulated the immune response, decreased fecal phosphorus and lessened the risk of environmental degradation. The different microbial phytases that have been characterized to date are physiologically active at wide temperature and pH levels, indicating that they have the potential to be incorporated in the feed during processing and could be estable in the gastrointestinal tract of the fish. Further areas of research in microbial phytases include the development of protocol for their large-scale production and the use of recombinant DNA and protein technologies through cloning of the gene encoding these enzymes and the production of these proteins using various expression systems.

Acknowledgements. The authors would like to thank the National Institute of Molecular Biology and Biotechnology (NIMBB), University of the Philippines Visayas and the Aquatic Animal Health Research Group, Faculty of Biosciences and Aquaculture, University of Nordland for the support provided in the preparation of the manuscript.

References

Ai Q., Mai K., Zhang W., Xu W., Tan B., Zhang C., et al., 2007 Effects of exogenous enzymes (phytase, non-starch polysaccharide enzyme) in diets on growth, feed utilization, nitrogen and phosphorus excretion of japanese seabass, *Lateolabrax japonicus*. Comp Biochem Physiol A Mol Integr Physiol **147**: 502-508.

Adeola O., Lawrence B. V., Sutton A. L., Cline T.R., 1995 Phytase-induced changes in mineral utilization in zinc-supplemented diets for pigs. J Anim Sci **73**: 3384–3391.

Alvi A. S., 1994 Adventitious toxins in plant origin feedstuffs: Quantification and tolerance level in fish. Masters dissertation, Aligarh Muslim University, Aligarh, India.

Baruah K. P., Sahu A. K., Jain N. P., Mukherjee K. K., Debnath S. C., 2005 Dietary protein level, microbial phytase, citric acid and their interactions on bone mineralization of *Labeo rohita* (Hamilton) juveniles. Aquacult Res **36**: 803-812.

Baruah K., Sahu N. P., Pal A. K., Debnath D., 2004 Dietary phytase: an ideal approach for a cost effective and low – polluting aquafeed. NAGA,World Fish Center Quarterly vol. N0.3 and 4 Jul – Dec, 2004.

Baitar K., Reinhold J. G., 1972 Phytase and alkaline phosphatase activities in intestinal mucosa of rat, chicken, calf, and man. Biochem Biophys Acta **268**: 442 – 452.

Cain K. D., Garling D. L., 1995 Pretreatment of soybean meal with phytase for salmonid diets to reduce phosphorus concentrations in hatchery effluents. The Progressive Fish Culturist **57**: 114-119.

Cao L., Wang W., Yang C., Yang Y., Diana J., Yakupitiyage A., et al., 2007 Application of microbial phytase in fish feed. Enz Microb Technol **40**: 497-507.

Cheng Z. J., Hardy R. W., 2002 Apparent digestibility coefficients of nutrients and nutritional value of poultry by-product meals for rainbow trout *Oncorhynchus mykiss* measured in vivo using settlement. J World Aquac Soc **33**: 458 – 465.

Cheryan M., 1980 Phytic acid interactions in food systems. Crit Rev Food Sci Nutr **13**: 297-335.

Chi H., Tiller G. E., Dasouki M. J., et al., 1999 Multiple inositol polyphosphate phosphatase: evolution as a distinct group within the histidine phosphatase family and chromosomal localization of the human and mouse genes to chromosomes 10q23 and 19. Genomics **56**: 324-336.

Copper J. R., Gowing H. S., 1983 Mammalian small intestine phytase. Br J Nutr **50**: 673-678.

Davies N. T., 1982 Effects of phytic acid on mineral availability. In: Vahoung G. V., Kritchevsky D. (eds), Dietary Fiber in Health and Disease. Plenum Press, New York.

Debnath D., 2003 Effect of dietary microbial phytase supplementation on growth performance and body composition of *Pangasius pangasius* fingerling. Central Institute of Fisheries Education, Versova, Mumbai, India. M.F. Sci. thesis.

Debnath D., Pal A. K., Sahu N. P., Jain K. K., Yengkokpam S., Mukherjee S. C., 2005 Effect of dietary microbial phytase supplementation on growth and nutrient digestibility of *Pangasius pangasius* (Hamilton) fingerlings. Aquacult Res **36**: 180-187.

Dechavez R. B., Serrano A. E. Jr., Nuñal S., Caipang C. M. A., 2011 Production and characterization of phytase from *Bacillus* spp. as feed additive in aquaculture. AACL Bioflux **4**: 394-403.

Dvorakova J., 1998 Phytase: sources, preparation and exploitation. Folia Microbiol **43**: 323-338.

Ferguson I. B., Bollard E. G., 1976 The movement of calcium in germinating pea seeds. Ann. Bot. (London) **40**: 1047-1051.

Forster I., Higgs D. A., Dosanjh B. S., Rowshandeli M., Parr J., 1999 Potential for dietary phytase to improve the nutritive value of canola protein concentrate and decrease phosphorus output in rainbow trout (*Oncorhynchus mykiss*) held in 11°C fresh water. Aquac Nutr **179**: 109-125.

Fretzdorff B., Brummer J. M., Rochen W., Greiner R., Konietzny U., Jany K. D., 1995 Reduktion des Phytinsaure-Gehaites bei der Herstellung von Backwaren und Getreidenahrmitteln. AID-Verhraucherdienst **40**: 12-20.

Freund W. D., Mayr G. W., Tietz C., Schultz J. E., 1992 Metabolism of inositol phosphates in the protozoan *Paramecium*. Eur J Biochem **207**: 359-367.

Fu S., Sun J., Qian L., Li Z., 2008 *Bacillus* phytases: present scenario and future perspectives. Appl Biochem Biotechnol **151**: 1-8.

Gibson D. M., Ullah A. H. J., 1988 Purification and characterization of phytase from cotyledons of germinating soybean seeds. Arch Biochem Biophys **260**: 503-513.

Graf E., Empson K., Eaton J. W., 1987 Phytic acid, a natural antioxidant. J Biol Chem **262**: 11647-11651.

Greaves M. P., Anderson G., Webley D. M., 1967 The hydrolysis of inositol phosphates by *Aerobacter aerogenes*. Biochim Biophys Acta **132**: 412-418.
Greiner R., Konietzny U., 2006 Phytase for food application. Food Technol Biotechnol **44**: 125-140.
Greiner R., Konietzny U., Jany K. D., 1993 Purification and characterization of two phytases from *Escherichia coli*. Arch Biochem Biophys **303**: 107–113.
Hara A., Ebina S., Kondo A., Funaguma T., 1985 A new type of phytase from pollen of *Typha latifolia* L. Agric Biol Chem 49: 3539 – 3544.
Hardy R. W., 1995 Current issues in salmonid nutrition. In: Lim, C. E., Sessa D. J. (eds), Nutrition and Utilization Technology in Aqauculture, AOCS Press, Campaign, IL. pp. 26-35.
Harland B. F., Morris E. R., 1995 Phytate: A good or a bad food component. Nutr Res **15**: 733-754.
Hassan S., Altaff K., Satyanarayana T., 2009 Use of soybean meal supplemented with cell bound phytase for replacement of fish meal in the diet of juvenile milkfish, *Chanos chanos*. Pak J Nutr **8**: 341-344.
Howson S. J., Davis R. J., 1983 Production of phytate-hydrolysing enzyme by fungi. Enzyme Microbiol Technol **5**: 377-382.
Hubel F., Beck E., 1996 Maize root phytase: purification, characterization, and localization of enzyme activity and its putative substrate. Plant Physiol **112**: 1429-1436.
Hughes K. P., Soares J. H. Jr., 1998 Efficacy of phytase on phosphorus utilization in practical diets fed to striped bass, *Morone saxatilis*. Aquac Nutr **4**: 133 -140.
Idriss E. E., Makarewicz O., Farouk A., Rosner K., Greiner R., Bochow H., Richter T., Borriss R., 2002 Extracellular phytase activity of *Bacillus amyloliquefaciens* FZB45 contributes to its plant-growth-promoting effect. Microbiology **148**: 2097-2109.
Inagawa J., Kiyosawa I., Nagasawa T., 1987 Effect of phytic acid on the hydrolysis of lactose with beta-galactosidase. Agric Biol Chem **51**: 3027-3032.
Irving G. C. J., Cosgrove D. J., 1971 Inositol phosphate phosphatase of microbial origin. Observations on the nature of the active center of a bacterial (*Pseudomonas* sp.) phytase. Austral J Biol Sci **24**: 1559-1564.
IUPAC-IUB (Commission on Biochemical Nomenclature), 1977 Nomenclature of phosphorus containing compounds of biochemical importance. Eur J Biochem **79**: 1-9.
Jackson L. S., Li M. H., Robinson E. H., 1996 Use of microbial phytase in channel catfish *Ictalurus punctatus* diets to improve utilization of phytate phosphorus. J World Aquac Soc **27**: 309–313.
Kaur P., Kunze G., Satyanarayana T., 2007 Yeast phytases: Present scenario and future perspectives. Crit Rev Biotechnol **27**: 93-109.
Kerovuo J., 2000 A Novel Phytase from Bacillus. Characterization and Production of the Enzyme. Academic Dissertation Danisco Cultor Innovation, Kantvik, Finland pp.69
Kim J. D., Knushik S. J., Breque J., 1998 Nitrogen and phosphorus utilization in rainbow trout (*Oncorhynchus mykiss*) fed diets with or without fish meal. Aquat Liv Res **11**: 261 – 264.
Konietzny U., Greiner R., Jany K. D., 1995 Purification and characterization of a phytase from oat spelt. J Food Biochem **18**: 165-183.
Laboure A. M., Gagnon J., Lescure A. M., 1993 Purification and characterization of a phytase (myo-inositol hexakisphosphate phosphohydrolase) accumulated in maize (*Zea mays*) seedlings during germination. Biochem J **295**: 413-419.
Lall S. P., 1991 Digestibility, metabolism and excretion of dietary phosphorus in fish. In: Cowey C. B., Cho C. Y. (eds), Nutritional Strategies and Aquaculture, University of Guelph, Ontario, Canada. pp 21-37.
Lazado C. C., Caipang C. M. A., Gallage S., Brinchmann M. F., Kiron V., 2010 Responses of Atlantic cod *Gadus morhua* head kidney leukocytes to phytase produced by gastrointestinal-derived bacteria. Fish Physiol Biochem **36**: 883-891.

Lei X. G., Ku P. K., Miller E. R., Yokoyama M. T., 1993 Supplementing corn-soybean meal diets with microbial phytase linearly improves phytate phosphorus utilization by weanling pigs. J Anim Sci **71**: 3359-3367.
Lei X. G., Stahl C. H., 2001 Biotechnological development of effective phytases for mineral nutrition and environmental protection. Appl Microbiol Biotechnol **57**: 474-481.
Li M. H., Robinson E. H., 1997 Phosphorus availability of common feedstuffs fed to channel catfish *Ictalurus punctatus* as measuered by weight gain and bone mineralization. J World Aquac Soc **27**: 297 – 302.
Mullaney E. J., Ullah A. H., 2003 The term phytase comprises several different classes of enzymes. Biochem Biophys Res Commun **312**: 179-184.
Nakamura Y., Fukuhara H., Sano K., 2000 Secreted phytase activities of yeasts. Biosci Biotechnol Biochem **64**: 841-844.
National Research Council, 1993 Nutrient Requirments of Fish. National Academy Press, Washigton, D.C.
Nayini N. R., Markakis P., 1986 Phytases. In: Phytic Acid: Chemistry and Application, Pilatus Press, Minneapolis.
Oliva-Teles A., Gouveia A. J., Gomes E., Rema P., 1994 The effect of different processing treatments on soybean meal utilization by rainbow trout, *Oncorhynchus mykiss*. Aquaculture **124**: 343 – 349.
Pallauf J., Rimbach G., 1996 Nutritional significance of phytic acid and phytase. Arch Anim Nutr **50**: 301-319.
Papatryphon E., Howell R. A., Soares J. H. Jr., 1999 Growth and mineral absorption of striped bass, *Morone saxatilis*, fed a plant feedstuff based diet supplemented with phytase. J World Aquacult Soc **30**: 161 – 173.
Pongamaneerat J., Watanabe T., 1992 Utilization of soybean meal as protein source in diets for rainbow trout . Nippon Suisan Gakkaishi **58**: 1983 – 1990.
Powar V. K., Jagannathan V., 1982 Purification and properties of phytate-specific phosphatase from *Bacillus subtilis*. J Bacteriol **151**: 1102-1108.
Rao D. E., Rao K. V., Reddy T. P., Reddy V. D., 2009 Molecular characterization, physicochemical properties, known and potential applications of phytases: an overview. Crit Rev Biotechnol **29**: 182-198.
Reddy N. R., Pierson M. D., Sathe S. K., Salunkhe D. K., 1989 Phytates in Cereals and Legumes. CRC Press, Inc. Boca Raton. FL, 438 pp.
Reddy N. R., Sathe S. K., Salunkhe D. K., 1982 Phytates in legumes and cereals. In: Chichester C. O., Mark E. M., Stewart G. F. (eds), Advances in foods research. Academic Press, New York, New York, USA. pp. 1-92.
Richardson N. L., Higgs D. A., Beams R. M., McBride J. R., 1985 Influence of dietary calcium, phosphorus, zinc and sodium phytate level on cataract incidence, growth and hispathology in juvenile Chinook salmon (*Oncorhynchus tshawytscha*). J Nutr **115**: 553 – 567.
Rodehutscord M., Pfeffer E., 1995 Effects of supplemental microbial phytase on phosphorus digestibility and utilization in rainbow trout (*Onchorhynchus mykiss*). Water Sci Technol **31**:141 – 147.
Roy T., Mondal S., Ray A. K., 2009 Phytase-producing bacteria in the digestive tracts of some freshwater fish. Aquacult Res **40**: 344-353.
Sano L., Fukuhara H., Nakamura Y., 1999 Phytase of the yeast *Arxula adeninivorans*. Biotechnol Lett **21**: 33-38.
Schafer A., Koppe W. M., Meyer–Burgdorff K. H., Gunther K. D., 1995 Effects of microbial phytase on utilization of native phosphorus by carp in diet based on soybean meal. Water Sci Technol **31**: 149 – 155.
Schwarz G., Hoppe P. P., 1992 Phytase enzyme to curb pollution from pigs and poultry. Feed Magazine **1**: 22–26.
Scott J. J., 1991 Alkaline phytase activity in nonionic detergent extracts of legume seeds. Plant Physiol **95**: 1298–1301.
Scott J. J., Loewus F. A., 1986 A calcium activated phytase from pollen of *Lilium longiflorum*. Plant Physiol **82**: 333-335.

Segueilha L., Lambrechts C., Boze H., Moulin G., Galzy P., 1992 Purification and properties of phytase from *Schwanniomyces castellii*. J Ferment Bioeng **74**: 7-11.
Selle P. H., Ravindran R., 2007 Microbial phytase in piggery nutrition. Anim Feed Sci Technol **135**: 1-41.
Shah V., Parekh L. J., 1990 Phytase from *Klebsiella* sp. No. PG-2: Purification and properties. Indian J Biochem Biophys **27**: 98-102.
Shieh T. R., Ware J. H., 1968 Survey of microorganisms for the production of extracellular phytase. Appl Microbiol **16**: 1348-1351.
Shimizu M., 1992 Purification and characterization of phytase from *Bacillus subtilis* (natto) N-77. Biosci Biotechnol Biochem **56**: 1266-1269.
Simell M., Turunen M., Piironen J., Vaara T., 1989 Feed and food applications of phytase. Lecture at 3rd Meeting on Industrial Applications of Enzymes, Barcelona, Spain.
Simons P. C. M., Versteegh H. A. J., Jongbloed A. W., Kemme P. A., Slump P., Bos K. D., Wolters W. G. E., Beudeker R. F., Verschoor G. J., 1990 Improvement of phosphorus availability by microbial phytase in broilers and pigs. Br J Nutri **64**: 525–540.
Singh M., Krikorian A. D., 1982 Inhibition of trypsin activity in vitro by phytase. J Agric Food Chem **30**: 799 – 800.
Storebakken T., Shearer K. D., Roem A. J., 1998 Availability of protein, phosphorus and other elements in fish meal, soy-protein concentrate and phytase – treated soy-protein –concentrate-based diets to Atlantic salmon, *Salmo salar*. Aquaculture **161**: 363-377.
Sutardi M., Buckle K. A., 1986 The characteristics of soybean phytase. J Food Biochem **10**: 197-216.
Tambe S. M., Kakli S. G., Kelkar S. M., Parekh L. J., 1994 Two distinct molecular forms of phytase from *Klebsiella aerogenes*; evidence for unusually small active enzyme peptide. J Ferment Bioeng **77**: 23-27.
Ullah A. H. J., 1988 Production, rapid purification and catalytic characterization of extracellular phytase from *Aspergillus ficuum*. Prep Biochem **18**: 443-458.
Ullah A. H. J., Sethumadhavan K., 1998 Myo-inositol hexasulfate is a potent inhibitor of *Aspergillus ficuum* phytase. Biochem Biophys Res Commun **251**: 260-263.
Usmani N., Jafri A. K., 2002 Influence of dietary phytic acid on the growth, conversion efficiency and carcass composition of Mrigal *Cirrhinus mrigala* (Hamilton) fry. J World Aquacult Soc **33**: 199 – 204.
Van Weerd J. H., Khalaf K. H. A., Aartsen F. J., Tijssen P. A. T., 1999 Balance trials with African catfish *Clarias gariepinus* fed phytase–treated soybean meal-based diets. Aquacult Nutr **5**: 135-142.
Vats P., Banerjee U. C., 2004 Production studies and catalytic properties of phytases (*myo*-inositolhexakisphosphate phosphohydrolases): an overview. Enzyme Microb Technol **35**: 3-14.
Vielma J., Ruohonen K., Gabaudan J., Vogel K., 2004 Top-spraying soybean meal-based diets with phytase improves protein and mineral digestibilities but not lysine utilization in rainbow trout, *Oncorhynchus mykiss* (Walbaum). Aquacult Res **35**: 955-964.
Vielma J., Makinen T., Ekholm P., Koskela J., 2000 Influence of dietary soy and phytase levels on performance and body composition of large rainbow (*Oncorhynchus mykiss*) and algal availability of phosphorus load. Aquaculture **183**: 349 – 362.
Vohra A., Rastogi S. K., Satyanarayana T., 2006 Amelioration in growth and phosphate assimilation of poultry birds using cell-bound phytase of *Pichia anomala*. World J Microbiol Biotechnol **22**: 553-558.
Vohra A., Satyanarayana T., 2002 Statistical optimization of the medium components by response surface methodology to enhance phytase production by *Pichia anomala*. Process Biochem **37**: 999-1004.
Volfova O., Dvorakova J., Hanzlikova A., Jandera A., 1994 Phytase from *Aspergillus niger*. Folia Microbiol **39**: 481-484.

Williams P. J., Taylor T. J., 1985 A comparative study of phytate hydrolysis in the gastrointestinal tract of the golden hamster (*Mesocricetus auratus*) and the laboratory rat. Brit J Nutr **112**: 1197–1202.

Wyss M., Brugger R., Kronenberger A., Remy R., Fimbel R., Oesterhelt O., 1999 Biochemical characterization of fungal phytases (*myo*-inositolhexakisphosphate-phosphohydrolases): catalytic properties. Appl Environ Microbiol **65**: 367-373.

Wyss M., Pasamontes L., Remy R., Kohler J., Kusznir E., Gadient M., Muller F., van Loon A., 1998 Comparison of the thermostability properties of three acid phosphatases from molds: *Aspergillus fumigatus* phytase, *A. niger* phytase, and *A. niger* pH 2.5 acid phosphatase. Appl Environ Microbiol **64**: 4446-51.

Yao B., Fan Y. L., 2000 Molecular biology and gene engineering of phytase. Chinese J Biotechnol **16**: 1-5.

Yoon S. J., Choi Y. J., Min H. K., Kim J. W., 1996 Isolation and identification of phytase producing bacterium, *Enterobacter* sp. 4 and enzymatic properties of phytase enzyme. Enz Microb Technol **18**: 449 – 54.

Zhang M., Zhou M., van Etten R. L., Stauffacher C. V., 1997 Crystal structure of bovine low molecular weight phosphotyrosyl phosphatase complexed with the transition state analog vanadate. Biochemistry **36**: 15-23.

Received: 02 June 2011. Accepted: 20 June 2011. Published online: 25 June 2011.
Authors:
Christopher Marlowe A. Caipang, Faculty of Biosciences and Aquaculture, University of Nordland, Bodø 8049, Norway, cmacaipang@yahoo.com.
Rande B. Dechavez, College of Fisheries, Sultan Kudarat State University, Kalamansig, Sultan Kudarat 9808, Philippines, jeanandrei01@yahoo.com
Mary Jane S. Apines-Amar, National Institute of Molecular Biology and Biotechnology, University of the Philippines Visayas, Miag-ao 5023, Iloilo, Philippines, mary_jane.amar@up.edu.ph
How to cite this article:
Caipang C. M. A., Dechavez R. B., Apines-Amar M. J. S., 2011 Potential application of microbial phytase in aquaculture. ELBA Bioflux **3**(1):55-66.