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Bacillus phytases: Current status and future prospects

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Phytases catalyze the hydrolysis of phytic acid in a stepwise manner to lower inositol phosphates, myo-inositol (having important role in metabolism and signal transduction pathways), and inorganic phosphate. These enzymes have been widely used in animal feed in order to improve phosphorus nutrition and to decrease pollution in animal waste. Compared to previously described phytases, the phytase (PhyL) from *Bacillus licheniformis* ATCC 14580 has attractive biochemical properties which can increase the profitability of several biotechnological procedures (animal nutrition, humain health. . .etc). Due to its amino acid sequence with critical substitutions, the PhyL could be a model to enhance other phytases features, in terms of thermal stability and high activity. Otherwise, an engineered PhyL, with low pH optimum, will represent a challenge within the class of β- propeller phytases.

Keywords: *Bacillus licheniformis*, biotechnological applications, enzyme engineering, phytase, protein biochemistry

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Phytate, the principle storage form of phosphate and inositol in cereals, legumes, oil seeds and nuts, strongly chelates charged proteins, minerals and amino acids whitin digestive tract.[1] Phytate phosphorus is largely unavailable to monogastric animals due to the luck or insufficient amount of phytate degrading enzymes in their gastrointestinal tract.[2] Degradation of phytate is catalysed by phytase (myo-inositol hexakisphosphate phosphohydrolase; EC 3.1.3.8 and EC 3.1.3.26) which releases a series of lowers isomers of myo-inositol phosphates.[3] This hydrolytic reaction plays an important role in energy metabolism, metabolic regulation and signal transduction pathways in biological system.[4] Hence, phytases have been studied intensively due to its potential application as feed additives, processing and manufacturing of human food to improve mineral nutrition.[5] In fact, β-Propeller phytases, a class to which belong *Bacillus* phytases were shown to entirely abrogates the ability of phytate to chelate metal ions.[6] In addition to that, phytase use protects the environment against phosphorus pollution.[7] This phosphohydrolase has different possible sources: plant phytases, microbial phytases (fungal and bacterial phytases), Mucosal phytases derived from small intestine and gut microfloral phytases.[8]

The phytate-hydrolysing enzyme has many applications in food industries. It has a potential for producing low phytin bread.[9] Addition of phytase improves also the nutritional value of bread through the reduction of phytate content and enhances the activation of endogenous alpha-amylase by making more calcium available.[10] Phytase can also be added for the production of phytate-free soymilk.[11] Phytase plays a crucial role for various inositol phosphate preparations, especially in immobilized forms.[12,13] The myo-inositol phosphates have various beneficial effects on health, as enzyme stabilizers,[14] inhibitors of enzymes and thus as potential drug blockers.[15] In animal nutrition, phytases are used in aquaculture feed and additive to ensure proper degradation of the phytate present in animal diets during digestion in the stomach.[16] For improved phosphorus utilization in animal agriculture, several transgenic plants overexpressing bacterial phytases were generated, including alfalfa, soybean, potato, rice and wheat.[17-21]

Given that phytases deliver economic benefits through their ability to replace added inorganic phosphorus, many works

were undertaken with the aim to improve phytase features and make it more suitable for industrial uses. Several studies were focused on the enhancement of thermal stability to preserve the enzyme activity during the heat step of feed pelleting under high temperature.[22] In this field, the PhyL is well suited due to its remarkable thermal stability. According to Farhat-Khemakhem et al. (2013)[23], it seems to be interesting to substitute the residue Ala 257 into Pro inside PhyL. This mutation should enhance more and more the thermal stability of PhyL.

The phytase of *Bacillus licheniformis* ATCC 14580 gathers the best features to be involved in animal feed formulation.[24] Its high specific activity toward phytic acid is a major parameter to be used for myo-inositol phosphates production.

Unlike other phytases from *Bacillus* strains, the low Ca^{2+} requirement of PhyL for its optimal activity seems to be explained by the fact that Ca^{2+}ions are not involved alone ovoid the on the maintain of the enzyme leading to an active state.[25]

Due to their atypical features compared to other phytases, the phyL from *Bacillus licheniformis* ATCC14580 could be promising to overcome the inhibitory effect of phytic acid and polyphenols as they chelate minerals in feeds. Such fact was demonstrated to be useful to limit zinc deficiency and ovoid the fortification process of cereals staples with zinc.[26] Supplementation of phyL in feeds should improve growth performance and nutrient digestibility as well as the increase of gene expression encoding for the peptide transporter.[27] In the same context, Zeng et al. (2014)[28] described the use of higher phytase amount produced from *E. coli* (having lesser interests then PhyL) to further improved mineral use, protein use and performance of young pigs.

Otherwise, the high thermal stability of PhyL compared to phytases from Bacteria, Fungi and Yeast (Table 1) is especially of interest for pelleting purposes. In fact, Park et al. (2003)[29] demonstrated the suitability of the phytase from *Bacillus amyloliquefaciens* (which is less thermostable compared to PhyL) in this field. Thereby, we believe that using PhyL in pelleting process is an attractive strategy.

On the basis on the works of Sanz-Penella et al. (2009)[30] and Haros et al. (2007)[31], it will be interesting to explore the PhyL genes within Bifidobacteria for bread fermentation technology. Taking into account the high specific activity of PhyL, such feature constitutes a promising way to reduce the content of InsP(6) in rich fiber products for human consumption, in favor of InsP(3) production. Dephosphorylation of other phosphorylated molecules could also be performed by using PhyL.

Engineered PhyL with low pH optimum can constitute a remarkable perspective since the obvious drawback of phytase from *Bacillus* is their inability to act at acidic conditions. Even so, no truly reliable methods for modifying the pH activity profile of an enzyme are yet available and the decrease of the pH optimum of phytases from *Bacillus* became a challenge. In this context, different strategies could be applied in order to modify the enzyme pH feature. The first is the mutation of ionizable groups that are implicated in substrate binding or catalysis by nonionizable ones or by amino acids having different charge or pK values.[32] The second is the replacement of residues interacting with Alanine residues by forming hydrogen bonds and/or salt bridges. Substitution of such residues may disturb the hydrogen- bonding network in the active site or alter the electronic environment of Alanine residues.[33,34] The third is the modification of the enzyme surface charge, which can be achieved by chemical modification of residues located at the protein surface.[35,36] In fact, making the surface more positively charged lowers the pKa values of ionizable groups and, thus the pH optimum. Such fact is favoured at low ionic strength.[36]

Table 1. The PhyL properties compared to those from other previously described phytases

Phytase source	Optimal temperature (°C)/ Activity at low temperature[a]	pH optimum	Specific activity ($U.mg^{-1}$)	Molecular weight (kDa)	Ca^{2+} demand (mM)	Reference
Bacillus						
B. licheniformis ATCC 14580	75/40% at 4°C	6.5–7.0	316	42	0.6	24
B. subtilis US417	55/50% at 37°C	7.5	25	41	1.0	40
B. subtilis 168	55/>5% at 25°C	7.0	36.9	44	5.0	37
B. licheniformis	65/>10% at 25°C	7.0	23,6	47	5.0	37
B. subtilis VTT E-68013	55/>20% at 37°C	7.0	88	43	1.0	41
B. sp MD2	67–73/-	6–7	39	47.5	2.0–5.0	38
B. laevolacticus	70/30% at 30°C	7.0–8.0	12.69	46	5.0	42
B. sp KHU-10	60/20% at 20°C	6.5–8.5	36	44	10.0	43
Fungi						
Buttiauxella sp. GC21	55/40% at 30°C	4.5	1180	45	No effect	44
Aspergillus ficuum NTG-23	67/40% at 30°C	1.3	150.1	65.5	No effect	45
Yeasts						
Hansenula fabianii J640	50/>20% at 20°C	4.5	25.67	49	No effect	46
Kodamaea ohmeri BG3	65/>20% at 30°C	5.0	16.5	51	No effect	47
S. cerevisiae CY	40/>20% at 20°C	3.6	71.06	55	inhibited	48
Debaryomyces castellii CBS 2923	60	4.0–4.5	182	51.2	—	49

[a]relative activity is indicated.

The inspection of the PhyL amino acid sequence in comparison with previously reported phytases from *Bacillus* genus showed some original substitutions. It was found that more than 40 substitutions were encountered inside the PhyL, compared to the most related phytase from *B. licheniformis* previously characterized by Tye et al. (2002).[37] In spite of their high sequence homology, the two phytases have significant differences in their specific activity, thermostability and efficiency at low temperature and requirement of Ca^{2+} ions. Among the 40 substitutions the N86/K, N139/S, N239/D, G251/D, D302/E could impact the performance of PhyL. Site-directed mutagenesis, crystallization and enzyme modeling procedures should certainly shed light on the role of these substitutions. These observations increasingly confirmed by the works of Tran et al. (2010)[38], which introduced the E229V and S283R mutations in phytase from *Bacillus* sp. MD2 and the recent work of Xu et al. (2015)[39] who concluded that the mutations D148E and S197E increased activity and thermostability of the phytase of *Bacillus amyloliquefaciens* DSM 1061. It is worthy to note that all newly introduced residues already existed or had their homologous ones inside PhyL amino acid sequence. Finally, amino acid sequence originality of PhyL gave it better physicochemical and kinetic properties, compared to phytases derived from bacterial, fungal and yeast species.[40-49]

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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