Beta-propeller phytases in the aquatic environment: characterization of a novel phytase from *Shewanella oneidensis* MR-1

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Although a substantial amount of phytate is carried from the terrestrial to aquatic systems, it is a minor component of organic P in coastal marine sediments **(**Suzumura and Kamatani, 1995). The ephemeral nature of phytate implies rapid hydrolysis of phytate under aquatic conditions. Among the four classes of known phytases identified from terrestrial organisms, only β-propeller phytase-like sequences can be identified in the aquatic environment. A putative β-propeller phytase gene (*physS*) from *Shewanella oneidensis* MR-1 shows ~30% sequence identities in protein level to *Bacillus* phytases (Tye *et al*., 2002). Culturing of *Shewanella oneidensis* in medium deficient in phosphate induced the expression of a ~62 kDa protein in the cell fraction (Figure 1). The full length *physS* protein has 661 amino acid residues, which is nearly twice the number of residues in *Bacillus* phytases (~380 aa). Alignment and homology modelling analyses (Figure 2) demonstrated that the *physS* protein consists of two domains that resemble the fold of the *Bacillus* phytase TS-phy. Domain II (323-661 aa) is structurally more similar to TS-phy than domain I (36-322 aa). Activity assays on recombinant domains I & II showed that while domain II is the catalytic domain, domain I is also required for full enzymatic activity (Table 1). The full length PhyS and its domain II subunit demonstrated similar biochemical properties, such as calcium dependent catalytic activity, neutral optimal pH and high specificity to phytate, to that of the *Bacillus* phytases. These results confirm that the *physS* gene encodes a functional β-propeller phytase.

The amino acid sequence of *physS* shares 80% identity with an environmental sequence that was collected from a seawater sample (Venter *et al*., 2004), which indicates that the *physS* gene is present in the other members of the *Shewanella* genus. Three *Shewanella* species have been isolated from the intestines of sea animals, including goby, black porgy, and saury (Satomi *et al*., 2003). Whether the phytases that are produced by these *Shewanella* species, if any, assist these animals in phytic acid assimilation, is a worthy subject for future study.

Phylogenetic analysis grouped *physS* and several homologs from *Caulobacter crescentus*, *Microbulbifer degradans*, *Azobacter vinelandii*, *Pseudomonas syringae* pv. *tomato*, and *Idiomarina loihiensis* (Figure 3). Sequence alignment shows the conservation of all of the amino acid residues that are involved in the phosphate and calcium binding sites of BPPs in these putative phytases. Like *physS*, these proteins consist of two domains that resemble the fold of the *Bacillus* phytase. Among these bacteria, *C. crescentus* and *M. degradans* have been isolated from freshwater and salt marsh grass. Some closely related strains of *C. crescentus* and *M. degradans* have also been isolated from sea animals: the hind gut of a millipede in the case of *C. crescentus*, and the gland of shipworm in the case of *M. degradans*.

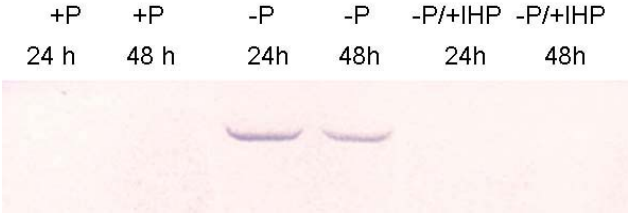

Figure 1. Western blotting of *Shewanella* proteins using an antisera against recombinant *PhyS*.

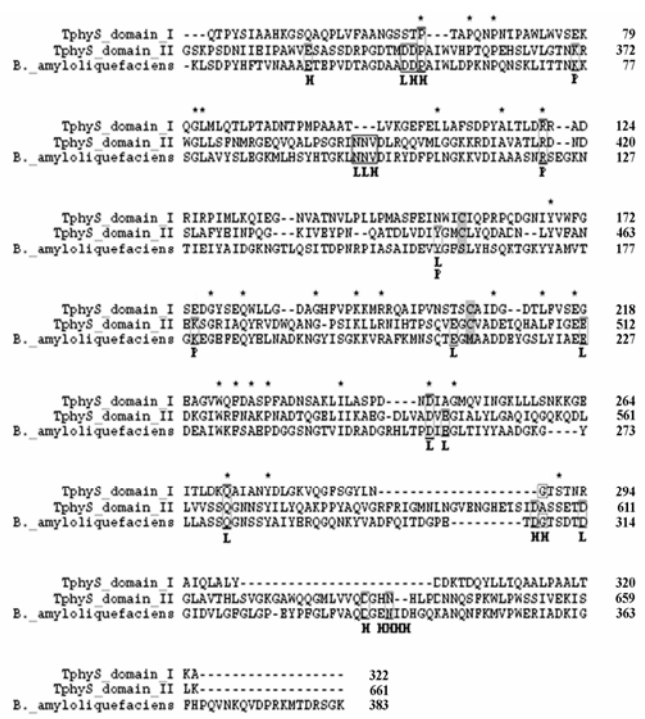

Figure 2. Alignment of the a.a. sequences of *PhyS* with BPP.

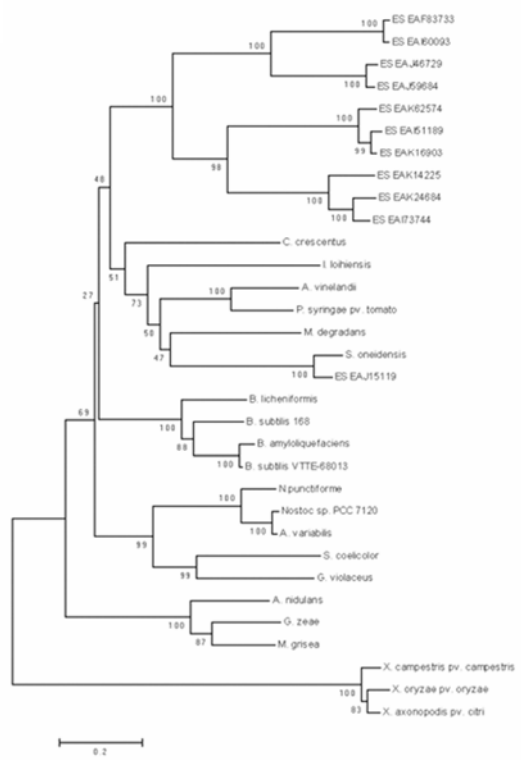

Figure 3. Phylogenetic analysis.

Table 1. Kinetic analysis with sodium phytate as substrate.

Enzyme[a]	K_m mM	K_{cat} min^{-1}	K_{cat}/K_m $mM^{-1}min^{-1}$
TphyS domain II	0.474 ± 0.041	10.65 ± 0.37	22.46 ± 0.79
His-tagged FLphyS	0.083 ± 0.007	175.92 ± 5.03	2130.51 ± 60.9
Bacillus phytase	0.198 ± 0.022	360.64 ± 29.1	1820.23 ± 146

[a] 37°C in 100 mM Tris-maleate buffer, pH 6.0 (n=3).

In summary, the presence of multiple sequences with a high similarity to *phyS* in environmental samples and the widespread occurrence of *Shewanella* species in water imply that the BPP family is the main class of phytases in the aquatic environment, which may play an important role in the mineralization of phytate in aquatic systems.

References

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