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# Production of Novel Functional Oligosaccharides by Novel Bacterial Phosphorylases

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## Research aims

Recently, a variety of oligosaccharides has been reported to have multiple functional bioactive properties such as prebiotic effects, antibacterial effects, and immunopotentiative action. These oligosaccharides are widely used as food additives and pharmaceuticals, and their functionality depends on their carbohydrate components and linkage types. Therefore, it would be worthwhile producing various oligosaccharides efficiently and specifically by suitable methods. Disaccharide phosphorylase catalyzes phosphorolysis of particular disaccharides to produce monosaccharide 1-phosphate. The phosphorolysis is reversible, making the phosphorylase a suitable catalyst for the synthesis of a particular disaccharide. So far, various oligosaccharides have been synthesized by reverse phosphorolysis using the corresponding sugar 1-phosphate as the donor and suitable acceptors. In addition, the reversibility makes it possible to produce the oligosaccharides from abundantly available commercial and/or natural sugar by using a single phosphorylase or the combined reaction of two phosphorylases that share the same monosaccharide 1-phosphate. However, utilization of disaccharide phosphorylase for the practical production of oligosaccharides is limited by the fact that there is little variation for substrate specificities, with only 21 phosphorylase activities known. Therefore, it would be beneficial to discover phosphorylases with previously unreported substrate specificities.

## Results

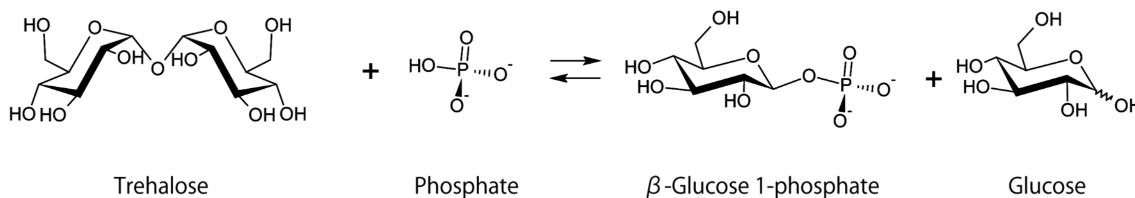
In this study, we focused on two proteins homologous to glycoside hydrolase family 65 (Bsel_1207 and Bsel_2816) that are encoded in the genomic DNA of *Bacillus selenitireducens* MLS10. Bsel_1207 exhibits high sequence similarity with the known trehalose phosphorylase. In contrast, Bsel_2816 could not be categorized into any of the known phosphorylases based on sequence similarity and phylogenetic tree analysis. Therefore, we expressed the two glycoside hydrolase family 65 homologs as His6-tag fusion proteins in *Escherichia coli* BL21 (DE3) to investigate their enzymatic properties.

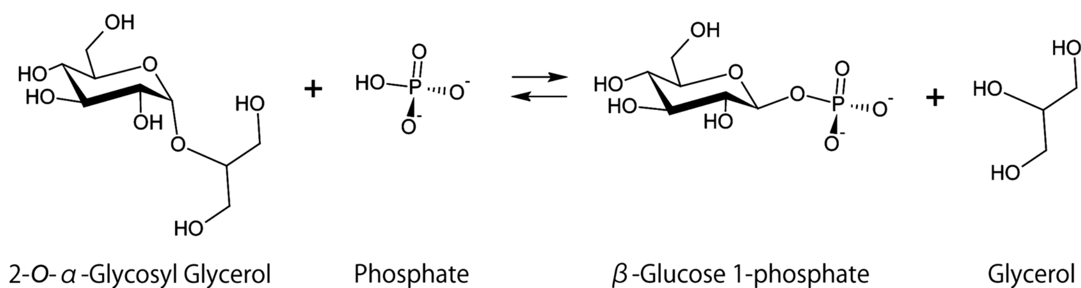
### 1. *Potassium ion-dependent trehalose phosphorylase (Bsel_1207)*

When tested in the presence of inorganic phosphate with several *α*-linked glucobioses such as trehalose (*α*1,*α*1), kojibiose (*α*-1,2), nigerose (*α*-1,3), maltose (*α*-1,4), and isomaltose (*α*-1,6) and including the typical substrates for glycoside hydrolase family 65 enzymes with inversion of the anomeric configuration releasing *β*-Glc1P and glucose, Bsel_1207 phosphorolyzed only trehalose, indicating that Bsel_1207 is a trehalose phosphorylase, which is in agreement with the results of its sequence analysis. Interestingly, Bsel_1207 strictly used potassium to regulate its enzymatic activity. Under high potassium ion concentrations, Bsel_1207 exists as an active dimeric form. Decreasing potassium ion concentrations significantly reduced the thermal and pH stability of Bsel_1207, causing it to form an inactive monomer. This is the first report of a potassium ion-dependent phosphorylase.

### 2. *2-O-α-glycosylglycerol phosphorylase (Bsel_2816)*

In the presence of inorganic phosphate, Bsel_1207 did not show obvious phosphorolytic activity with *α*-linked glucobioses such as trehalose (*α*1,*α*1), kojibiose (*α*-1,2), nigerose (*α*-1,3), maltose (*α*-1,4), and isomaltose (*α*-1,6), including the typical substrates for glycoside hydrolase family 65 enzymes. Therefore, the detail acceptor specificity in the reverse phosphorolysis was investigated. It was found that only a synthetic reaction with glycerol as the acceptor gave a single product efficiently. The NMR spectra indicated that the product was a 2-*O*-*α*-glycosylglycerol. In addition, we confirmed that Bsel_2816 catalyzed phosphorolysis of 2-*O*-*α*-glycosylglycerol in the presence of inorganic phosphate. Therefore, we propose 2-*O*-*α*-D-glucopyranosylglycerol: phosphate *β*-D-glucosyltransferase as the systematic name and 2-*O*-*α*-glucosylglycerol phosphorylase as






the short name for Bsel_2816. This is the first report of a phosphorylase utilizing a polyol as the real acceptor molecule.

## Conclusion

We discovered two kinds of novel phosphorylases belonging to glycoside hydrolase family 65 from the halophilic strain *B. selenitireducens* MLS10. One is a potassium ion-dependent trehalose phosphorylase. The other is a 2-*O*-*α*-glucosylglycerol phosphorylase.

## References

1) Nihira T., Saito Y., Chiku K., Kitaoka M., Ohtsubo K., Nakai H. (2013) Potassium ion-dependent trehalose phosphorylase from halophilic *Bacillus selenitireducens* MLS10. *FEBS Lett.* (2013) **587**: 3382–3386.

2) Nihira T., Saito Y., Ohtsubo K., Nakai H., Kitaoka M. (2013) 2-*O*-α-D-glucosylglycerol phosphorylase from *Bacillus selenitireducens* MLS10 possessing hydrolytic activity on β-D-glucose 1-phosphate. *PLOS ONE* (2013) accepted.