Molecular cloning of a gene encoding the sucrose phosphorylase from *L. mesenteroides* B-742 and the expression in *Escherichia coli*

Jin-Ha Lee[1], Mi Young Seo[2], Dae Sung Hwang[3], Seyong Kim[3], Do Won Kim[4], and Doman Kim[1,5]

[1]Engineering Research Institute, [2]Department of Materials and Biochemical Engineering, Chonnam National University, Gwang-Ju 500-757, Korea, [3]Department of Physics, Sejong University, Seoul 143-747, Korea, [4]Department of Physics, Kangnung National University, Kangnung 210-702, Korea, [5]School of Biological Sciences and Technology, Chonnam National University, Gwang-Ju 500-757, Korea.
TEL:+82-62-530-0874, FAX:+82-62-530-1869

Abstract

Leuconostoc mesenteroides NRRL B-742 sucrose phosphorylase (SPase) gene, 742*sp*, was isolated and characterized. It is composed of 1,458 bp nucleotides and encodes a 1149SPase of 485 amino acid residues with a calculated molecular mass of 55.3 kDa. It has unique C-terminal amino acid sequences (^{439}TVETPSEHDIKITRTDHSGDNIAILLANAKTRTFVITAMGKTVL QNK^{485}). 742*sp* was expressed in *Escherichia coli* and the purified 742SPase specific activity was 7.94 U/mg for sucrose. The optimum temperature and pH were 37℃ at pH 6.7 and it showed K_m of 3.11 mM for sucrose. It had a broad range of acceptor specificity and transferred the glucosyl moiety of sucrose or glucose-1-phosphate to various acceptors.

References

1. Kawasaki, H., N. Nakamura, M. Ohmori, K. Amari, and Sakai, T. Screening for bacteria producing sucrose phosphorylase and characterization of the enzymes. (1996a) *Biosci. Biotechnol. Biochem.* **60**: 319-321.
2. Van den Broek, L.A.M., E.L. Van Boxtel, R.P. Kievit, R. Verhoef, G. Beldman, and A.G.J. Voragen. Physico-chemical and transglucosylation properties of recombinant sucrose phosphorylase from *Bifidobacterium adolescentis* DSM20083. (2004) *Appl. Microbiol. Biotechnol.* **65**: 219-227.