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# Cloning and Characterization of UDP-Glucose Pyrophosphorylases from Lactobacillus kefiranofaciens and Rhodococcus wratislaviensis

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**Abstract :** Uridine-5’-diphosphate (UDP)-glucose is one of the most versatile building blocks within the metabolism of prokaryotes and eukaryotes, serving as an activated sugar donor during the glycosylation of natural products. It is formed by the enzyme UDP-glucose pyrophosphorylase (UGPase) using uridine-5′-triphosphate (UTP) and α-d-glucose 1-phosphate as a substrate. Herein, two UGPase genes from Lactobacillus kefiranofaciens ZW3 (LkUGPase) and Rhodococcus wratislaviensis IFP 2016 (RwUGPase) were identified through genome mining approaches. The LkUGPase and RwUGPase have 299 and 306 amino acids, respectively. Both UGPase has the conserved UTP binding site (G-X-G-T-R-X-L-P) and the glucose -1-phosphate binding site (V-E-K-P). The LkUGPase and RwUGPase were cloned in E. coli, and SDS-PAGE analysis showed the expression of both enzymes forming about 36 KDa of protein band after induction. LkUGPase and RwUGPase have an activity of 1549.95 and 671.53 U/mg, respectively. Currently, their kinetic properties are under investigation.

**Keywords :** UGPase, LkUGPase, RwUGPase, UDP-glucose, glycosylation
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