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Description of a Putative Oligosaccharyl:S-Layer Protein Transferase from the Tyrosine *O*-Glycosylation System of *Paenibacillus alvei* CCM 2051^T

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ABSTRACT

Surface (S)-layer proteins are model systems for studying protein glycosylation in bacteria and simultaneously hold promises for the design of novel, glyco-functionalized modules for nanobiotechnology due to their 2D self-assembly capability. Understanding the mechanism governing S-layer glycan biosynthesis in the Gram-positive bacterium *Paenibacillus alvei* CCM 2051^T is necessary for the tailored glyco-functionalization of its S-layer. Here, the putative oligosaccharyl:S-layer protein transferase WsfB from the *P. alvei* S-layer glycosylation gene locus is characterized. The enzyme is proposed to catalyze the final step of the glycosylation pathway, transferring the elongated S-layer glycan onto distinct tyrosine *O*-glycosylation sites. Genetic knock-out of WsfB is shown to abolish glycosylation of the S-layer protein SpaA but not that of other glycoproteins present in *P. alvei* CCM 2051^T, confining its role to the S-layer glycosylation pathway. A transmembrane topology model of the 781-amino acid WsfB protein is inferred from activity measurements of green fluorescent protein and phosphatase A fused to defined truncations of WsfB. This model shows an overall number of 13 membrane spanning helices with the Wzy_C domain characteristic of *O*-oligosaccharyl:protein transferases (*O*-OTases) located in a central extra-cytoplasmic loop, which both compares well to the topology of OTases from Gram-negative bacteria. Mutations in the Wzy C motif resulted in loss of WsfB function evidenced in reconstitution experiments in *P. alvei* ΔWsfB cells. Attempts to use WsfB for transferring heterologous oligosaccharides to its native S-layer target protein in *Escherichia coli* CWG702 and *Salmonella enterica* SL3749, which should provide lipid-linked oligosaccharide substrates mimicking to some extent those of the natural host, were not successful, possibly due to the stringent function of WsfB. Concluding, WsfB has all features of a bacterial *O*-OTase, making it the most probable candidate for the oligosaccharyl:S-layer protein transferase of *P. alvei*, and a promising candidate for the first *O*-OTase reported in Gram-positives.

KEYWORDS

Bacterial Glycosylation; S-Layer; Oligosaccharyl Transferase; Tyrosine-*O*-Glycosylation; Trans-Membrane Topology

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