Summary

Glycosylation is one of the most abundant and important post-translational modification of proteins. The occurrence of glycosylated proteins is equally acknowledged in eukaryotes and prokaryotes, now. The enzyme instrumental in protein glycosylation is commonly termed as a glycosyltransferase. Based on the type of linkage catalysed, primarily three types of protein glycosyltransferases are known namely, N-, O- and S- glycosyltransferases. Accordingly, in recent years a number of new bacterial protein glycosyltransferases (GTs) have been characterized. CAZy is a database that provides sequence based listing and grouping of glycosyltransferases that includes both predicted as well as characterized glycosyltransferases. Among all the families mentioned in CAZy (at present 103), GT-2 is the largest family, yet represents only two characterized bacterial protein glycosyltransferases, as of now. Bacterial protein GTs are fascinating, primarily for their versatility as compared to their eukaryotic counterparts. They show a great amount of diversity in their substrate specificities, catalytic linkages, structures and action mechanisms. They play a significant role in catalyzing various biological processes. Accordingly, these enzymes are seen as a potential tool for various applications in protein engineering. In this context, glycosyltransferases namely, SunS and ThuS are two known bacteriocin modifying GTs characterized recently, in bacteria. These enzymes can selectively glycosylate their acceptor substrates employing S-glycosylation. In four known glycobacteriocins (bacteriocins modified with glycosylation) namely, Sublancin 168 (*B. subtilis* 168), Glycocin F (*L. plantarum* KW30), Enterocin F4-9 (*E. faecalis* F4-9) and *in vitro* glycosylated Thurandacin peptide (*B. thuringiensis* BGSC 4AW1), glycosylation is suggested to play a role in the bacteriostatic activity. The study presented here describes yet another peptide modifying GT, named EntS that has hitherto unknown dual abilities of transferring and extending the glycan onto its acceptor peptide substrate. EntS is capable of di-glycosylating a previously known

bacteriocin, Enterocin 96 *in vitro*. EntS modifies Ser/Thr/Cys residue of leaderless Enterocin 96 peptide (EC peptide) at 33rd position with two hexoses (Glc/Gal) sugars, thereby catalysing O- and S-linked glycosylation. The presence of mono-glycosylated species during the early phase of assay reaction confirmed that EntS transfers two sugars in a sequential manner. EntS is a metal-dependent GT and site-directed mutagenesis studies suggest that DXD motif constitutes an essential part of the active site. Site-directed mutagenesis of DXD motif in EntS abolished both mono-glycosylation and di-glycosylation activities of EntS. Hence, EntS exploits single active to perform the dual actions of transferring and extending the glycan. Accordingly, EntS is identified as a glycosyltransferase that acts in iterative and dissociative manner. EntS engineers two sugars on to the acceptor substrate by catalysing two different types of linkages, wherein the proximal linkage is defined as O- and or S- linkage and terminal is identified as β (Hex-Hex). This study further provides a method of glycodiversification and creation of glycovariants of acceptor substrates by employing differential donor substrate specificity of EntS. The *in vitro* glycosylated EC peptide displays antimicrobial activity against *L. monocytogenes* EGD-e, a food borne pathogen. The glycovariants generated using EntS show differential bioactivity wherein the number and nature of glycan play an important role in the antimicrobial activity. As the antimicrobial peptides are used in a variety of applications such as therapeutics, cosmetics, probiotics and agriculture, EntS can be an interesting and potential glyco-engineering tool to generate glycovariants.