A New Strategy for the Improved Production of Class I Lanthipeptides in *Escherichia coli*

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RiPPs, which are ribosomally synthesized and post-translationally modified peptides, are a diverse class of natural products generated by various microorganisms.[1] They exhibit a wide range of biological activities, including antimicrobial, antifungal, antiviral, and virulence effects. Lanthipeptides are one of the most extensively studided class of RiPPs and distinguished by their internal thioether bonds known as lanthionine (Lan) or methyl-lanthionine (MeLan).[2] These bonds are formed through the dehydration of Ser/Thr residues followed by intramolecular thia-Michael addition of Cys to the resulting dehydroamino acids. Class I lanthipeptides, specifically, employ LanB dehydratases that utilize glutamyl-tRNA to activate Ser/Thr residues and introduce (Me)Lan structures.

While heterologous expression of class I lanthipeptides in genetically tractable hosts like *E. coli* has proven beneficial for generating desired compounds, this approach encounters two significant challenges.[3] Firstly, incomplete dehydration of Ser/Thr residues has often been observed due to the limited compatibility between *E. coli* $tRNA^{Glu}$ and LanB efficiency. Second, electrophilic dehydroamino acids in lanthipeptides can undergo undesirable *C*-*S* bond formation with glutathione (GSH). In light of these challenges, we present a new system that involves co-expression of the producing organism's glutamyl synthetase (GluRS) and $tRNA^{Glu}$ pair within the pEVOL vector.[4] Additionally, we showcase the capability of human LanCL enzymes to remove GSH adducts from *C*-glutathionylated peptides with DL- or LL-lanthionine stereochemistry. These advancements will greatly assist in the exploration of new lanthipeptides through synthetic biology-driven genome mining efforts.

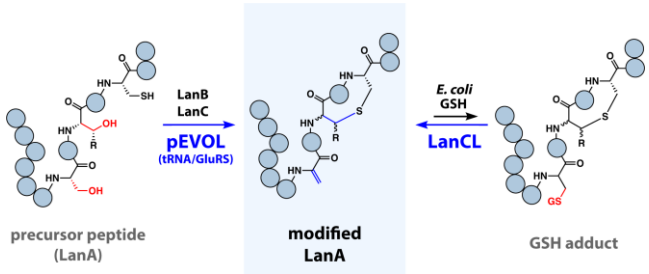

Figure 1. Improved production of class I lanthipeptides in *E. coli* using pEVOL platform and LanCL enzymes.

References

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