Synopsis of Original Research Paper

Construction of Bacterial Synthesis System for peptide gemini surfactants Usable in Cosmetics Substrates

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Our aim is to create a bacterial synthesis system for the peptide gemini (PG)-surfactants, previously known for their antibacterial properties. We focused on the Polymyxin B synthesis enzyme, structurally similar to our target PG-surfactants. This enzyme, part of the non-ribosomal peptide synthesis (NRPS) family, comprises ten modules, each with an A domain for substrate amino acid adenylation, a T domain for conversion to thioester form, and a C domain for peptide chain elongation. To adapt this enzyme for PG-surfactant synthesis, we needed to modify the A domain's substrate amino acids to cysteine derivatives with alkyl chains. Prior studies suggested A domain mutation allows this conversion, yet details on the Polymyxin B A domain structure and crucial amino acids were scarce. In our study, we cloned and sequenced the ten Polymyxin B enzyme modules from *P. polymyxa* NBRC3020, expressed them in *E. coli*, purified the proteins, and tested each A domain's adenylation activity. We confirmed their ability to adenylate the respective substrate amino acids. Next, to activate modified cysteine derivatives with C1, C2, or C3 alkyl chains to SH groups, we introduced mutations in the 10th module's A domain. Our results showed these mutations successfully enabled the adenylation of these modified cysteine derivatives.