

## Biosynthetic pathway of benzoic polyene acid (BPA): Characterization of the enzymes involved in BPA biosynthesis

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### Abstract

Cinnamoyl-containing nonribosomal peptides (CCNPs) are a small group of secondary metabolites exhibiting a broad range of biological activities. A previous study proposed that the biosynthesis of CCNP natural product, skyllamycin A (skyA) which is a platelet-derived growth factor (PDGF) inhibitor and has anti-cancer activity. The cinnamoyl moiety formation in skyA biosynthesis was reported that polyene respectively undergoes isomerization and cyclization via the putative isomerase Sky5 or Sky27 and the putative oxidoreductase Sky4 or the phytoene dehydrogenase-like Sky28 in skyllamycin biosynthesis to generate an aromatic structure. However, no *in vivo* and/or *in vitro* characterization of the benzene ring formation have been reported so far, which limits the understanding of the enzymes involved in this biosynthesis and their catalytic mechanisms. This study focuses on the biosynthesis of the benzoic polyene acid (BPA), which is a lookalike of the cinnamoyl moiety. We sequenced and identified a *spe* gene cluster, of which the encoded enzymes participate in BPA production. *In vivo* studies, we have confirmed that genes 3736 and 3735 in the *spe* gene cluster participate in the aromatic ring formation in BPA biosynthesis. Thus far, the three-dimensional structure of 3736 and it in complex with glutathione (GSH) have been determined. 3736 is a glutathione-dependent isomerase and the structural comparison of 3736 to its homologs, it possesses a loop near the binding pocket, which is longer than the others. The long loop is suggested to play an important role in protein-protein interaction with 3735. Besides, 3736 appears to bind GSH in different ways compared to homologous protein. Interestingly, according to the NCBI database, the protein encoded by 3735 is a novel enzyme, whose function is still a mystery. Our results provide insights into novel enzymes involved in the formation of BPA aromatic ring.