FUNCTIONAL CHARACTERIZATION OF TWO NOVEL PRENYLTRANSFERASES TRANSFERRING DIMETHYLALLYL OR GERANYL MOIETIES TO 1,6-DIHYDROXYPHENAZINE

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Introduction. Aromatic prenyltransferases (PTases) transfer isoprenyl moieties onto an aromatic acceptor molecule, usually catalyzing an electrophilic substitution of the aromatic ring under formation of C-C bonds. This transfer leads to an astounding diversity of primary and secondary metabolites in plants, fungi and bacteria.
Many *Streptomyces* strains produce prenylated phenazines as secondary metabolites. However, only two phenazine PTases, PpzP from *Streptomyces anulatus* 9663 (1) and EpzP from *Streptomyces cinnamonensis* DSM 1042 (2), have been characterized so far. Both enzymes catalyze the reaction of the isoprenoid dimethylallyldiphosphate (DMAPP) with 5,10-dihydrophenazine 1-carboxylate (dihydro-PCA).
The marine strain *Streptomyces* sp. SpC080624SC-11 produces the prenylated 1,6-dihydroxyphenazine derivatives JBIR-46, -47 and -48 (3), and we speculated that the PTase in this pathway may utilize a phenazine substrate different from dihydro-PCA.
The objective of this work was to identify new phenazine PTase genes in the marine actinomycetes *Streptomyces* sp. SpC080624SC-11 and *Streptomyces* sp. CNQ-509, to express the encoded enzymes and to characterize their function by biochemical assays.

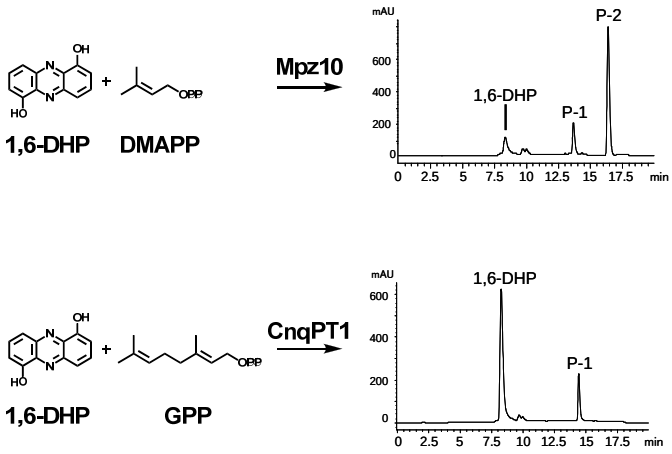

Fig. 1. HPLC analysis of the PTase reactions catalyzed by the novel prenyltransferases Mpz10 and CnqPT1.

Methods. Draft genome sequences of *Streptomyces* sp. SpC080624SC-11 and *Streptomyces* sp. CNQ-509 were obtained and bioinformatically analyzed. Putative PTase genes were expressed in *E. coli*, and the resulting proteins were incubated with different isoprenoid and phenazine substrates. Product formation was analyzed by HPLC-UV and HPLC-MS.

Results. We identified the gene cluster for the biosynthesis of JBIR-46, -47 and -48 from *Streptomyces* sp. SpC080624SC-11. The cluster contains one PTase gene, *mpz10*, besides the genes coding for phenazine biosynthesis and for the mevalonate pathway.
An *in silico* BLAST search with the sequence of Mpz10 against the genome sequence of *Streptomyces* sp. CNQ-509 revealed a gene with 44% identity (amino acid level) to *mpz10*. This gene was termed *cnq*PT1.
Expression of Mpz10 and CnqPT1 in *E. coli* and biochemical investigation of the enzymes clearly showed that 1,6-dihydroxyphenazine (1,6-DHP) and not dihydro-PCA is the genuine aromatic substrate of both enzymes. Mpz10 utilizes DMAPP as isoprenoid substrate and forms two different products, whereas CnqPT1 utilizes geranyldiphosphate (GPP) and forms a single product (Fig. 1). The structure elucidation of all reaction products by MS and NMR experiments will be presented.

Conclusions. We were able to identify two novel phenazine PTases from marine actinomycetes. Both enzymes utilize 1,6-DHP as phenazine substrate. Therefore 1,6-DHP represents a new genuine substrate for aromatic PTases.

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