

The pine *O*-methyltransferase involved in stilbenoid biosynthesis

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Pine wilt diseases gave severe damages on Japanese pine forests, and it has been gradually decreasing after 1980s. Meanwhile, they are spread into other East Asian countries. The diseases are caused by pine wood nematode, which is transmitted by a pine sawyer. The pesticides and nematicides have been applied for the disease control. However, it has been criticized from an environmental point of view. Moreover, the disease is not completely controlled by these efforts because of the wide target area in the forests and mountains. On the basis of these backgrounds, alternative control strategies for pine wilt diseases are now expected.

In the genus *Pinus*, the stilbenoids, pinosylvin and its monomethyl ether are major constitutive phenolic constituents in the heartwood. Both compounds accumulate upon biotic and abiotic stress, which may function as phytoalexins in the tree. Especially, pinosylvin monomethylether showed strong nematicidal activity, of which LD₅₀ was at 4ppm [1]. This is about 10-fold high comparing with the case of pinosylvin, suggested that pinosylvin monomethylether biosynthesis is a good target to control pine wilt diseases. This reaction would be catalyzed by an *O*-methyltransferase (OMT), which transfer methyl group from *S*-adenosyl-L-methionine to pinosylvin, to produce pionsylvin monomethylether [2].

We had already cloned five *O*-methyltransferase (OMT) cDNAs from the seedlings of *Pinus densiflora*. They belong to a type II OMT family, and are divided into two sub-groups. For the OMT cDNAs, we have established a pET32Xa/LIC expression system with *E. coli* OrinagmiB (DE3) pLysS. They can be produced in a soluble form probably because of thioredoxin tag. The OMTs are purified with a good yield and with a single band on SDS-PAGE gel through a Ni-NTA agarose column. The OMTs are finally obtained in soluble forms after removal of the attached tag. OMT is rich in SH groups and easily miss-folded without activity in some cases. For example, loblolly pine OMT with the activity has hardly obtained in *Escherichia coli* and form inclusion body [3]. Here, the purified soluble OMTs with mass production will provide insight of the folding studies for the 3D structure and the activity. Further studies on the cDNA translates will elucidate their functional roles.

REFERENCES

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