Reconstitution of Biosynthetic Machinery for Indole-Diterpene Nodulisporic acids in *Aspergillus oryzae*

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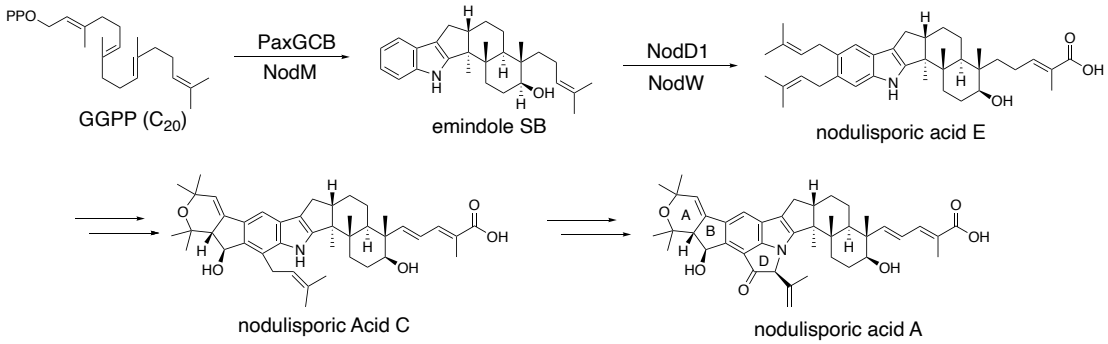
Indole-diterpenes (IDTs) with paspaline scaffold are one of the important class of fungal secondary metabolites with various biological activities. Among them, nodulisporic acids (NAs) produced by *Hypoxylon pulicicidum* have shown potent insecticidal activity without adverse effects in mammals[1]. NAs have a characteristic A/B-bicyclic system as well as a D-ring on the indole ring. Although, the biosynthetic gene cluster (nod) was reported on 2018[2], the modification reactions of emindole SB to synthesize the A, B, D rings remains unclear. In this study, we applied the recently established hotspot knock-in strategy[3] to elucidate the biosynthetic pathway of NAs.

Based on the previous biosynthetic studies of emindole SB, we initially introduced four genes, *paxG, paxC, paxB,* and *nodM,* into *Aspergillus oryzae* NSPlD1 by CRISPR/Cas9-based genome editing method and construct a transformant AO-*paxGCB/nodM*. Metabolite analysis showed the production of emindole SB. Subsequently, we incorporated two genes, *nodW* (cytochrome P450) and *nodD1* (prenyltransferase) to construct AO-*paxGCB/nodMD1W*. The transformant produced nodulisporic acid E, a key biosynthetic intermediate of NAs. Functional analysis of other modification enzyme genes is in progress. Details will be discussed in the presentation.



References

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