Construction of biosynthetic machinery for polyketides with a bis-tetrahydrofuran moiety

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Research aims

Natural products containing a bis-tetrahydrofuran moiety are reported to have attractive biological activities.[1, 2] For example, asteltoxin (**1**), a polyketide found in *Emericella variecolor*, is a potent ATPase inhibitor. In its production, a hexaene-fused α-pyrone (**2**) is formed by polyketide synthase (PKS), followed by epoxidation and hydration steps that result in a bis-tetrahydrofuran formation (Fig. 1).[3] The regio-selective bis-tetrahydrofuran formation and potential biological activity prompted us to investigate the asteltoxin biosynthetic machinery, which could lead to the preparation of asteltoxin derivatives as drug candidates. The draft genome analysis of *E. variecolor* IFM42010 found two candidate asteltoxin gene clusters, namely Ev460 and Ev152 (Fig. 2A, 3A). We previously determined that Ev460PKS produces a hexaene-fused β-keto lactone (**4**), whose side-chain is the same as that of (**2**) (Fig. 2B). Herein, we attempted the identification of the gene cluster responsible for the biosynthesis of asteltoxin based on gene disruption and biochemical analysis of the initial step of asteltoxin biosynthesis.

Methods

The *Agrobacterium tumefaciens*-mediated transformation (ATMT) method was utilized to generate *E. variecolor* IFM42010 knockout mutants. The disruption cassette containing *hph* flanked by 2 kb of Ev460PKS gene at each end were cloned into the T-DNA region of pRI201AN to construct the disruption plasmid pRI201AN-hph-Ev460PKS. Agrobacteria carrying the disruption plasmid were incubated with conidia of *E. variecolor* on a nylon filter placed on a co-cultivation plate with acetosyringone. The transformant of *E. variecolor* was then selected using a PDA plate containing hygromycin and cefotaxime.

Aspergillus oryzae NSAR1, a quadruple auxotrophic mutant, was transformed by the protoplast-PEG method with a fungal expression plasmid harboring an α-amylase promotor system.

Results

1 Disruption of the genes in the Ev460 cluster

The hygromycin-resistant transformants were analyzed via PCR to confirm whether Ev460 genes were replaced with a *hph* cassette. To date, only one disruptant per 45 candidate transformants was obtained using the ATMT method. HPLC analysis of the ethyl acetate extract from the Ev460PKS disruptant revealed that Ev460PKS was not required for the asteltoxin production (Fig. 2C). Furthermore, the ΔEv460TF2 mutant maintained asteltoxin production. We therefore concluded that the Ev460 gene cluster is not involved in asteltoxin biosynthesis.

2 Heterologous expression of Ev152PKS

Genes in the Ev152 cluster are similar to those in the aurovertin biosynthetic gene cluster.[4)] When the *A. oryzae* transformant with pTA-Ev152PKS was cultured in rice medium, a new peak was detected which was absent in the control transformant (*A. oryzae* transformant with pTAex3) in the ethyl acetate extract after HPLC analysis (Fig. 3B). LC-MS analysis of the extract suggested that the molecular formula of the new product ($C_{22}H_{26}O_3$) was the same as that of the hexaene-fused α-pyrone (**2**) precursor to asteltoxin (Fig. 3C). Currently, co-expression experiments of other genes in the Ev152 cluster with Ev152PKS are underway.

Conclusion

In this study, we attempted to identify the gene cluster responsible for asteltoxin biosynthesis and to construct biosynthetic machinery for polyketides with a bis-tetrahydrofuran moiety. A gene disruption experiment showed that the Ev460 cluster was not involved in asteltoxin biosynthesis. Heterologous expression of Ev152PKS suggested that the Ev152 cluster, which is similar to that of aurovertin, could be the actual asteltoxin biosynthetic gene cluster. Further functional analyses of the Ev152 gene cluster (Fig. 3A) could provide useful information for the reconstitution of biosynthetic machinery to produce a bis-tetrahydrofuran moiety in polyketides.

References

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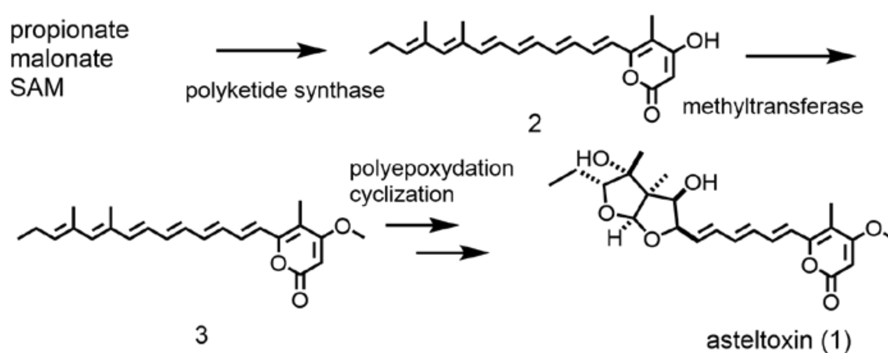

Fig. 1.　Putative biosynthetic pathway of asteltoxin

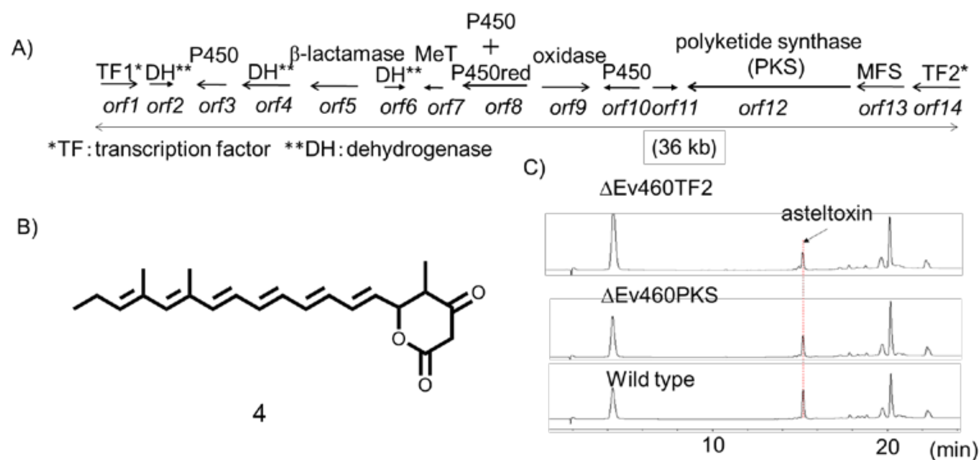

Fig. 2. The 460 gene cluster and HPLC analysis of Ev460PKS disruptant

A) Ev460 cluster in *E. variecolor* IFM42010 B) Ev460PKS product C) HPLC analysis of ethyl extract from ΔEv460 transformant

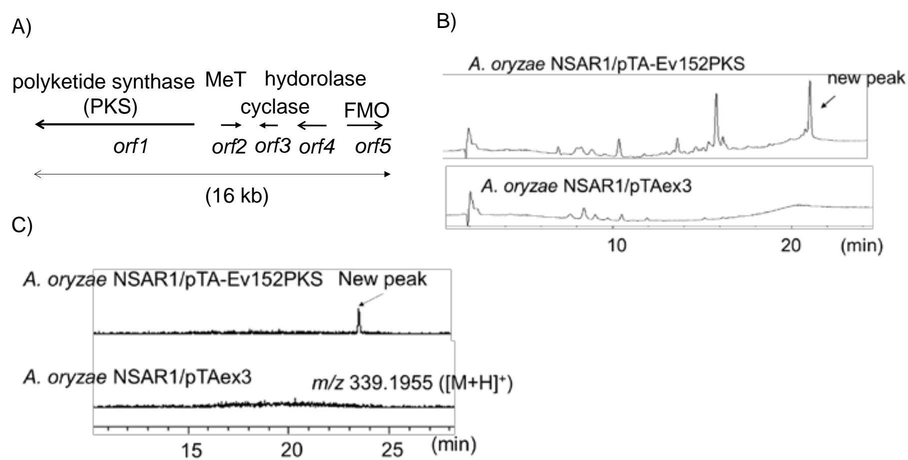

Fig. 3. The Ev152 gene cluster and HPLC analysis of *A. oryzae* with Ev152PKS

A) Ev152 cluster in *E. variecolor* IFM42010 B) HPLC analysis of *A. oryzae* NSAR1/pTA-Ev152PKS C) Mass chromatogram with *m/z* 339.1955 of the extract