Screening of secondary metabolites by genome shuffling

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Research aims

In recent years, it has become feasible to artificially synthesize secondary metabolites by introducing biosynthetic genes for natural products into microorganisms. However, no effective method has yet been established to create new secondary metabolites by modifying biosynthetic gene clusters. In order to achieve such modifications, it is necessary to gain a deep understanding of biosynthetic gene clusters, but their fundamental genetic associations are still poorly understood. In this work, natural products were biosynthesized to artificially activate biosynthetic gene clusters, and then, in order to create various non-natural products, we attempted to randomly combine gene groups by genome shuffling.

Methods and results

We selected sesterterpenoids as targets for the artificial activation of biosynthetic gene clusters. Terpenes are natural products derived from C_5 isoprene units. They include a huge number of compounds, with many different backbone skeletons, that have been used in the development of important pharmaceuticals. Sesterterpenoids are a group of terpenoid natural products that are primarily biosynthesized via cyclization of the C_{25} linear substrate. There are relatively rare reports on the isolation of sesterterpenoids. However, various organisms such as bacteria, fungi, and plants produce sesterterpenoids as well as other terpenoids. Therefore, in order to obtain sesterterpenoids, several putative terpene synthases were heterologously expressed in *Aspergillus oryzae* NSAR1, as this strain is suitable for the characterization of fungal biosynthetic enzymes. The genes selected and introduced in this study were identified by the genome analysis of two *Penicillium* species, *P. brasilianum* NBRC 6234 and *P. verruculosum*, which harbor putative terpene synthases.

Transformants were cultivated in induction medium and the resulting mycelial metabolites were analyzed by high resolution mass spectrometry and nuclear magnetic resonance. As a result, we identified two new sesterterpenes: preasperterpenoid A, derived from *P. verruculosum* preasperterpenoid A synthase (PvPS) and sesterbrasiliatriene, derived from *P. brasilianum* sesterbrasiliatriene synthase (PbSS) (Figure 1). [1)] In addition, copalyl diphosphate was also obtained from *P. verruculosum* copalyl diphosphate synthase (PvCPS) (Figure 1). [2)]

Meanwhile, an oligopeptide possessing an isoprenylated tryptophan residue, ComXnatto pheromone, was also obtained by means of heterologous overexpression (Figure 2). [3)]

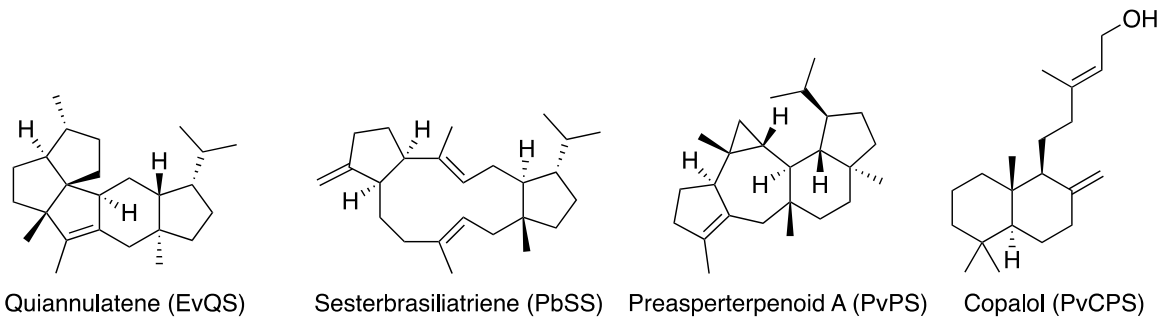

Figure 1. Chemical structures of sesterterpenes and diterpene (copalol).

Next, we attempted to randomly combine gene groups by genome shuffling. As a model study, we conducted a polyethylene glycol (PEG)-mediated cell fusion between a quiannulatene [4)]-overexpressing *A. oryzae* strain and an auxotrophic *A. oryzae* strain (*ΔpyrG* or *A. ΔadeB*). As a result, we obtained 5 fusion cells, but no new products were detected from the resulting mycelial metabolites thus far. A further cell fusion study was conducted by using *P. islandicum Sopp* NBRC 6964 and *P. rugulosum Thom* NBRC 696. As a result, we obtained five phenotypically distinct fusion cells. We are currently analyzing their mycelial metabolites.

In addition, the heterologous expression of enzymes derived from *Rhododendron dauricum* plants and *Stachybotrys bisbyi* fungi mediated the three-step combinatorial biosynthesis of (+)-daurichromenic acid, which is a meroterpenoid with highly potent anti-HIV activity from *R. dauricum*. Additional introduction of a halogenase from *Fusarium* sp. fungi into the strain resulted in the biosynthesis of the non-natural product (+)-5-chlorodaurichromenic acid, which exceeds the antibacterial activity of the original compounds (Figure 2). [5)]

Conclusion

Heterologous expression of putative terpene synthases derived from a filamentous fungus enabled the artificial synthesis of several novel secondary sesterterpenes and diterpenes. Furthermore, cell fusion was successfully carried out using the strains. Unfortunately, new secondary metabolites were not obtained from the fusion cells, but other fusion cells were obtained with distinct phenotypes.

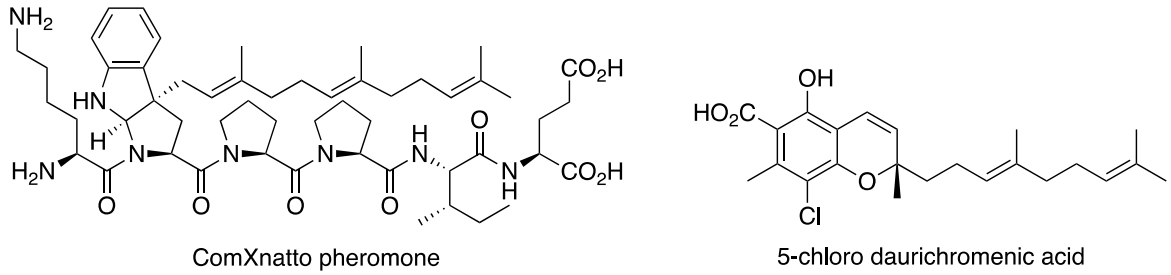

Figure 2. Chemical structures of the ComXnatto pheromone and (+)-5-chlorodaurichromenic acid.

Acknowledgments

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References

1) Mitsuhashi, T., Okada, M., and Abe, I. (2017) Identification of chimeric αβγ diterpene synthases possessing both type II terpene cyclase and prenyltransferase activities. ChemBioChem 18: 2104–2109.
2) Mitsuhashi, T., Rinkel, J., Okada, M., Abe, I., and Dickschat, J. S. (2017) Mechanistic characterization of two chimeric sesterterpene synthases from *Penicillium*. Chem. Eur. J. 23: 10053–10057.
3) Sugita, T., Okada, M., Nakashima, Y., Tian, T., and Abe, I. (2018) A tryptophan prenyltransferase with broad substrate tolerance from *Bacillus subtilis* subsp. *natto*. ChemBioChem, 19: 1396–1399.
4) Okada, T., Matsuda, Y., Mitsuhashi, T., Hoshino, S., Mori, T., Nakagawa, K., Quan, Z., Qin, B., Zhang, H., Hayashi, F., Kawaide, H., and Abe, I. (2016) Genome-based discovery of an unprecedented cyclization mode in fungal sesterterpenoids biosynthesis. J. Am. Chem. Soc. 138: 10011–10018.
5) Okada, M., Saito, K., Wong, C. P., Li, C., Wang, D., Iijima, Taura, F., Kurosaki, F., Awakawa, T., and Abe, I. (2017) Combinatorial biosynthesis of (+)-daurichromenic acid and its halogenated analogue. Org. Lett. 19: 3183–3186.