Discovery of cryptic fungal natural products using genome rearrangement

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Research objective

Filamentous fungi are important microbial resources that have contributed to the development of numerous pharmaceuticals including penicillin and lovastatin. The genome of filamentous fungi contains a large number of unused biosynthetic genes, many of which are known to be silent genes whose expression is not sufficiently induced under normal culture conditions to produce substances. These silent genes are expected to be valuable troves of novel natural products that can be used as seeds for new drug development, and methods to activate silent genes are being actively pursued. We established a method to induce the expression of silent genes by artificially modifying epigenetic regulation using small-molecule compounds and have discovered novel natural products. However, the methods developed thus far can only induce the production of natural products derived from one or two silent biosynthetic gene clusters; these gene resources have not yet been fully exploited. In this study, we applied the TAQing system, a large-scale genome reorganization technique, to filamentous fungi and developed a method for activating diverse silent genes in a single species.

Methods

The TAQing system is a method of introducing the restriction enzyme TaqI (activated at high temperature) from a highly thermophilic bacterium into the cell and causing a transient temperature shift, which simultaneously causes multiple double-strand breaks in the genomic DNA, followed by recombination repair, resulting in various genomic reorganizations such as end joining, homologous recombination, translocation, and copy number variation.[1] In filamentous fungi, many secondary metabolite biosynthetic gene clusters are located near the telomeres at the chromosome ends, and the location of these biosynthetic gene clusters plays a major role in their expression. In this study, we established an optimal protocol using *Aspergillus niger,* a model filamentous fungus for secondary metabolism studies. A plasmid vector was created by introducing *taqI* downstream of the *amyB* and *enoA* promoters. Pyrithiamine resistance was used to select the transformants. This plasmid was introduced into

A. niger using the protoplast-PEG method to obtain a *taqI*-expressing strain. As the introduced TaqI shows high activity at high temperatures, we examined various heat shock conditions for the transformants and confirmed the appearance of transformed strains by repeated subculture at temperatures between 30°C and 37°C, where the restriction enzyme activity of TaqI is moderately observed. Morphologically altered strains were then observed under these conditions. These morphologically altered strains were isolated, the production profiles of secondary metabolites were investigated, and genomic analysis was performed.

Results

Through repeated subculture of the transformants with *taqI* at 30°C to 37°C, we succeeded in obtaining diverse morphologically altered strains (Fig. 1). There was no significant difference in the expression frequency of the morphologically altered strains between the *amyB* and *enoA* promoters, suggesting that a strong promoter that induces constant expression is sufficient for this method. Most of the morphologically altered strains were found to have reduced sporulation ability and mycelial growth compared to those of the parental strain; however, as this was detected by visual morphological changes, obtaining different transformed strains may be possible using other selection methods.

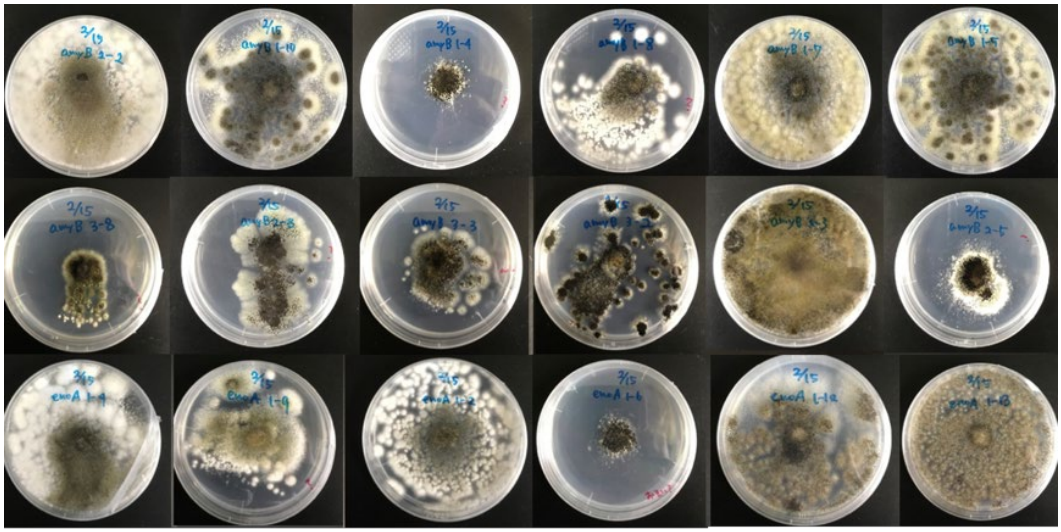

Fig. 1. Examples of morphologically altered strains isolated in this study

The production profiles of the secondary metabolites of the morphologically altered strains obtained in this study were examined by HPLC after cultivation, and the results showed that the secondary metabolic profiles were different from those of the parental strains. The metabolic profiles differed among the morphologically altered strains, indicating that this study successfully activated diverse silent genes as intended (Fig. 2). In addition, decoding of

the genomes of the morphologically altered strains revealed large-scale gene deletions and translocations near the TaqI recognition site, suggesting that genome reorganization occurred owing to gene cleavage by TaqI.

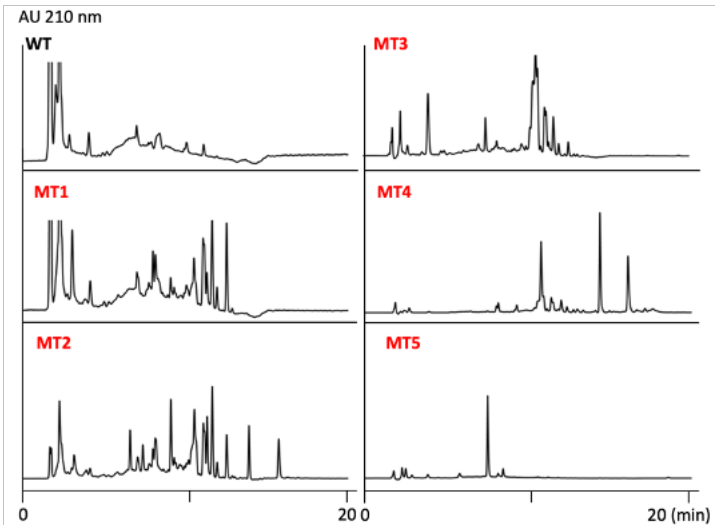

Fig. 2 HPLC profiles of wild type (parental strain) and some morphologically altered strains

In summary, this study showed that heterologous expression of TaqI in filamentous fungi and subculture at 30°C to 37°C can provide morphologically altered strains. However, the difficulty in maintaining traits and improving selection methods to identify more diverse traits are issues for future research. Currently, we are continuing our research to solve these challenges and expanding our research to various other filamentous fungi to obtain diverse new natural products.

References

1. Muramoto, N., Oda, A., Tanaka, H., Nakamura, T., Kugou, K., Suda, K., Kobayashi, A., Yoneda, S., Ikeuchi, A., Sugimoto, H., Kondo, S., Ohto, C., Shibata, T., Mitsukawa, N., Ohta, K. Phenotypic diversification by enhanced genome restructuring after induction of multiple DNA double strand breaks. *Nature Commun*. *9*:1995. doi: 10.1038/s41467-018-04256-y. (2018).