Activation of cryptic secondary metabolisms in thermophilic streptomycetes for discovery of new natural products

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Research objective

Streptomyces are promising microorganisms for producing a wide variety of bioactive compounds as secondary metabolites. The accumulation of actinomycete genomic information has revealed that the *Streptomyces* genome is rich in biosynthetic capacity, with approximately 20–40 biosynthetic gene clusters of secondary metabolites per strain. However, only a few secondary metabolites have been detected and identified in each strain, and many unique secondary metabolites remain unknown. If the production of these cryptic secondary metabolites are activated, natural products with novel structures can be obtained. To date, several methods have been developed to activate the production of cryptic compounds, but they all target known streptomycete strains, and the number of new compounds discovered has plateaued. The discovery of new streptomycetes will increase the hit rate of new natural products.

Streptomyces that grow at 28°C have generally been used for discovering new natural products. Interestingly, thermophilic streptomycetes with a growth temperature of around 50°C have been isolated, but their potential for producing secondary metabolites remains unclear. In recent years, new compounds have been identified in some *Streptomyces* strains by shifting the cultivation temperature from 28°C to 45°C.[1,2] However, these strains were isolated at 28°C, suggesting they might be thermotolerant. Conversely, we hypothesized that thermophilic streptomycetes would contain unusual genetic elements, as their growth environment differs from that of thermotolerant strains.

Here, we aimed to discover new natural products by activating the cryptic secondary metabolism of thermophilic actinomycetes using several methods and to develop a genetic tool to analyze the molecular mechanism of activation.

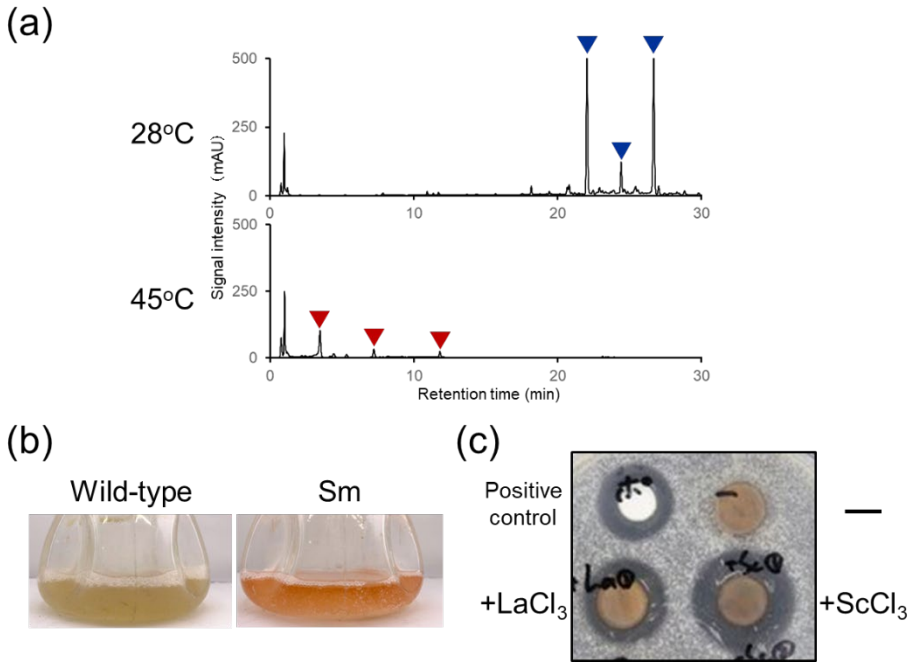
Methods

Actinomycetes (14 *Streptomyces* species and one *Actinomadura* species) deposited at the NITE Biotechnology Centre (NBRC) at a growth temperature of 45°C or higher were used in this study. They were cultivated at 45°C and 28°C to analyze their metabolite profiles and antimicrobial activity. The culture broth of these thermophilic strains was extracted with *n*-butanol and analyzed using reversed-phase HPLC. Mutant strains resistant to rifampicin, streptomycin (Sm), or ofloxacin were obtained from thermophilic actinomycetes. In addition, thermophilic actinomycetes were cultivated in media supplemented with Sc or La, and their metabolites were analyzed using reversed-phase HPLC. To rapidly analyze the molecular mechanisms that activate cryptic secondary metabolism, a base-editing vector was constructed.

Results

1. Production of compounds dependent on cultivation temperature

The antimicrobial activity of thermophilic actinomycetes was determined by a bioassay using culture agar pieces, showing that the anti-*Escherichia coli* activity of strain A was induced by a cultivation temperature of 45°C. Reversed-phase HPLC analysis demonstrated that some compounds were specifically produced at the cultivation temperature of 45°C, while the production of other compounds was activated by the cultivation temperature of 28°C (Fig. a). These findings suggest that the secondary metabolism of thermophilic actinomycetes changes when cultured at different temperatures.



2. Activation of cryptic secondary metabolism by ribosomal engineering

Ribosomal engineering is a valuable method for activating cryptic secondary metabolism in *Streptomyces* by modifying transcriptional and translational systems with antibiotics. We then obtained a Sm-resistant thermophilic actinomycete strain B. The culture broth of the Sm-resistant strain was orange in color, whereas that of the parental strain was brownish (Fig. b). Reverse-phase HPLC analysis of the *n*-butanol extract from the culture broth revealed that the production of some compounds increased with increasing Sm resistance. Similar phenomena have been observed in other strains, suggesting that ribosomal engineering activates cryptic secondary metabolism in thermophilic actinomycetes.

3. Activation of cryptic secondary metabolism by rare earth element

The addition of rare earth elements to streptomycetes cultures leads to the activation of cryptic secondary metabolism. Thermophilic actinomycetes were cultured in the presence of scandium chloride or lanthanum chloride. In the thermophilic actinomycete strain C, anti-*E. coli* activity was induced by adding scandium chloride or lanthanum chloride during solid cultivation (Fig. c), whereas, in liquid culture, the production of several compounds is induced by the addition of those rare earth elements. Similar production-inducing phenomena were observed in other strains, and the physical properties of the compounds whose production was activated by earth elements were being analyzed.

4. Construction of a base-editing plasmid pLK101

The biosynthetic enzymes involved in the secondary metabolism of thermophilic actinomycetes may be thermostable and have potential industrial applications. Thus, we attempted to develop a base-editing plasmid for the rapid analysis of gene function, and constructed pLK101, a Target-AID vector for C-to-T base substitutions. Gene inactivation using pLK101 allowed the analysis of actinorhodin and indigoidine production. Finally, the biosynthetic genes for polyene macrolide compounds were successfully identified in *S. lavendulae* FRI-5. [3)]

Conclusion

In this study, we investigated thermophilic actinomycetes as novel biological resources for the discovery of new natural products. Many new compounds have been produced by activating cryptic secondary metabolism using several methods. Thus, these compounds are attractive targets for drug development; however, their structures must be identified. This study provides valuable information regarding the characteristics of thermophilic actinomycetes.

References

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