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Analysis of the regulatory genes encoded on a *Streptomyces* linear plasmid and its application for antibiotic production

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Research aims

The filamentous soil bacteria *Streptomyces* are characterized by the ability to produce a wide variety of secondary metabolites including antibiotics. In many *Streptomyces* species, small diffusible signaling molecules, such as A-factor and virginia butanolide, control antibiotic production and morphological differentiation.

Streptomyces rochei strain 7434AN4 carries three linear plasmids (pSLA2-L, -M, and -S) and produces two structurally unrelated polyketide antibiotics: lankacidin (LC) and lankamycin (LM) (Fig. 1). The biosynthetic gene clusters of these antibiotics are located on the giant linear plasmid pSLA2-L.[1] In addition, many regulatory genes have been identified on pSLA2-L, including a biosynthetic gene (*srrX*), six *tetR*-type repressor genes (*srrA-F*), and three SARP (*Streptomyces* antibiotic regulatory protein) genes (*srrY*, *srrZ*, and *srrW*). We revealed that *srrX* and *srrA* constitute the signaling molecule/receptor system in *S. rochei*.[2–4] In this study, we analyzed the following topics: (1) structural diversity of the signaling molecules termed "SRBs (Streptomyces rochei butenolides)" that induce LC and LM production at nanomolar concentrations; (2) genome mining of cryptic biosynthetic gene clusters by genetic manipulation of the regulatory genes.

Methods

Antibiotic production was analyzed by high performance liquid chromatography (HPLC) and thin layer chromatography (TLC). Metabolites were purified by Sephadex LH-20 and/or silica gel chromatography. NMR spectra were recorded on a JEOL LA-500 spectrometer equipped with a field gradient accessory. Electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) mass spectra were measured using a LTQ Orbitrap XL mass spectrometer (Thermo Fisher Scientific, USA).

Results

1. *Structural diversity of the signaling molecules SRBs*

Our group has determined the structure of two novel signaling molecules, SRB1 and SRB2, which induce LC and LM production in *Streptomyces rochei*. Based on ESI-MS and extensive NMR analyses, SRB1 and SRB2 were determined to be a 2-(1′-hydroxyl-6′-oxo-8′-methylnonyl)-3-methyl-4-hydroxybut-2-en-1,4-olide and 2-(1′-hydroxyl-6′-oxo-8′-methyldecyl)-3-methyl-4-hydroxybut-2-en-1,4-olide, respectively (Fig. 2).[5] In this study, we synthesized the structural variants of these SRBs (2,3-dihydro-SRBs and 6′-deoxo-SRBs) to analyze their biological activity. We found that 2,3-dihydro-SRBs were unable to induce LC and LM production, suggesting the importance of the C2-C3 double bond for activity. On the other hand, 6′-deoxo-SRBs exhibited induced LC and LM production, suggesting that the C-6′ ketone group in SRBs is not directly related to activity. Several potential genes for SRB biosynthesis were found clustered with *srrX* (*orf85*) on pSLA2-L (Fig. 2): an NAD-dependent dehydrogenase gene, *srrG* (*orf81*); a phosphatase gene, *srrP* (*orf83*); a P450 hydroxylase gene, *srrO* (*orf84*); and a thioesterase gene, *srrH* (*orf86*). Comprehensive mutational analysis to confirm the identity and function of the SRB biosynthetic genes is in progress in our laboratory.

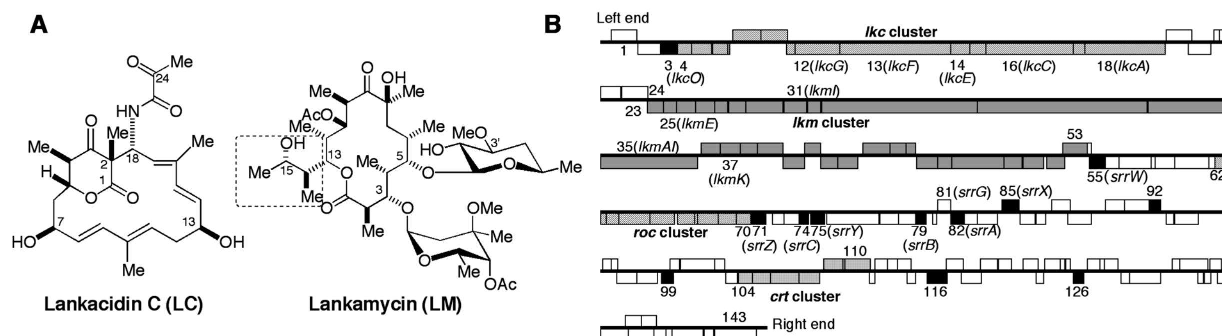

Fig. 1. (A) Chemical structure of lankacidin C (LC) and lankamycin (LM) isolated from *Streptomyces rochei* 7434AN4. (B) Gene organization of a linear plasmid pSLA2-L.

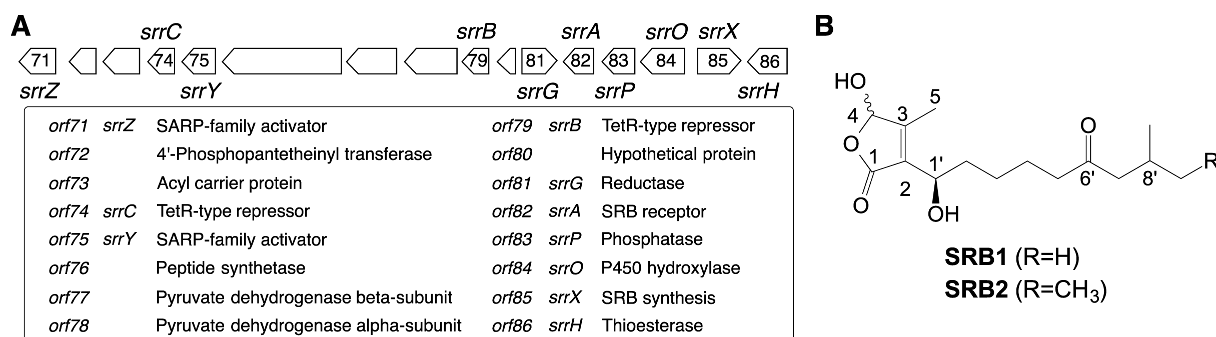

Fig. 2. (A) The biosyntheic gene cluster for the signaling molecules SRB1 and SRB2 on pSLA2-L. (B) Structure of SRB1 and SRB2 isolated from *S. rochei*.

2. *Genome mining of cryptic biosynthetic gene clusters by genetic manipulation of the regulatory genes*

Mutation of *srrB*, a *tetR*-type transcriptional repressor gene, resulted in the overproduction of both LC and LM, suggesting that *srrB* has a negative role in antibiotic biosynthesis. This result led us to develop a genome mining approach in *S. rochei* to discover new secondary metabolites by activation of silent cryptic gene clusters. A triple mutant with an *srrB* mutation and blocked in the biosynthetic pathway of both LC and LM accumulated moderate amounts of a UV-active compound, which was not present in the parent strain or in any of the single/double mutants. This compound contains a unique azoxyalkene chromophore, whose biosynthetic pathway is under investigation.

Conclusion

In general, the *Streptomyces* genome contains over 20 genes or gene clusters associated with the biosynthesis of secondary metabolites. Under normal laboratory conditions, nearly 70–80% of biosynthetic genes are transcriptionally silent. Genetic manipulation of regulatory genes might be a powerful tool to identify bioactive secondary metabolites of cryptic biosynthetic pathways.

References

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