Functions of a novel signal molecule inducing sporulation in *Streptomyces* species

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Research aims

Surugamides A–E are cyclic octapeptides isolated from a marine-derived actinomycete, *Streptomyces* sp. JAMM992. We have revealed that other marine *Streptomyces* isolated from sea sediment around Japan and several terrestrial *Streptomyces* produced surugamide A; Thorson et al. reported that a terrestrial *Streptomyces* isolated from soil collected at a coal mine fire site in Kentucky also produced surugamide A. The ubiquity of biosynthetic genes for surugamides led us to speculate that it has some physiological function in the producers. We set out to identify the biosynthetic gene cluster for surugamide A to generate a mutant strain that lacks surugamide A productivity and then elucidated the physiological function of surugamide A using the mutant strain. Our study on the biosynthetic gene cluster for surugamide A revealed that surugamides A-E were produced by two NRPS genes, which were separated by two other NRPS genes; the central two genes were responsible for the biosynthesis of an unrelated peptide, termed as surugamide F. The formation of aerial mycelium was notably delayed in the Δ*surA* strain, which was recovered by treatment with surugamide A in a concentration- dependent manner. In this study, we identified the putative target protein of surugamide A and revealed that surugamide A promotes aerial mycelium formation.

Methods

1. Preparation of mutant strains based on the biosynthetic gene cluster of surugamides.

   Surugamides A-E are biosynthesized by a combination of two non-ribosomal peptide synthetases (NRPS) SurA and SurD in the four successive NRPS genes, SurA-D. The amino acid sequence in surugamide F, biosynthesized by the other NRPS SurB and SurC, is much different from that in surugamides A-E. To analyze the physiological function of surugamides A and F, mutant strains of Δ*surB* and Δ*surA*, and Δ*surB* were generated by in-frame deletion of the A-domain in the first module of each gene.

2. Identification of the protein function to promote aerial mycelium formation.

   An enzyme enhanced by surugamide A was isolated by bioassay-guided fractionation

from the culture media of *Streptomyces* sp. JAMM992. The amino acid sequence at the N-terminus of the enzyme was analyzed by a protein-sequencer. With the sequence in hand, the complete amino acid sequence of the enzyme was obtained from the draft genome sequence. Recombinant SJP was then expressed in *Escherichia coli* and purified with Ni-Sepharose.

3. Comparison of amino acid sequences in SJP homologs among surugamide-producing and non-producing *Streptomyces*.

Genomes derived from several *Streptomyces* were sequenced using Illumina Hiseq, followed by read mapping to the sequence of SJP. A phylogenic tree was constructed using the neighbor-joining method.

Results

With the sequence of the biosynthetic gene cluster for surugamide A, the mutant strain Δ*surA* was prepared by in-frame deletion of the A-domain in the first module of surA. Sporulation of the mutant strain was clearly delayed, and was recovered by the addition of SA in a dose-dependent manner. Because SA and SF are biosynthesized by different genes in the same gene cluster, we hypothesized that SF also plays an important role in the strain. Therefore, we prepared Δ*surA* as well as Δ*surB*, and a double mutant, Δ*surA*Δ*surB*. Delayed sporulation similar to that in Δ*surA* was observed in both Δ*surB* and the double mutant. Addition of SF also recovered the delay in sporulation. In case of the double mutant, addition of SA recovered the delay in sporulation partially, whereas addition of both SA and SF recovered the sporulation completely (Figure 1). These results suggest that SA and SF are implicated in aerial mycelium formation.

Next, we embarked on elucidating the molecular mechanisms to promote aerial mycelium formation. Because SA inhibits cathepsin B activity, we hypothesized that SA affected one of the proteases in the strain. After preparation of a crude extract from the surugamide producer, we checked its enzyme activity *in vitro*. Eventually we found that SA activated one of the protease. The crude extract was then separated based on activation of enzyme activity to obtain a purified protease. The amino acid sequence at the N-terminus of the protease was analyzed by Edman degradation, and the complete amino acid sequence was obtained from genomic data. In order to confirm that SA activated the protease, we prepared a recombinant protein of the mature protease with His-tag, expressed in *E. coli*. Finally, we concluded that activation of the protease promoted aerial mycelium formation. On the other hand, SF did not activate the recombinant protease, which implied the presence of another molecular target for SF.

To the best of our knowledge, this is the first case where aerial mycelium formation was promoted through protease activation. Therefore, we performed a survey on the activation

specificity for the homologues of the protease from several *Streptomyces* species. To our surprise, SA only activated the protease derived from the surugamide-producing *Streptomyces*, but not the one from non-producers (Figure 2). Amino acid sequences of these proteases were obtained from draft genome data. The amino acid sequence of the protease from the producer was obviously different compared to those from non-producers despite relatively high homology. We concluded that SA activated only specific proteases derived from surugamide producers.

Conclusion

In this study, we found a novel mechanism wherein surugamides enhance aerial mycelium formation *via* activation of one protease by the peptides. Even though a few compounds are known to control morphological changes by inhibiting extracellular proteases, this is the first case where the aerial mycelium formation was promoted through protease activation. We therefore consider that surugamides are a novel type of signaling molecules.

References

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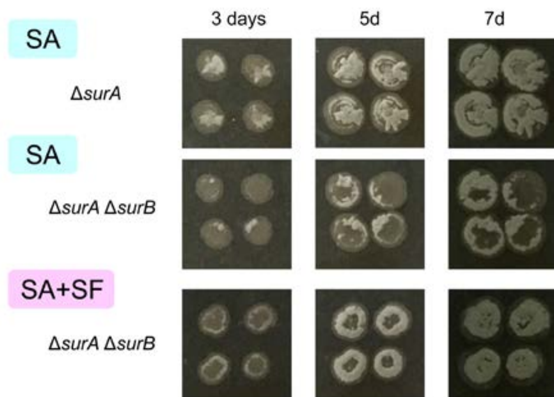

Figure 1. Recovery of sporulation in the mutants. (upper) addition of SA in Δ*surA* (middle) addition of SA in Δ*surA* Δ*surB* (lower) addition of SA and SF in Δ*surA* Δ*surB*

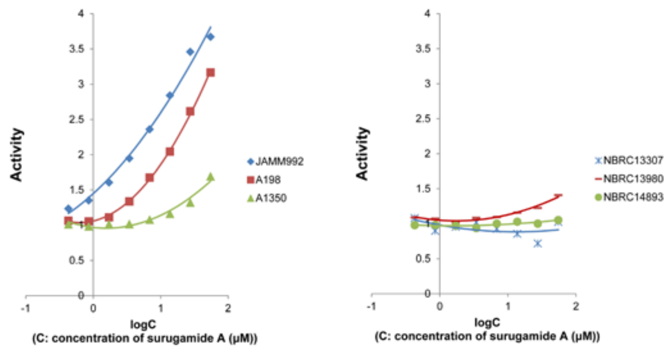

Figure 2. Enzyme activity of proteases after the treatment of SA. (left) proteases from Streptomyces producing surugamides (right) proteases from Streptomyces not producing surugamides