Effect of Different Biosynthetic Precursors on the Production of Nargenicin Aₐ from Metabolically Engineered Nocardia sp. CS682

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Nargenicin Aₐ is a 28-membered polyketide macrolide, with antibacterial activity against methicillin-resistant Staphylococcus aureus, produced by Nocardia sp. CS682. In this study, the production of nargenicin Aₐ was improved by enhancing the supply of different biosynthetic precursors. In Nocardia sp. CS682 (KCTC11297BP), this improvement was ~4.62-fold with the supplementation of 30 mM methyl oleate, 4.25-fold with supplementation of 15 mM sodium propionate, and 2.81-fold with supplementation of 15 mM sodium acetate. In Nocardia sp. metK18 and Nocardia sp. CS682 expressing S-adenosylmethionine synthetase (MetK), the production of nargenicin Aₐ was improved by ~5.57-fold by supplementation with 30 mM methyl oleate, 5.01-fold by supplementation with 15 mM sodium propionate, and 3.64-fold by supplementation with 15 mM sodium acetate. Furthermore, supplementing the culture broth of Nocardia sp. ACC18 and Nocardia sp. CS682 expressing acetyl-CoA carboxylase complex (AccA2 and AccBE) with 30 mM methyl oleate, 15 mM sodium propionate, or 15 mM sodium acetate resulted in ~6.99-, 6.46-, and 5.58-fold increases, respectively, in nargenicin Aₐ production. Our overall results showed that among the supplements, methyl oleate was the most effective precursor supporting the highest titers of nargenicin Aₐ in Nocardia sp. CS682, Nocardia sp. metK18, and Nocardia sp. ACC18.

Keywords: Methyl oleate, Nocardia sp. CS682, nargenicin Aₐ, polyketide, precursor

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and a global regulatory gene, \( \text{afsR} \), \([10, 12, 16, 21, 28, 29, 30]\).

In microorganisms, the polyketides are assembled by a series of decarboxylative condensations of simple carboxylic acid precursors catalyzed by polyketide synthase complexes, following a mechanism similar to that utilized in fatty acid synthesis \([6]\). As polyketides are assembled using several common biosynthetic precursors, which include malonyl-CoA, methylmalonyl-CoA, and ethylmalonyl-CoA, regardless of their structural diversity \([3, 6, 9]\) one of the strategies is to increase the respective precursor pool for enhancing the production of polyketides. There has been considerable interest in enhancing the yield of polyketides by increasing the supply of precursors, because intracellular availability of biosynthetic precursors is a key factor determining the productivity of secondary metabolites. \([8, 13, 18–20, 24–26]\).

\( \text{Nocardia} \) species are partially acid-fast stainable and their complex cell walls cannot easily be disrupted by standard chaotropic solutions used for the rapid lysis of other bacteria \([5]\). Even though genetic manipulation in \( \text{Nocardia} \) species has been limited by available microbiological methods, in our previous study, we successfully carried out the metabolic engineering of \( \text{Nocardia} \) sp. CS682 and showed that transcriptional activator genes and precursor genes from \( \text{Streptomyces} \) strains could be expressed to enhance the production of antibiotics in \( \text{Nocardia} \) species. To enhance the production of nargenicin \( \text{A}_1 \), we separately overexpressed \( \text{S}-\text{adenosylmethionine synthetase (MetK1-sp)} \) from \( \text{S. peucetius} \) and the acetyl-CoA carboxylase complex (\( \text{AceA2} \) and \( \text{AceBE} \)) from \( \text{S. coelicolor} \) \( \text{A3 (2)} \) in \( \text{Nocardia} \) sp. CS682 \([15]\).

Fig. 1. Diagrammatic sketch showing various approaches for the enhancement of nargenicin \( \text{A}_1 \) production.

Nargenicin \( \text{A}_1 \) aglycon has been shown to be derived from common precursors (acetate and propionate) by a series of incorporation experiments employing \([1-\text{\textsuperscript{13}}\text{C}]\)-, \([2-\text{\textsuperscript{13}}\text{C}]\)-, and \([1,2-\text{\textsuperscript{13}}\text{C}]-\text{acetate and } [1-\text{\textsuperscript{13}}\text{C}]-\text{ and } [2-\text{\textsuperscript{13}}\text{C}]\)-propionate \([1]\). In the present study, we investigated the influence of different biosynthetic precursors, including sodium acetate, sodium propionate, and methyl oleate on nargenicin \( \text{A}_1 \) biosynthesis by \( \text{Nocardia} \) sp. CS682 and its metabolically engineered strains. When the optimal concentrations of these precursors were determined, the effect of each precursor concentration was further observed. Our study showed that the production of nargenicin \( \text{A}_1 \) in \( \text{Nocardia} \) strains was enhanced to different levels by increasing the intracellular pools of different biosynthetic precursors. To our best knowledge, this is the first study to investigate the influence of precursors of short-chain fatty acids on the biosynthesis of nargenicin \( \text{A}_1 \) in \( \text{Nocardia} \) strains.

**Materials and Methods**

**Chemicals and Reagents**

Methyl oleate (Acros Organics), sodium propionate (Sigma), and sodium acetate, anhydrous (Amresco) were used for feeding experiments. All the other chemicals and reagents used were of analytical grade.

**Bacterial Strains and Growth Conditions**

\( \text{Nocardia} \) sp. CS682 and its metabolically engineered strains, \( \text{Nocardia} \) sp. metK18 and \( \text{Nocardia} \) sp. ACC18, were employed to study the influence of different biosynthetic precursors on nargenicin \( \text{A}_1 \) biosynthesis by \( \text{Nocardia} \) strains. \( \text{Nocardia} \) sp. NV18 was used