An indigoidine biosynthetic gene cluster from *Streptomyces chromofuscus* ATCC 49982 contains an unusual IndB homologue

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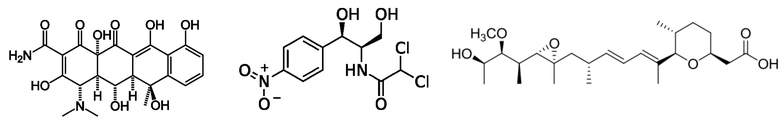
Introduction

Streptomyces is well-known for the production of structurally diverse natural products, including many industrially important bioactive molecules, such as oxytetracycline and chloramphenicol. *Streptomyces chromofuscus* ATCC 49982 is the producer of the anti-cholesterol polyketide natural product herboxidiene. To better understand this pharmaceutically important strain, we sequenced the genome of *S. chromofuscus* ATCC 49982 and identified a noniterative type I polyketide biosynthetic gene cluster that is responsible for the biosynthesis of herboxidiene.



oxytetracycline chloramphenicol herboxidiene

Indigoidine is a powerful radical scavenger which enables phytopathogens to tolerate oxidative stress, organic peroxides and superoxides during the plant defense response Recently, indigoidine has also been found to possess antimicrobial activity. In this work, we identified a biosynthetic gene cluster which is responsible for the biosynthesis of a natural blue pigment, indigoidine. This 9.4-kb biosynthetic gene cluster (**Fig. 1**) contains five open reading frames (ORFs), including a putative indigoidine synthetase gene, designated *Sc-indC*. We functionally identified *Sc-IndC* and developed an efficient production and extraction process for this blue pigment. We also found that Sc-IndB is a novel helper protein, which can increase the yield of indigoidine by more than 40%.

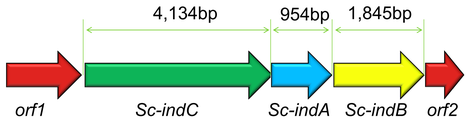
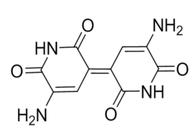


Fig. 1 The indigoidine biosynthetic gene cluster in *S. chromofuscus* ATCC 49982.



Indigoidine

Materials and Methods

Bacterial strains, vectors, and culture conditions: *E. coli* XL1-Blue and pJET1.2 were used for DNA cloning and sequencing. *E. coli* BAP1 and pET28a were used for protein expression and pACYCDuet-1 was used for the co-expression experiments. *S. coelicolor* CH999 was routinely grown in R5 medium at 30°C. *E. coli* cells were grown in Luria-Bertani (LB) medium.

Expression of *Sc-indC* in *S. coelicolor* CH999:The *Sc-indA, Sc-indB and Sc-indC* genes were amplified by PCR from the genome of *S. chromofuscus* ATCC 49982 (**Fig. 2**).The *Sc-indC* gene was ligated into pRM5 to generate pDY49 (**Fig. 3**). The plasmid was introduced into *S. coelicolor* CH999

Expression of *Sc-indC* in *E. coli* BAP1:The *Sc-indC* gene was inserted into the pET28a to generate pJV6. The plasmid was transformed into *E. coli* BAP1.

Co-expression of *Sc-indC* with *Sc-indA* and/or *Sc-indB* in *E. coli* BAP1: The amplified *Sc-indA* gene was inserted into MCS2 of the pACYCDuet-1 vector to yield pDY52. The *Sc-indB* was inserted into MCS1 of the pACYCDuet-1 vector to yield pDY53. The *Sc-indA* gene was ligated into MCS2 of pDY53 to afford pDY54. Each of these pACYCDuet-1 derived plasmids (pDY52, pDY53 and pDY54) was co-transformed with pJV6 into *E. coli* BAP1.

Results and Discussion

1. Functional characterization of *Sc-indC*

1.1 Expression of Sc-IndC in *S. coelicolor* CH999

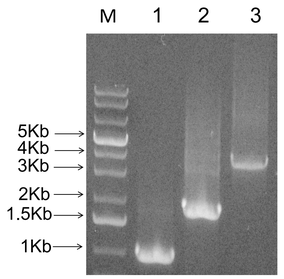


Fig. 2 PCR amplification of *Sc-indA*, *Sc-indB* and *Sc-indC*. M: DNA ladder;1: *Sc-indA*; 2: *Sc-indB*; 3: *Sc-indC*.

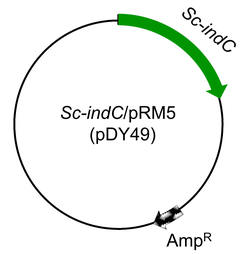


Fig. 3 Plasmid constructed for the expression of *Sc-IndC* in *S. coelicolor* CH999.

The engineered strain of *S. coelicolor* CH999/pDY49 was grown in R5 medium at 30°C and was found to produce a blue pigment after 6 days (**Fig. 4A** and **4B**). The pigment was extracted and re-dissolved in DMSO, which showed a bright blue color (**Fig. 4C** and **4D**). The pigment was identified as indigoidine by LC-MS analysis (**Fig. 4E**), thus confirming that Sc-InC is an indigoidine synthetase.

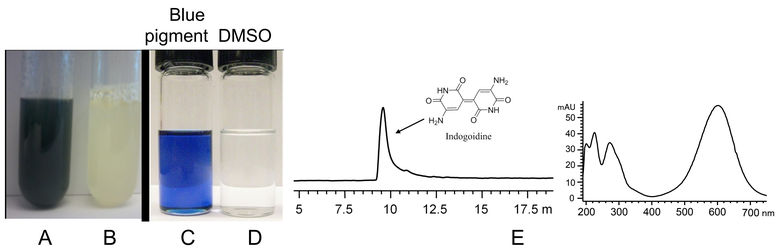


Fig. 4 A: Production of the blue pigment by *S. coelicolor* CH999/pDY49; B: Control; C: Blue pigment extracted from *S. coelicolor* CH999/pDY49 and redissolved in DMSO; D:DMSO; E: LC-MS analysis of the extracted pigment at 600 nm.

1.2 Expression of Sc-IndC in *E. coli* BAP1

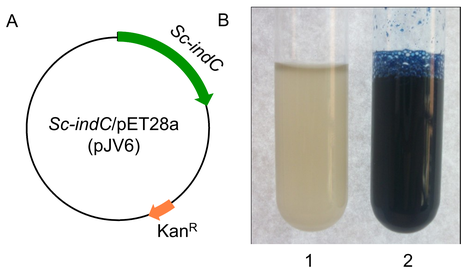


Fig. 5 A:Plasmid constructed for the *E. coli* BAP1 transformation; B: (1) control; (2) Production of indigoidine by *E. coli* BAP1/pJV6.

As expected, the *E. coli* BAP1 cells transformed with pJV6 (**Fig. 5A**) produced indigoidine (**Fig. 5B**). Compared to *S. coelicolor* CH999, the synthesis of this blue pigment in *E. coli* was much faster. The blue color could be easily observed in the *E. coli* culture 30 min after IPTG induction.

2. The yield of indigoidine

In *S. coelicolor* CH999, the maximum yield of indigoidine achieved is 593.5 mg/l after 6 d of cultivation;

The optimal production conditions in *E. coli* were studied. The best fermentation condition:OD_{600} = 0.6 (**Fig. 6A**); Temperature = 18°C (**Fig. 6B**) Time = 28 hours (**Fig. 6B**). The yield reached 2.78 g/l.

The pigment is more stable at lower temperatures (**Fig. 6C**)

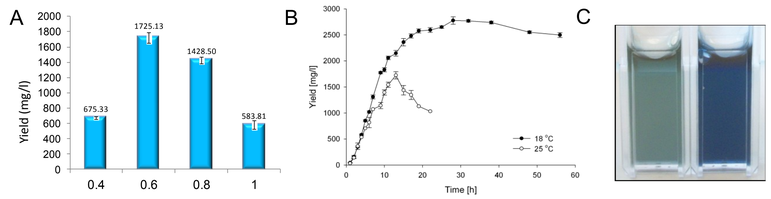


Fig. 6 A: Effect of the OD_{600} values with IPTG induction on the yield of indigoidine; B: Time-course analysis of indigoidine production at 18°C and 25°C. C: Effect of temperature on the stability of indigoidine. The pigment was stored at room temperature (left) and 4°C (right) in cell-free LB medium for 2 d.

3. Co-expression of *Sc-IndC* with *Sc-IndA* and/or *Sc-IndB* in *E. coli* BAP1

All the proteins can be expressed in *E. coli* (**Fig. 7A**). Sc-InA has no effects on indigoidne biosynthesis, but Sc-IndB can significantly increased the yield of the pigment to 3.93 g/l (**Fig. 7B**).

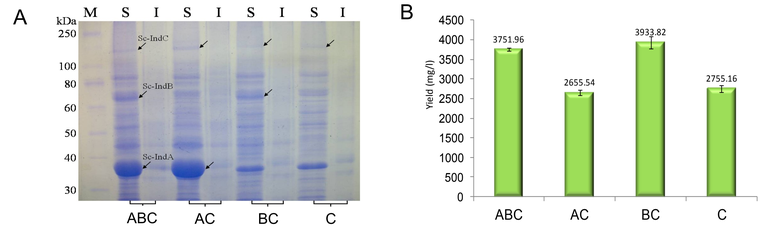


Fig. 7 A: SDS-PAGE analysis of co-expression of *Sc-IndA* and/or *Sc-IndB* with *Sc-IndC* in *E. coli* BAP1 at 18°C; B: The yield of indigoidine in *E. coli* BAP1 with or without co-expression of *Sc-IndA* and/or *Sc-IndB*.

Conclusion

- Discovered a new indigoidine biosynthetic gene cluster.
- Indigoidine biosynthesis was reconstituted in both *S. coelicolor* CH999 and *E. coli* BAP1.
- Under the optimal fermentation conditions, the yield of indigoidine reached 2.78 g/l in *E. coli* BAP1/pJV6.
- The presence of Sc-IndA had no obvious effects on the production of the blue pigment.
- Co-expression of *Sc-IndB* with *Sc-IndC* drastically increased the yield of indigoidine by 41.4% (3.93g/l). Thus, Sc-IndB plays a role of helper in indigoidine biosynthesis.

Acknowledgements

Funding support: American Heart Association (National Scientist Development Grant 09SDG2060080); National Natural Science Foundation of China (31170763).

We thank Prof. Chaitan Khosla, Stanford University for kindly providing *S. coelicolor* CH999 and *E. coli* BAP1.Thanks to Prof. Zhan and Dr. Yu. Thanks to all the members of the Metabolic Engineering Laboratory!