Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2014, 6(6):508-511

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Identification of *Aurj3M* as a positive regulator of aureofuscus biosynthesis in *Streptomyces aureofuscus*

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ABSTRACT

The production of aureofuscin is very low in the wild-type strain. We attempt to increase the production of aureofuscin by over-expression of a controlling gene in the wild-type strain. Recombinant bacterial strains were constructed by transforming SYAU0709 with an expression plasmid (pBJJ3M) that contained aurj3M, thereby increasing the number of aurj3M gene copies. The fermentation results showed aurJ3M gene could promote Aureofuscin production. Specifically, the recombinant strain produced approx. 600% more aureofuscin, as quantified by high-performance liquid chromatography analysis. The recombinant strains have the stable heritage.

Key words: *Streptomyces aureofuscus* ; Aureofuscin ; aurj3M gene; production

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INTRODUCTION

Aureofuscin is a tetraene macrolides antifungal antibiotic produced by *Streptomyces aureofuscus* isolated from the soil in China. The structure of aureofuscin is similar to natamycin (natamycin = pimaricin)[1-2]. Both antibiotics exhibit high activity against human pathogens such as mycetes, yeast, and mycelial fungus, and low activity against bacterium[1-4].

In addition, the study of IPRS (1975) indicates that aureofuscin can be easily extracted from fermentation broth and has almost no toxicity to mammals. Such regulatory cascades are consistent with the need of *Streptomyces* spp[5-9]. These studies have established the rationale for enhancing aureofuscin- production by over- expression of a gene in the aureofuscin biosynthesis gene cluster. However, to our knowledge, this approach has not been tried before and few genes in the aureofuscin pathway have been cloned and characterized. In this study, we attempt to increase the production of aureofuscin by over-expression of a controlling gene in the wild-type strain and construct gene engineering strains. Therefore, if the yield of aureofuscin could be increased, it could be a promising antifungal agent to be used industrially as a food preservative.

EXPERIMENTAL SECTION

S.aureofuscus was incubated at 29◦C. *E. coli* strains DH5*α* and *JM109* were used as hosts for constructs made with vector pMD19 for general cloning and subcloning. *E. coli* ET12567 (pUZ8002) was used for intergeneric conjugal transfer of DNA from *E. coli* to *Streptomyces* [10-11]. 2×YT medium, yeast extract/malt extract liquid medium (YEME), and agar minimal medium (MM) were prepared as described [12-13]. Yeast extract/amidulin (YSA) medium [14] was used for aureofuscin fermentation. *E.coli* strains were cultured in liquid or on solid Luria–Bertani (LB) medium at 37◦C[12].

Plasmid pBJJ3M containing the *S. aureofuscus aurj3M* gene was constructed by the following steps. Primers P1 (5’-GTGGATCCTCACTTCACGAAGTCGTCCA-3’) and P2 (5’-GTGATATCATGGC GAGCCTTGATAGAA C-3’) with *Bam*HI and *Eco*RV restriction sites underlined were used to amplify the *aurj3M* gene. The PCR product

was digested with *Bam*HI and *Eco*RV then ligated into the *Bam*HI and *Eco*RV sites of pBJERM. The resulting plasmid was named pBJJ3M (Fig.1) and introduced into wild-type *S.aureofuscus* SYAU0709 via *E.coli-Streptomyces* conjugation.

High Performance Liquid Chromatography (HPLC) analysis was performed using a Waters C18 column as the stationary phase and methanol-water-glacial acetic acid (48:32:1, v/v) as the mobile phase. The flow rate was 1.0 ml/min, UV wavelengh at 302.5 nm. A known, pure sample of aureofuscin was used as the internal standard.

RESULTS AND DISCUSSION

Construction of recombinant *S.aureofuscus* strains with *aurj3M* gene

According to our former research, a DNA fragment of approximate 579 bp was amplified from *S. aureofuscus* SYAU0709 by PCR method. The amplified new gene was named *aurj3M* [15]. Further, the AURJ3M was registered (GenBank Accession EU697915).In this study, a high-copy-number plasmid pBJJ3M from pBJERM containing a promoter region-*ermEp* [16] and the *S. aureofuscus aurj3M* gene (Figure 1) was constructed. The plasmid was introduced into wild-type *S.aureofuscus* SYAU0709 via *E.coli-Streptomyces* conjugation. Recombinant strains were obtained using apramycin resistance as a selector and further confirmed by replica screening [17]. Resistance of the resulting strains to apramycin indicated the incorporation of the constructs pBJJ3M into the chromosome of S. aureofuscus SYAU0709.

Next, whether the *aurj3M* gene was constructed into pBJJ3M was examined. PCR amplification proof results of plasmid PBJJ3M (Figure 2A) shows that the *aurj3M* gene (579 bp) was successfully constructed into pBJJ3M in No.J2, J3, J4. Figure 2B shows that plasmid PBJJ3M contain *aurj3M* gene by restriction analysis proof of plasmid PBJJ3M.

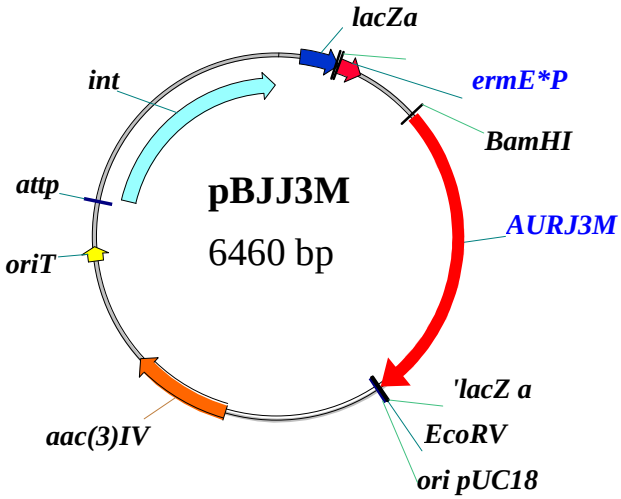

Figure 1 Construction of plasmid PBJJ3M

Plasmid pBJJ3M with ~740bp DNA fragment containing the *aurj3M* gene and its promoter region *ermE*P*(Jin Huiyi and Zhang Huizhan,2006) cloned into the multi-cloning sites (mcs) of vector pSET152(Ling Huayun *et al*,2006).

Arrow indicates the coding sequence of the inserted gene; aac (3) IV, apramycin resistance gene.

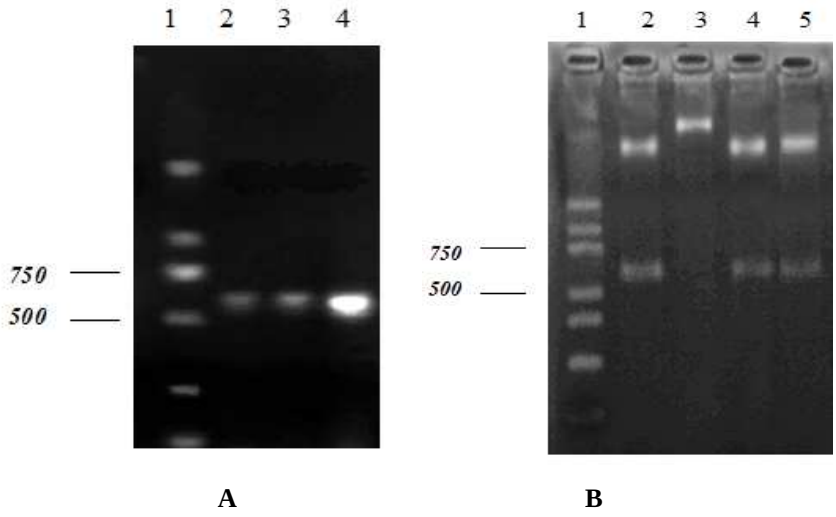

Figure 2. A,PCR amplification proof results of plasmid PBJJ3M
Lanes: 1, DL2000 DNA Ladder Marker; 2, No.2; 3, No. 3; 4, No. 4; B, Restriction analysis proof of plasmid PBJJ3M. Line 1, DL2000; Line 2, PBJJ3M/BamHI+EcoRV(No. 2).; Line 3, PBJJ3M; Line 4-5, PBJJ3M/BamHI+EcoRV(No. 3、4).

Effect of over-expression of *aurj3M* on aureofuscin production

To analyze aureofuscin, a portion of the culture broth was extracted with methanol by shaking vigorously, and then supernatant portion was obtained by high speed centrifugation. Aureofuscin was extracted and HPLC analysis indicated that its production in wild-type and recombinant strain was ~700 and ~4000 μg ml^{-1} respectively. This shows that the production of aureofuscin from the recombinant strain was almost six-fold than that of the wild-type strain (Figure 3).

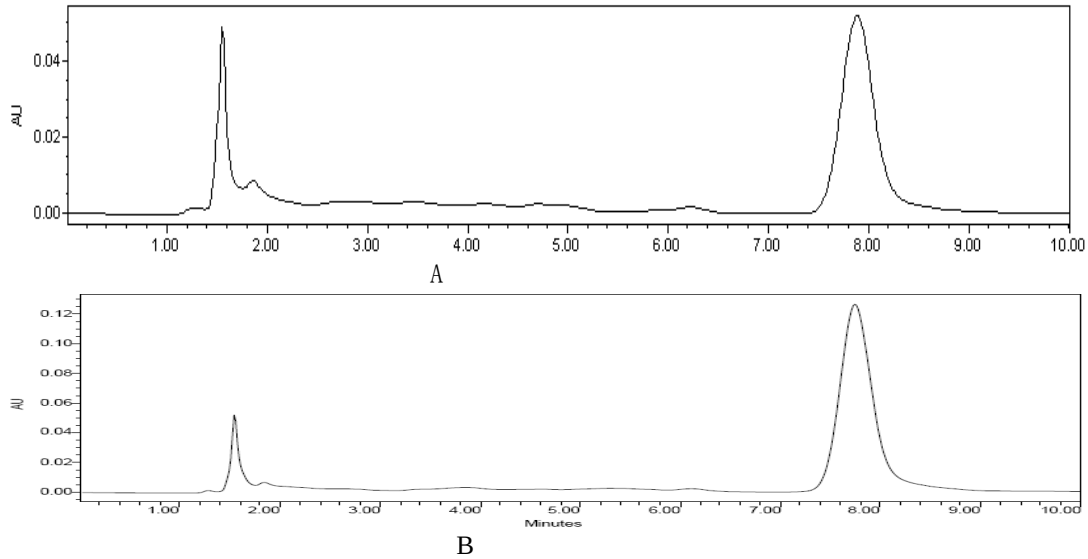

Figure 3. HPLC identification of Aureofuscin

Aureofuscin samples were prepared after incubation in YSA medium for 5 days. A, culture filtrates from wild-type *S. aureofuscus* SYAU0709;B, culture filtrates from the recombinant strain containing plasmid pBJJ3M.

Retention time of Aureofuscin is 7.957min.

Next, whether the *aurj3M* gene had an effect on colony phenotype was examined. The wild-type strain and the recombinant strain were grown in YSA medium, and mycelia were harvested at various growth phases. Figure 4 shows that the production of aureofuscin (yellow color) in the recombinant strain was remarkably higher and increased quickly, consistent with the over-expression of the AURJ3M protein. The concentrations of aureofuscin were rapidly increased when we determined to use plate cultivation with fixed concentrations at the same time.

Thus, it is concluded that the enhanced production of aureofuscin is mainly due to the over-expression of the

aurj3M gene and AURJ3M played the positive role.

(1) (2) (3) (4) (5)

Figure 4

(1) Growth information of wild-type strain SYAU0709. Strains were inoculated on YSA medium, followed by incubation for 4 days at 29°C. White colony in strains. (2) Growth information of wild-type strain SYAU0709. Strains were inoculated on YSA medium, followed by incubation for 6 days at 29°C. Grey spore in strains. (3) Growth information of wild-type strain SYAU0709. Strains were inoculated on YSA medium, followed by incubation for 8 days at 29°C. Yellow production in strains. (4)Growth information of the recombinant strain. Strains were inoculated on YSA medium, followed by incubation for 2 days at 29°C. Massive yellow production(Aureofuscin) in strains. Aureofuscin production was detected as described previously.(5)Aureofuscin production of wild-type *S. aureofuscus* SYAU0709 and recombinant strain. Strains were inoculated in YSA liquid medium, followed by incubation for 3 days at 29°C. Massive yellow production (Aureofuscin) in recombinant strain(Left).

CONCLUSION

These findings have demonstrated that yield enhancement of an important anti-fungal compound can be accomplished through the isolation and manipulation of a stimulatory factor. This approach was validated in a relatively low-producing wild-type strain and also in a semi-industrial strain. Thus, this methodology can be further used by microbiologists and biotechnologists to improve the abilities of numerous industrial strains.

Acknowledgements
This work was supported by grants from Science and Technology Government Department Fund in Liaoning province, the National Natural Science Foundation of China. We thank Prof. Song Yuan (China Agricultural University) for providing plasmids and apramycin.

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