Enhancement of Avermectin Production by Introduction of a Foreign Regulatory Gene in *Streptomyces avermitilis*

황용순[1], 이재영[1], 김상수[1], 김응수[2], 최차용[1*]
서울대학교 응용화학부, 생물공학실험실[1], 한국외국어대학교 환경학과, 환경생물공학 실험실[2]
전화 (02) 880-7079, FAX (02) 888-1604

ABSTRACT

Production of eight avermectin components was improved in *Streptomyces avermitilis* wild type strain (ATCC31267) and high producing mutant strain (ATCC31780) when transformed with a foreign regulatory gene, *afsR2* of *Streptomyces lividans*. Wild type and the high producing strain of *S. avermitilis* transformed with multiple copies of *afsR2* improved total avermectin productions by 2.3 fold and 1.5 fold, respectively. In both of wild type and the high producing transformants carrying *afsR2*, glycerol was proved to be the best carbon source for the stimulation of avermectin production.

INTRODUCTION

afsR2 was originally isolated from *Streptomyces lividans* and proved to induce overproduction of at least two structurally unrelated antibiotics, actinorhodin and undecylprodigiosin in *S. lividans* and its close relative *Streptomyces coelicolor* [1]. The *afsR2* gene encodes a 63 amino-acid protein which has been shown to be a transcriptional activator of the pathway-specific regulatory genes of the two antibiotics in *S. lividans* [1]. To apply this stimulatory ability of *afsR2* to the avermectin production, we introduced the multiple copies of *afsR2* into wild type strain and high producing mutant strain of *S. avermitilis*. In this study, we show that total avermectin production is increased by the introduction of the heterogeneous regulatory gene, *afsR2* and the increasing effect of this gene is stimulated by the use of glycerol as a sole carbon source in both wild type and high producing mutant strain of *S. avermitilis*.

MATERIALS AND METHODS

Bacterial strains and plasmids

S. avermitilis ATCC31267 and ATCC31780 were used as *afsR2* acceptor and plasmid pMOV532, pIJ487 containing *afsR2* was used for the transformation of *S. avermtilis* and the fermentation for the stimulation of avermectin production.

Media and culture conditions

S. lividans TK21 strain was grown on R2YE or in liquid YEME medium. In the case of

S. avermitilis, the inocula were cultivated for 72 hr in a complex medium containing (g/ℓ): glucose, 30; soybean meal, 5; yeast extract, 30; pepton, 5; malt extract, 3. The following production minimal media containing various carbon sources were used for avermectin production (g/ℓ): each carbon source, 30; $(NH_4)_2SO_4$, 2; $CaCO_3$, 5; NaCl, 3; $FeSO_4$, 0.05; $MnSO_4$, 0.05; $MgSO_4$, 0.1. The culture media were inoculated with a 5% (v/v) and incubated for 10 days.

Analysis of avermectin production

For the detection of avermectin, samples of the fermentation broth (2 mℓ) were brought to 80% saturation with methanol (v/v) and shaken vigorously (300 rpm) for 15 min in a shaking incubator. The solids were removed by centrifugation, and avermectin in the supernatant was determined by high-pressure liquid chromatography (HPLC) on a Waters C-18 column with methanol-water (85:15, v/v) as the mobile phase [2-4].

RESULTS AND DISCUSSION

Effect of exogenous afsR2 *on avermectin production*

Introduction of the multiple copies of *afsR2* into the wild type ATCC31267 strain and the high producing mutant ATCC31780 caused the overproduction of avermectins by 2.3 fold and 1.5 fold, respectively. The variation of overall avermectin production was only showed because the eight major avermectin components were increased or decreased in the almost same ratio for the entire culture period. Although overall avermectin production was slightly decreased in both *S. avermitilis* ATCC31267 and ATCC31780 carrying only pIJ487, the growth of all transformants was not severely affected by the plasmid carriage or the addition of thiostrepton at the concentration of 1 μg/mℓ. The pMOV532-cured strain obtained by streaking the transformants on R2YE plates without thiostrepton produced the same amount of overall avermectin as those of the wild type strain. As expected, the retransformation of the plasmid-cured strain with pMOV532 regained the avermectin overproduction capability. These results confirmed that the introduction of the multiple copies of *afsR2* caused the enhancement of the total avermectin production in both the wild type and the high producing mutant of *S. avermitilis*.

Effect of various carbon sources on avermectin production in S. avermitilis *transformants carrying afsR2*

S. lividans TK21 without the extra copies of *afsR2* was proved to stimulate the production of the two pigmented antibiotics in a specific physiological condition such as a minimal medium containing glycerol as a sole carbon source [5]. These results suggested that the growth in a glycerol-containing minimal medium would stimulate the

expression of *afsR2* on the chromosome of *S. lividans* TK21. In order to investigate the effect of glycerol as well as other carbon sources on the expression of the exogenous *afsR2* on avermectin production in *S. avermitilis*, the transformants carrying pMOV532 were cultured in the minimal medium containing each carbon source. Among all carbon sources tested, the glycerol was found to be the best for the production of avermectin in both *S. avermitilis* ATCC31267 and ATCC31780 transformants with pMOV532 (Fig. 1). These results demonstrate that the exogenous *afsR2* seemed to be stimulated by glycerol in the transformed *S. avermitilis* such as *S. lividans* [5], and its stimulatory effect on avermectin biosynthesis might induce the transformants to increase the production of avermectin.

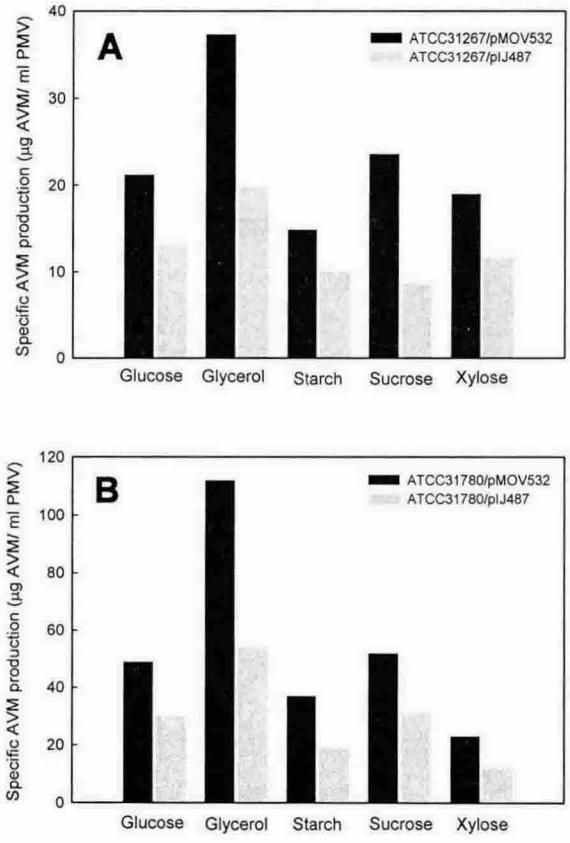

Fig. 1. The effect of various carbon sources on avermectin production in transformed *S. avermitilis* wild type ATCC31267 (A) and high producing strain ATCC31780 (B) carrying multiple copies of *afsR2* (pMOV532).

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