БІОЛОГІЧНІ НАУКИ

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THE IMPROVEMENT OF OXYTETRACYCLINE BIOSYNTHESIS BY INTRODUCTION OF EXTRA COPY OF OXYTETRACYCLINE RESISTANCE GENE *OTRB*

Streptomyces spp. are filamentous Gram-positive aerobic soildwelling bacteria that belong to the family *Streptomycetaceae* and the order *Actinomycetales*. *Streptomyces* spp. and closely related genera have the ability to coordinate the production of various secondary metabolites during morphological development [1]. Many of these secondary metabolites have antibiotic properties.

Current methods employed to increase the antibiotic productivity of industrial microorganisms range from classical random mutagenesis studies performed in conjunction with the optimization of large-scale industrial fermentations. Metabolic engineering is a common method used by researchers to regulate the production of many antibiotics. For example, genetic modifications of primary metabolic fluxes can lead to increases in the productivity of antibiotic synthesis [1, 2], since the availability of biosynthetic precursors is a key factor that determines their production. To date, many studies have reported the improvement of antibiotic production by engineering the availability of certain precursors in the producer organisms, such as the optimization of fermentation conditions, etc [1].

The aromatic polyketide antibiotic oxytetracycline (OTC) is produced by *Streptomyces rimosus* as an important secondary metabolite. There are three resistance genes in the OTC biosynthesis cluster, namely *otrA*, *otrB* and *otrC*, of which *otrA* and *otrB* are located at either end of this cluster. *otrA* changes the conformation of the 30S ribosome non-covalently and prevents the binding of OTC [1; 2]. Furthermore, *otrA* may be a substitute for the regulatory elongation factor, and *otrB* encodes a membrane transport protein that aids the transportation of OTC out of the cell. The *otrB* sequence shares great similarity with other transport genes, including *tetA* from Tn10 [1; 2].

However, the function of *otrC* remains to be elucidated. A traditional mutation program has resulted in the improvement of OTC production from 2 $g \times 1^{-1}$ to 80 $g \times 1^{-1}$, and OTC production can also be improved by disrupting the zwf (coding glucose-6-phosphate dehydrogenase) gene [1, 2]. Nevertheless, there is no information concerning the effects of *otrA*, *otrB* and *otrC* on OTC production.

Materials and Methods: The strains and plasmids used in this present study are listed in *Table 1*. Organisms were grown at 37°C in Luria-Bertani medium (1% tryptone, 1% NaCl, 0.5% yeast extract), and standard procedures were used for transformations. *E. coli* transformants were selected with ampicillin (100 mg×ml^{-1}), apramycin (50 mg×ml^{-1}), kanamycin (10 mg×ml^{-1}) or chloramphenicol (25 mg×ml^{-1}). *SRI* (*S. rimosus* M4018) was grown and manipulated as described previously [3].

Table 1

Strains and plasmids used in this present study

Strains or plasmids	Functions
E. coli Top10	Plasmid amplification
E. coli ET12567(pUZ8002)	Plasmid demethylation
Industrial *S. rimosus* (*SRI*)	OTC producer
pMD19T pMD19T-*otrA* pMD19T-*otrB*	Gene amplification
pSET152	Gene integration
pSET152-*otrA* pSET152-*otrB*	Gene integration

Reference: [3]

Construction of recombinant plasmids: The *otrA* (2.1 kb) and *otrB* (1.7 kb) gene fragments were amplified using primers P1 and P2 or P3 and P4 which are listed in *Table 2*. Genomic DNA of *SRI* was used as the template. The fragments were cloned into the pMD19T vector to yield pMD19T-*otrA*/ *otrB*, and then transformed into competent cells of *E. coli* Top10 [3]. Recombinant clones were screened by white-blue plaque selection and recombinant plasmids were analyzed by both single and double restriction enzyme digestion. pMD19T-*otrA*/*otrB* were digested with *Nde*I and *Xba*I, and then cloned into pSET152, which was digested with the same restriction enzymes to give pSET152-*otrA*/*otrB*. Then, the plasmids were transferred into *E. coli* ET12567 for demethylation and stored at -20°C until later use [3].

Table 2

Primers used in this present study

Primer	Sequence	Digestion site
P1(*otrA*-F) P2(*otrA*-R)	5' CGCCATATGATGAACAAGCTGAATCTGGG 3' 5' GGAAGCTTTCTAGATCACACGCGCTTGAGC 3'	*Nde*I *Xba*I
P3(*otrB*-F)	5' CGCCATATGGTGTCATCCGCAAATCCG 3'	*Nde*I
P4(*otrB*-R)	5' CCAAGCTTGCTCTAGATCAGGCGTCCGACGC 3'	*Xba*I
P5(*attB*-F)	5’ GTTCACCAACAGCTGGAGGC 3’	
P6(*attB*-R)	5’ CGTCATGCCCGCAGTGACC 3’	

Reference: [3]

Gene enhancement and identification of mutants: Demethylated pSET152-*otrA*/*otrB* were electroporated into *SRI* competent cells at 2 kV, 25 µF and 400Ω. Exconjugants were selected on tryptone soy agar plates containing apramycin 500 µg×mL^{-1} and incubated at 30°C for 4–6 days. Mutants were confirmed by polymerase chain reactions (PCR) using *apr*F (P5) and *apr*R (P6) as primers [3].

Fermentation experiments: A spore suspension was inoculated into 30 ml of seed medium containing glucose (10 g×l^{-1}), yeast extract (0.5 g×l^{-1}), tryptone (15 g×l-1), sucrose (2.8 g×l^{-1}) and calcium carbonate (0.1 g×l^{-1}). The first seed cultures were grown for 3 days at 260 rpm and 30°C. Then, 2 ml of the first seed culture was inoculated into 50 ml of fermentation medium in a 500-ml shaking flask with a spring. The second cultures were grown for 8 days at 260 rpm and 30°C [3].

For determination of dry cell weight, 5-ml samples of each culture were collected every 24 h and dried at 105°C to constant weight. OTC production *in vivo* and *in vitro* was analyzed by high performance liquid chromatography according to the reference [3].

Results: In this present study, we aimed to investigate the influence of *otrA* and *otrB* on OTC production by introducing extra copies of these resistance genes into the genome of the industrial strain of *S. rimosus* (*SRI*) [3]. Production of OTC was increased by 67% in one *SRI*-B mutant compared with the parent strain, suggesting that the enhancement of resistance gene *otrB* in the antibiotic producer is an effective way to improve OTC biosynthesis. However, introduction of extra copy of *otrB* could retard growth of mutant cells [3].

References:

1. Thompson C.J. Principles of microbial alchemy: insights from the Streptomyces coelicolor genome sequence. – New York, 2002. – 1020 p.
2. Nielsen J. The role of metabolic engineering in the production of secondary metabolites. – 1998. – P. 330–336.
3. Sambrook J. Molecular cloning: a laboratory manual / J. Sambrook, E.F. Fritsch, T. Maniatis. – New York: Cold Spring Harbor Laboratory Press, 1989.

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АНТРОПОГЕННИЙ ВПЛИВ НА СУЧАСНУ ЕКОЛОГІЧНУ КРИЗУ

Людина на певному етапі розвитку цивілізації почала активно перетворювати природу, а її вплив на довкілля збільшувався з кожним сторіччям, доки не став провідним екологічним фактором – антропогенним. Перелік екологічних проблем, породжених діяльністю людини, досить великий