Manufacturing of the Acylated Macrolide Antibiotic AIV by Bioconversion Method and Construction of a Genetically-Engineered Strain for Direct Fermentative Production of AIV

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AIV (3-*O*-acetyl 4"-*O*-isovaleryltylosin; Fig. 1) is an industrially important 16-membered macrolide antibiotic which is used as a veterinary medicine and is known to inhibit growth of tylosin-resistant bacteria. AIV is produced by bioconversion of tylosin which is produced by *Streptomyces fradiae*, using *Streptomyces thermotolerans* as a converter.

We obtained a strain of *S. thermotolerans* which has high conversion activity but lacks carbomycin production by NTG mutagenesis, and also determined optimum temperature (32°C) and optimum pH (pH 7.3 for 3-*O*-acetylation, pH 6.2 for 4"-*O*-isovalerylation) for the acylconversion. D-Glucose and L-leucine were important components for providing acetyl-CoA and isovaleryl-CoA intracellularly, because the AIV production increased when they were fed into the bioreactor. After improvement studies concerned with conditions for the better productivity of AIV, this bioconversion has been scaled up to the industrial level.

To resolve problems on cost-performance in the bioconversion method, we tried to generate a genetically-engineered strain which produces AIV in the single step fermentation. We have cloned three genes involved in the macrolide acylation from *S. thermotolerans*. These were identified to be structural genes for 3-*O*-acyltransferase (*acyA*) and 4"-*O*-acyltransferase (*acyB1*, equally *carE*), and a regulatory gene (*acyB2*) which activates expression of the both structural genes. A tylosin producer *S. fradiae* transformed with a plasmid carrying these genes directly produced 3-*O*-acetyltylosin and 3-*O*-acetyl 4"-*O*-acyltylosins including AIV as a minor component (56 µg/ml, 7.1% of total macrolide). L-Leucine supplemented at latter term of the culture contributed to accumulation of AIV (602 µg/ml, 71.6% of total macrolide) in great preference to the other tylosin-analogues. This fact suggests that the high level of the endogenous pool of isovaleryl-CoA is quite important for the selective production of AIV.

Fig. 1. Structure of AIV