PRODUCTION OF ANTIBIOTICS IN *RHODOCOCCUS*

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Key words: Rhodococcus, antibiotics, bioproduction

Introduction. The genus *Rhodococcus* is a non-spore-forming, high GC Gram-positive bacterium, a member of phylum *Actinobacteria*. Research studies for *Rhodococcus* have been mainly focused on its great ability to degrade recalcitrant chemicals such as poly-chlorinated biphenyls and dioxins (1, 2). Also, their pathogenic features have been intensively studied in limited species. However, only a few antibiotic producing *Rhodococcus* has been reported so far. In our recent study, numbers of strains showing the production ability were discovered for the first time (3, 4). It indicates that *Rhodococcus* is a good resource for new antibiotics and its biosynthesis genes, and also indicates that *Rhodococcus* might be a good host microorganism for the production of antibiotics.
In this study, we tried to improve host-vector system and to develop antibiotic production system in *Rhodococcus*.

Methods. In order to obtain new *Rhodococcus* host strain, nearly 100 strains were collected and tested for its plasmid stability in their cells. The stability test was demonstrated by checking the existence of intact plasmid after repeated subculture with or without selective drugs. To develop a new expression vector, a cryptic plasmid was isolated from *Rhodococcus* sp. strain R09 and then *E. coli—Rhodococcus* shuttle vector was constructed. Biosynthesis genes of an antibiotic aurachin RE (*rau* genes) were cloned and ligated into the expression vector and its production was tested under induced or un-induced conditions in the candidate host strain. The antibiotic production was confirmed by bioassay using an *Arthrobacter* strain as the indicator, and also confirmed by HPLC analysis.

Results. In some cases, *Rhodococcus* strain showed plasmid instable nature such as partial deletion of internal nucleotides (i.e. plasmid size change). A new host strain was needed to overcome this problem, and therefore numbers of *Rhodococci* were screened for the plasmid stability. As results, one preferable strain was isolated showing high plasmid stability, high growth rate and also low cellular aggregation nature. These characteristics are suitable for host strain. Then, we selected it for the new candidate host and named D32. All the other strains had some difficulty with plasmid stability and/or cellular aggregation. In order to understand the genetic background of this nature, genome DNA sequences of three *Rhodococcus* strains including D32 was analyzed (in progress).
A cryptic plasmid isolated from R09 was genetically modified to construct new expression vector, pBcf-XA (*E. coli—Rhodococcus* shuttle vector). This new expression vector contained newly obtained *Rhodococcus* replicon (replicon X) from the cryptic plasmid and contained constitutive promoter from benzoate catabolic gene cluster. The vector is compatible with previously developed vectors, pTip-QC and pNit-RT series (5). Using these vectors, up to three different vectors can be co-existed in *Rhodococcus* cells.
Aurachin RE is a quinoline antibiotic isolated from *R. erythropolis* JCM 6824 (4). Biosynthesis gene cluster of aurachin RE (*rau*) was identified from the strain and cloned into the expression vectors. The resultant vectors were then introduced to the strain D32, by electroporation. Using the host-vector system, aurachin RE was successfully produced in D32.

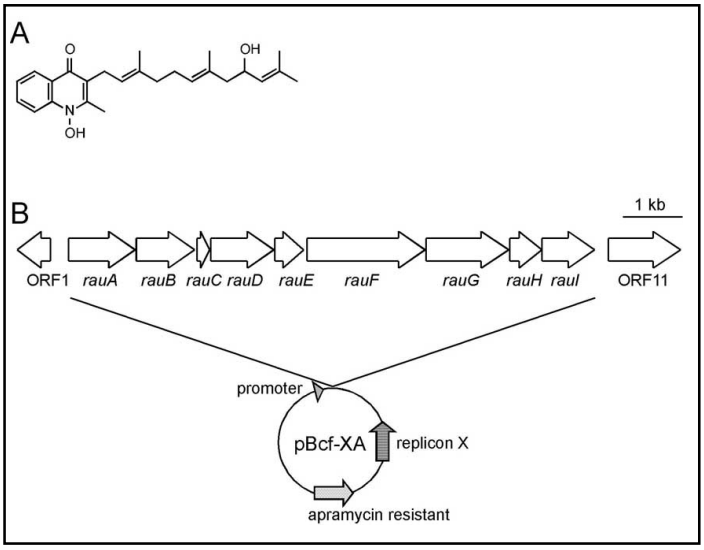

Fig.1 (A) Structure of aurachin RE. **(B)** Biosynthesis genes of aurachin RE (*rau* genes) were cloned into the expression vector pBcf-XA.

Conclusions. We have developed host-vector system in *Rhodococcus*. Using with the new host strain and new expression vector, antibiotic production was achieved in *Rhodococcus*. Our system might be a good alternative host-vector system for production of bioactive compounds.

References.

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