Self-resistance mechanism in *Streptomyces peucetius*: Overexpression of *drrA*, *drrB* and *drrC* for doxorubicin enhancement

Sailesh Malla, Narayan Prasad Niraula, Kwangyoung Liou, Jae Kyung Sohng*

Institute of Biomolecule Reconstruction (IBR), Department of Pharmaceutical Engineering, SunMoon University, # 100 Kalsan-ri, Asansi, Chungnam 336-708, Republic of Korea

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Summary

The resistance genes *drrABC* from *Streptomyces peucetius* ATCC 27952 were cloned into the pIBR25 expression vector under a strong *ermE* promoter to enhance doxorubicin (DXR) production. The recombinant expression plasmids, pDrrAB25, pDrrC25 and pDrrABC25, were constructed to overexpress *drrAB*, *drrC* and *drrABC*, respectively, in *S. peucetius* ATCC 27952. The recombinant strains produced more DXR than the parental strain: a 2.2-fold increase with pDrrAB25, a 5.1-fold increase with pDrrC25 and a 2.4-fold increase with pDrrABC25. We also studied the relative ratios of doxorubicin, daunorubicin and e-rhodomycinone produced in these recombinant strains.

Introduction

*Streptomyces* produce various bioactive natural products and possess resistance systems for these metabolites, which are co-regulated with antibiotic biosynthesis genes. Microorganisms require one or more self-resistance determinants to produce antibiotics; these encode proteins that inactivate the antibiotic, facilitate its export, or modify the host to render it insensitive to the antibiotic (Cundliffe 1989). Multiple resistance mechanisms are often found; in such cases, it is not known whether any one resistance mode is sufficient to ensure survival or antibiotic production.

*Streptomyces peucetius* ATCC 27952 produces two anthracycline-type antitumor compounds, daunorubicin (DNR) and doxorubicin (DXR) (Arcamone et al. 1969). The four *drr* genes in the DXR

Like that of other secondary metabolites in *Streptomyces* species, biosynthesis of DNR and DXR in *S. peucetius* is believed to be tightly regulated, thereby limiting DXR production (Stutzman-Engwall et al. 1992). Production inhibition is often a regulatory feature in secondary metabolism and can be a form of negative feedback. The DoxA P450 enzyme catalyzes three oxidation steps in the late stage of DXR biosynthesis (Figure 1A) (Dickens et al. 1997), but its activity is inhibited with increased DXR concentration (Walczyk et al. 1999) (Figure 1B). In addition, the cytotoxic nature of DXR acts against the producing strain itself and causes rapid cell death.

Figure 1. (A) Abbreviated biosynthetic pathways doxorubicin (DXR), Daunorubicin (DNR) and ε-Rhodomycinone (RHO) from propionyl-CoA, malonyl-CoA and glucose-1-phosphate. Open arrows indicate multiple steps between the precursor and the product shown. (B) Schematic representation of DoxA inhibition by DXR. (I) Conversion of 13-deoxy-DNR into DXR at low concentration of DXR and (II) inhibition of DoxA activity at high concentration of DXR.