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

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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 ε-rhodomycinone produced in these recombinant strains.
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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

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biosynthetic gene cluster from *S. peucetius*, *drrA*, *drrB* (Guifoile and Hutchinson 1991), *drrC* (Lomovskaya et al. 1996) and *drrD* (Ali and Hutchinson, unpublished), provide self-resistance to these drugs. DrrA and DrrB together form an ATP-dependent efflux pump for transporting DXR and DNR out of the cell, thereby imparting resistance (Guifoile and Hutchinson 1991; Kaur 1997). DrrC provides self-resistance to the cell through excisional repair of DNA by DNR–DXR-induced formation of hydroxyl radicals. Expression of DrrC in the *Escherichia coli* (*E. coli*) UNC523 *uvrA* mutant significantly increased its DNR resistance (Lamovskaya et al. 1996).

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 (Walczak et al. 1999) (Figure 1B). In addition, the cytotoxic nature of DXR acts against the producing strain itself and causes rapid cell death.

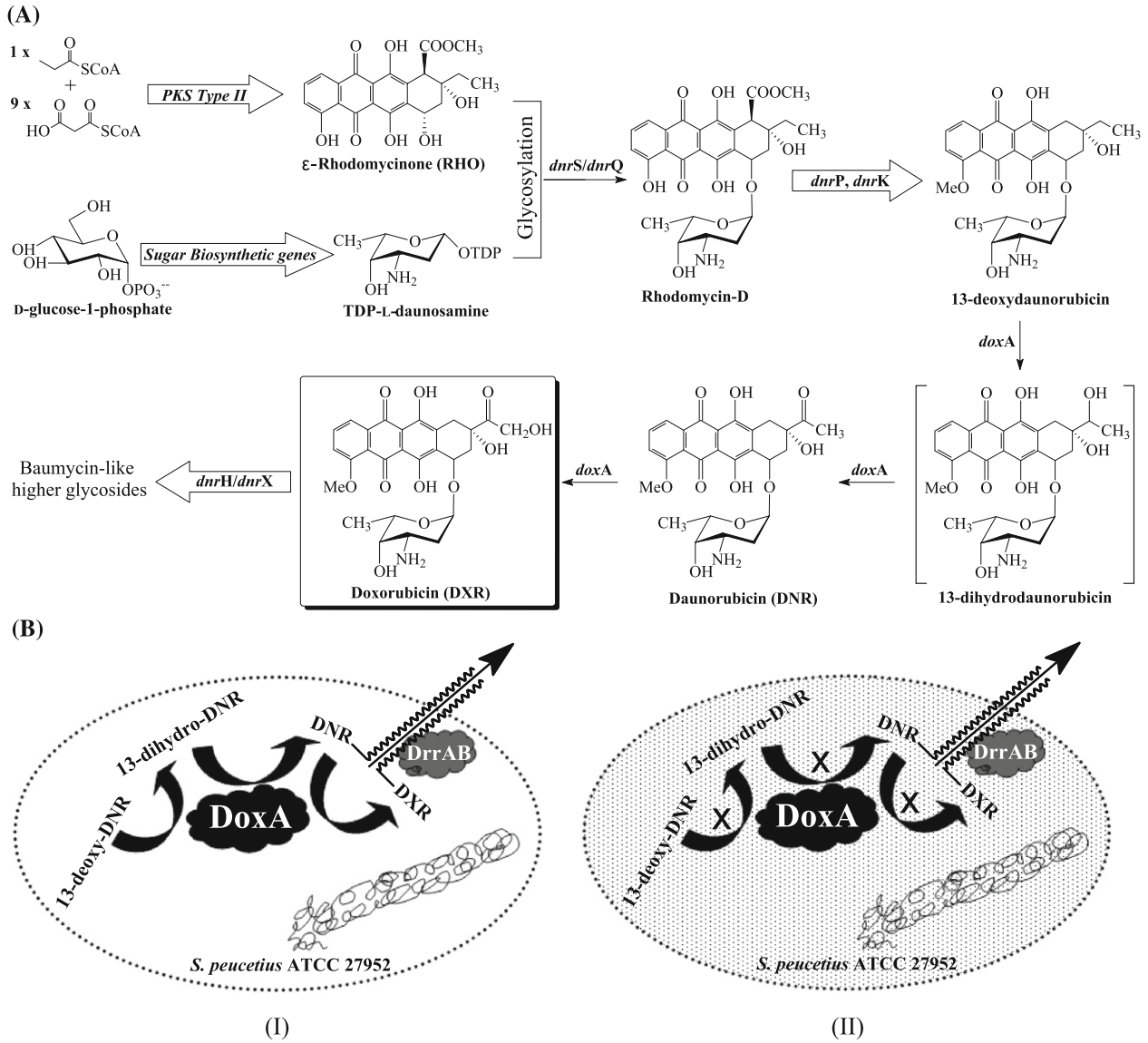

Figure 1. (A) Abbreviated biosynthetic pathways doxorubicin (DXR), Daunorubicin (DNR) and ε-Rhodomycinone (RHO) from propionyl-CoA, malonyl-CoA and glucose-1-phosphate. *Open arrows indicate multiple steps between the precursor and the product shown*. (B) Schematic representation of DoxA inhibition by 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.

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