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# Surprising production of a new urdamycin derivative by *S. fradiae* Δ*urdQ/R*

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**Abstract**

A strain (*S. fradiae* Δ*urdQ/R*) with mutations in *urdQ* and *urdR* encoding a dTDP-hexose-3,4-dehydratase and a dTDP-hexose-4-ketoreductase, respectively, produces a new urdamycin analogue (urdamycin X) with changes in the polyketide structure. The structure of urdamycin X has been elucidated by NMR spectroscopy. Urdamycin X was not detectable, even in small amounts, in either *S. fradiae* Δ*urdQ*, in *S. fradiae* Δ*urdR* or in *S. fradiae* A0, a mutant lacking all glycosyltransferase genes. Complementation of *S. fradiae* Δ*urdQ/R* restored urdamycin A production indicating that the mutations did not cause any polar effect.


*Keywords:* *Streptomyces fradiae*; Secondary metabolism; Urdamycins; Deoxysugar biosynthetic enzymes

## 1. Introduction

Urdamycin A (Fig. 1), produced by *Streptomyces fradiae* Tü2717 (*S. fradiae* Tü2717), is an angucycline antibiotic with some anticancer activity (Drautz et al., 1986). It has been used as model substance for biosynthetic studies focusing especially on the biosynthesis of the sugar side chain (Hoffmeister et al., 2000; Trefzer et al., 2000). In addition, mutants of *S. fradiae* Tü2717 have been used as hosts for the production of hybrid natural products obtained by combinatorial biosynthesis (Trefzer et al., 2001; Luzhetskyy et al., 2005).

Combinatorial biosynthesis has been described as an efficient implement for drug discovery and development, once the enzymology of the pathway being manipulated and the physiology of the organisms used for production is perfectly understood (Floss, 2006; Reeves, 2003). The enzymology for natural product biosynthesis ranges from single catalytic steps to multistep assemblies. Examples for single step reactions are glycosylation, hydroxylation and methylation, examples for multistep assemblies are polyketide synthases or non-ribosomal peptide syntethases (Reeves, 2003). Inactivation of a gene encoding a multifunctional protein very often leads to a non producing strain while inactivation of a gene responsible for a single step reaction very often leads to simple structural changes and these changes are often predictable. For example, the inactivation of a sugar biosynthetic gene generates a mutant producing a novel compound with changes in the glycan structure, and the inactivation of a gene involved in modifying the polyketide backbone results in a mutant producing a compound with changes in the aglycon structure (Reeves, 2003; Rix et al., 2002).

In this article, surprisingly and for the first time, we have observed that a mutant with modified sugar biosynthetic genes produces a new compound with changes in the polyketide structure.

*Abbreviations:* PCR, polymerase chain reaction; dTDP, deoxythymidine phosphate; NMR, nuclear magnetic resonance; NAD(H), nicotinamide adenine dinucleotide; NADP(H), nicotinamide adenine dinucleotide phosphate; PMP, pyridoxamine 5′-phosphate

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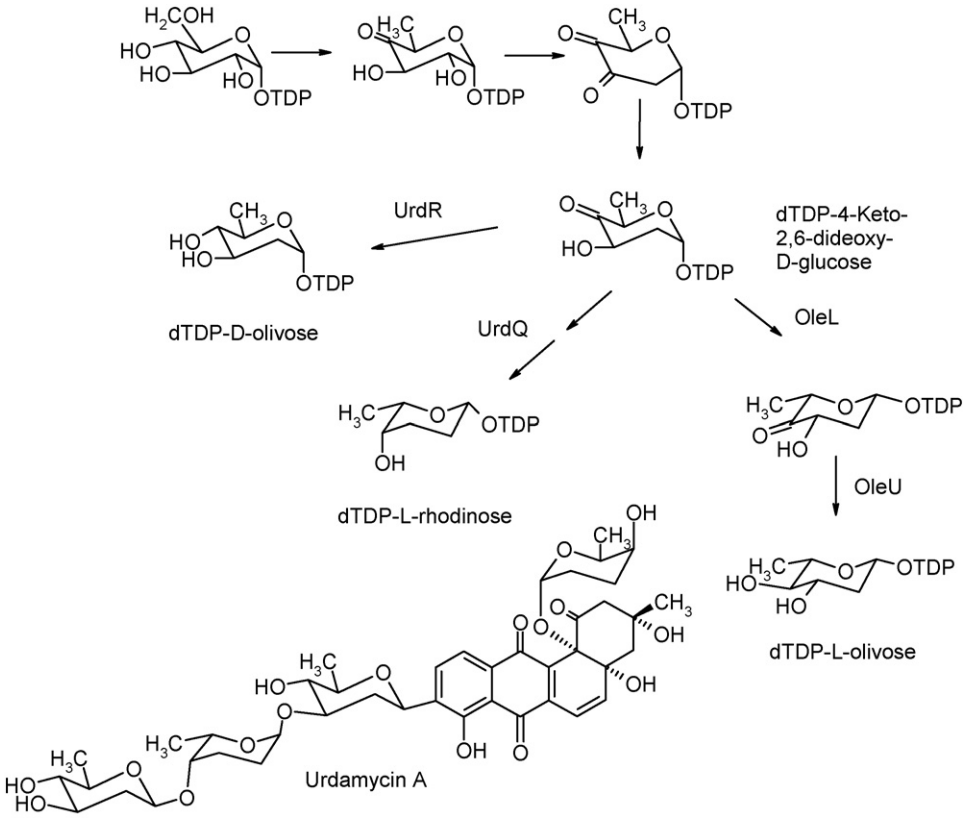


Fig. 1. Biosynthetic pathway to dTDP-D-olivose, dTDP-L-rhodinose as sugar components of urdamycin A. A pathway to dTDP-L-olivose catalyzed by OleL and OleU is also shown.

## 2. Materials and methods

### 2.1. Baterial strains, plasmids and culture conditions

*S. fradiae* Tü2717 (Drautz et al., 1986), *S. fradiae* Δ*urdR* (Hoffmeister et al., 2000) and all mutants were grown on 1% malt extract, 0.4% yeast extract, 0.4% glucose and 1mM $CaCl_2$, pH adjusted to 7.2 (HA medium) at 28 °C. DNA manipulation was carried out using *Escherichia coli* XL-1 Blue MRF′ (Strategene) as host strain. Plasmids pBluecript SK− was from Stratagene. Plasmid pKC1132 and pKC1218ermE were kind gifts from Eli Lilly and Company. For gene expression (complementation experiments) plasmid pUWL201 (Trefzer et al., 2000) and pIJ2925 (Kieser et al., 2000) were used. The construction of the gene inactivation plasmid pKurdQpm has been described (Hoffmeister et al., 2000). Plasmids pLR234Δ7 and pUC18U containing *oleL* and *oleU* (Aguirrezabalaga et al., 2000; Lombo et al., 2004; Salas and Mendez, 2005) were a kind gift of Prof. Dr. J.A. Salas, Oviedo, Spain. *E. coli* was grown on Luria-Bertani (LB) agar or liquid medium containing the appropriate antibiotic. For urdamycin production *S. fradiae* and all mutants were grown in NL111V medium as described (Trefzer et al., 2000).

### 2.2. General genetic manipulation

Standard molecular biology procedures were performed as described (Kieser et al., 2000). Isolation of *E. coli* DNA and DNA restriction, were performed by the protocols of the manufactures of kits, enzymes, and reagents (Amersham, Pharmacia, Boehringer, Mannheim, Promega, Stratagene).

### 2.3. Generation of S. fradiae Δ*urdQ/R*, a mutant lacking UrdQ and UrdR

Intergeneric conjugation between *E. coli* and *S. fradiae* Δ*urdR* was performed as described earlier (Luzhetskyy et al., 2006) using the plasmid pKurdQpm. After conjugation apramycin resistant colonies were obtained. Numerous colonies were grown on plates containing no apramycin to select for loss of resistance. Six apramycin-sensitive colonies were obtained suggesting that they were the consequence of a double crossover. One mutant named *S. fradiae* Δ*urdQ/R* was further examined. PCR fragments obtained from *S. fradiae* Δ*urdQ/R* using primers Q1: 5′-GGAACCACCGAATTCTGGCCGTCC-3′ and Q2: 5′-CTAGCCACAAGCTTCGACGAACTCCTTGAT-3′ were analyzed by restriction analysis. Fragments could not be restricted by *Nco*I which was the endonuclease employed for frame shifting. In contrast, PCR fragments amplified with the wt-DNA as template were digestible.

### 2.4. Construction of gene expression (complementation) plasmids

The construction of gene complementation plasmids containing either *urdQ* or *urdR* has been described (Hoffmeister