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Overexpression of a pathway specific negative regulator enhances production of daunorubicin in *bldA* deficient *Streptomyces peucetius* ATCC 27952

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ABSTRACT

The *dnrO* gene is the first regulator to be activated in the daunorubicin (DNR) biosynthesis pathway of *Streptomyces peucetius* ATCC 27952. DnrO is known for its self-repression capability while it activates rest of the DNR biosynthesis pathway through cascades of regulatory events. *S. peucetius* was found to contain no functional copy of *bldA*-tRNA while a detailed examination of *dnrO* codons reveals the presence of TTA codon, which is rarely encoded by *bldA*-tRNA. Therefore, for evaluating the role of *dnrO* in DNR production, multiple engineered strains of *S. peucetius* were generated by heterologously expressing *bldA, dnrO* and combination of *bldA* and *dnrO*. Using these strains, the effects of heterologously expressed *bldA* and overexpressed *dnrO* were evaluated on pathway specific regulators, mycelial densities and production of DNR. The results showed that the transcription level of *dnrO* and master regulator *dnrI*, was found to be elevated in *bldA* containing strain in comparison to *dnrO* overexpressed strain. The *bldA* containing strain produces 45.7% higher DNR than *bldA* deficient wild type strain from culture broth with OD_{600} of 1.45 at 72 h. Heterologous expression of *bldA*–tRNA is accounted for increased transcription levels of the DNR pathway specific regulators and enhanced DNR production.

1. Introduction

Streptomyces peucetius ATCC 27952 is a Gram positive soil bacterium which produces anthracycline antibiotics such as doxorubicin (DXR) and daunorubicin (DNR) (Bao et al., 1999). The antibiotics of anthracycline family function by intercalating into DNA. Therefore, intracellular concentration of anthracycline in the bacterium is maintained below the lethal dose (Vasanthakumar et al., 2013) by various regulators. *Streptomyces* species possess a large diversity of regulators encoding transcription factors, sigma factors and small regulatory RNAs (sRNAs) whose regulation and signal transduction mechanism is controlled by availability or depletion of signaling molecules (Pokhrel et al., 2015; Chaudhary et al., 2015a; Chaudhary et al., 2015b). Beside resistant determinants and regulatory proteins the major unit responsible for the production of secondary metabolites is a biosynthetic gene cluster (Chaudhary et al., 2013). Thus, the production of such secondary metabolites can be controlled by "rewiring" the genetic network of biosynthetic and regulatory genes (Koju et al., 2012; Dhakal et al., 2015; Dhakal and Sohng, 2015).

In *S. peucetius*, biosynthesis of DNR and self-resistance is activated due to the activation of an efflux pump that is regulated by pathway specific transcriptional regulators (Furuya and Hutchinson, 1998). The major transcriptional regulator DnrO contains a helix-turn-helix DNA binding domain at the N-terminal region (Otten et al., 2000), a signature of the DeoR family of transcriptional repressors (Valentin-Hansen et al., 1985). DnrO is required for the expression of the pathway specific *dnrN* transcriptional activator which in turn activates *dnrI* (Otten et al., 2000). DnrI then binds to the multiple regions of polyketide synthases (PKSs) (Madduri and Hutchinson 1995) and efflux regulatory genes of the DNR biosynthetic pathway providing evidence of being the master

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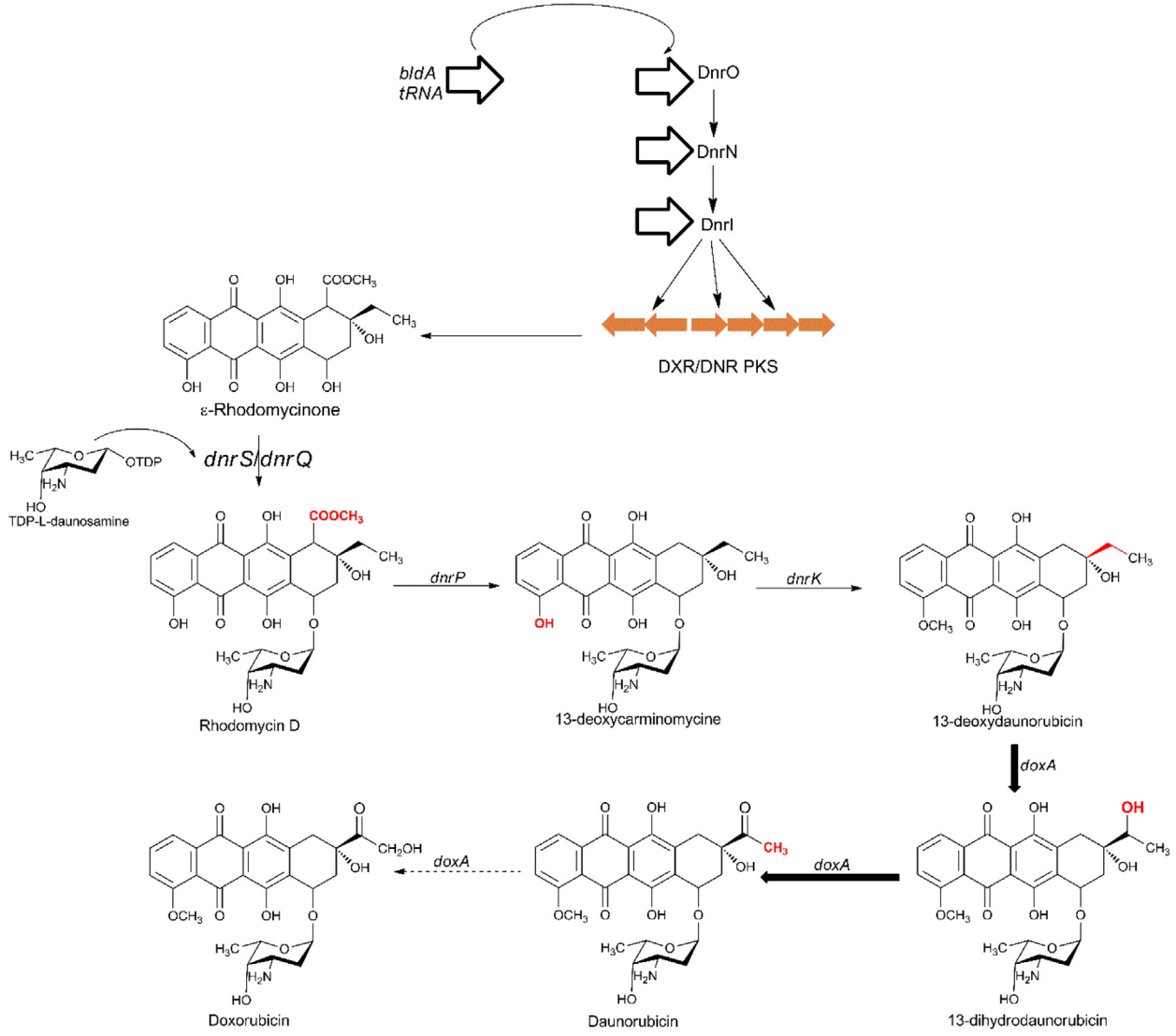

Fig. 1. The pathway specific regulatory scheme and biosynthesis pathway of DNR.

regulator (Tang et al., 1996) (Fig. 1). However, DnrO is considered a pathway specific negative regulator (Lei and Parekh, 2005) most likely due to its self-repression phenomena. In addition, mutation in *dnrO* has also been shown to affect the expression of *dnrN* and *dnrI* (Otten et al., 2000). An attempt has been made to overexpress *dnrI* and *dnrN* for production of DNR (Otten et al., 1995a, 1995b), however overexpression of *dnrO* has not been attempted for DNR production (Malla et al., 2010). Interestingly, a closer look at *dnrO*, the key activator of DNR biosynthetic pathway of *S. peucetius* reveals the presence of a single TTA codon. Among the six codons that encode leucine, UUA is very rare in *Streptomyces* as only 2–3% of the protein-coding genes in *Streptomyces* contain TTA codon(s) (Chater and Chandra, 2008; Zaburanny et al., 2009). However, extensive scanning of *S. peucetius* genome provided an evidence of no functional copy of *bldA*, the only tRNA species that read the UUA codon efficiently in *Streptomyces* (Lawlor et al., 1987; Li et al., 2007; Chater and Chandra, 2008).

Although *bldA* is non-essential for the survival of *Streptomyces* species, it plays an important role in secondary metabolism mostly due to the presence of TTA codons in regulatory genes. Genome sequencing (Bentley et al., 2002), coupled with codon-anticodon recognition rules (Crick, 1966), indicates that no other tRNA in the genome should be able to read the UUA codon. The first *Streptomyces* developmental gene to be cloned (Piret and Chater, 1985) was non-functional *bldA* mutants defective in the expression of several TTA containing genes. However, replacing TTA codon by a synonymous codon resulted in full expression in such mutants. However, there is apparent variation in the extent to which introduced genes and native genes containing a TTA codon are expressed in the mutants (Leskiw et al., 1991). Several studies have investigated the role of TTA codon on antibiotic biosynthesis. For instance, the landomycin biosynthesis in *Streptomyces globisporus* is regulated by *lndI* (Rebets et al., 2006), puromycin by *pur6* and *pur10* in *Streptomyces alboniger* (Tercero et al., 1998), avermectin by two of the pathway-specific genes, *aveA3* and *aveR* in *Streptomyces avermitilis* (Tao et al., 2007). Though, the pathway specific activator *ccaR* of cephamycin C and clavulanic acid contains a TTA codon, dependence to *bldA* for activation of antibiotic production was not apparent (Perez-Llarena et al., 1997; Trepanier et al., 2002). However, the complementation by functional copy of *bldA* in *bldA* deficient *Streptomyces clavus* led to activation of a cryptic polyene biosynthetic gene cluster (Kalan et al., 2013).

In the present study, we determine the effect of *bldA* on pathway specific regulation of DNR biosynthesis in *S. peucetius*. Engineered strains of *S. peucetius* were generated with overexpressed *bldA* (bldA-25), *dnrO* (dnrO-25) and combination of *bldA* and *dnrO*