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Superoxide dismutase (SOD) genes in *Streptomyces peucetius*: Effects of SODs on secondary metabolites production

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Abstract

Two superoxide dismutase (SOD) genes; *sod1* and *sod2*, from *Streptomyces peucetius* ATCC 27952 show high similarity to other known SODs from *Streptomyces coelicolor* A3(2) and *Streptomyces avermitilis* MA-4680. These *sod1* and *sod2* were cloned into pIBR25 expression vector under a strong *ermE** promoter to enhance secondary metabolites from *Streptomyces* strains. The recombinant expression plasmids; pIBR25SD1 and pIBR25SD2, were constructed to overexpress *sod1* and *sod2* respectively to enhance production of doxorubicin (DXR) in *S. peucetius*, clavulanic acid (CA) in *Streptomyces clavuligerus* NRRL 3585 and actinorhodin (ACT) and undecylprodigiosin (Red) in *Streptomyces lividans* TK24. Biomass variation, antibiotics production and transcriptional analysis of regulatory genes in recombinant strains have been studied to understand the effect of *sod1* and *sod2*. The cell growth analysis shows that life span of all recombinant strains was found to be elevated as compared to wild type cells. In *S. peucetius*, overexpression of *sod1* and *sod2* was not effective in DXR production but in case of *S. clavuligerus*, CA production was increased by 2.5 and 1.5 times in *sod1* and *sod2* overexpression, respectively while in case of *S. lividans*, ACT production was increased by 1.4 and 1.6 times and Red production by 1.5 and 1.2 times upon *sod1* and *sod2* overexpressions, respectively as compared to the corresponding wild type strains.

Abbreviations: DNR, daunorubicin; DXR, doxorubicin; SOD, superoxide dismutase; CA, clavulanic acid; ACT, actinorhodin; Red, undecylprodigiosin.

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1. Introduction

Streptomyces, filamentous soil-dwelling bacteria, are industrially important microorganisms since they produce numerous novel secondary metabolites, including antibiotics. *S. peucetius* produces two important chemotherapeutic polyketide type II anthracyclic compounds, daunorubicin (DNR) and doxorubicin (DXR) (Arcamone et al., 1969; Dorshow 1986). It is itself resistant to these antitumor agents (Guilfoile and Hutchinson, 1991). The minor structural difference (hydroxylation at C-14) between DNR and DXR plays a major role in their activities. DXR is most widely used as a chemotherapeutic drug in human cancer therapy and has the broadest spectrum anti-tumor effect (Weiss, 1992).

In normal metabolism and stress conditions, most aerobic organisms are exposed to reactive oxygen species (ROS) such as superoxide anion, hydrogen peroxide, and hydroxyl radical as byproducts (Kang et al., 2006, 2007; Lee et al., 2006). Therefore, living organisms are protected against these reactive oxygen species by complex oxidative defense and repair systems (Halliwell and Gutteridge, 1989). Superoxide dismutase (SOD) is one of the key enzymes involved in the oxidative defense system, which catalytically disproportionate superoxide anion (O_2^-) to hydrogen peroxide (H_2O_2) and molecular oxygen (O_2) through reduction and oxidation of their active site metal ions.

All of the known SODs require a redox active transition metal in the active site in order to catalytically breakdown the superoxide anion. A general mechanism for the metalloenzyme-dependent dismutation consists of two steps. The metal cofactors catalyze both a one-electron oxidation (first step) and a one-electron reduction of (second step) separate superoxide anions to give the overall disproportionation reaction. These reactions typically require no external source of redox equivalents and are thus self-contained components of the antioxidant machinery. This allows SODs to function in a variety of intracellular and extracellular environments.

Various groups of superoxide dismutases containing different metals have been characterized. Bimetallic CuZnSOD and FeZnSOD, and the group of MnSOD, FeSOD or cambialistic SOD (using Fe or Mn depending on environmental metal availability) (Martin et al., 1986) have already been reported. A third group of nickel-containing SODs has been described in the actinobacterial genus *Streptomyces* (Youn et al., 1996a,b). Now, it is known that cytoplasmic nickel-dependent SOD is a general feature of the genus *Streptomyces* (Leclere et al., 1999).

The role of superoxide dismutase enzymes in aging and life span regulation has been studied in model systems including *Drosophila melanogaster*, *Saccharomyces cereviseae* and *Mus musculus* (mouse) (Spencer et al., 2003; Fabrizio et al., 2003; Harris et al., 2003). So, we were interested to observe the effect in *Streptomyces* as well.

Two superoxide dismutases genes; *sod1* and *sod2*, have been found in the genome of *S. peucetius*. Here, we studied overexpression of these genes in the parental strain, in *S. clavuligerus* NRRL 3585 and in *S. lividans* TK24 and their effect on life span and secondary metabolites [doxorubicin (DXR), clavulanic acid (CA), actinorhodin (ACT) and undecylprodigiosin (Red)] production by those recombinant strains.

2. Materials and methods

2.1. Bacterial strains and culture conditions

Escherichia coli (*E. coli*) strains were cultured in Luria Bertani (LB) medium at 37 °C. *E. coli* XL1-Blue MRF (Stratagene, La Jolla, CA) and *E. coli* ET12567 (John Innes Centre, UK), a non-methylating (*dam*$^-$ *dcm*$^-$ *hsdS*$^-$ Cm^r) host, were used for DNA manipulation and demethylation of plasmid DNA for transformation into *Streptomyces* strains (except *S. lividans*), respectively. *S. peucetius* ATCC 27952 was cultured at 28 °C in R2YE medium (Kieser et al., 2000) for 2–3 days for the preparation of genomic DNA. *S. peucetius* cultures were grown in APM medium (6% glucose, 2% malt extract, 1.5% MOPS, 0.2% sodium chloride, and 0.8% yeast extract) prior to reverse transcriptase polymerase chain reaction (RT-PCR). To prepare protoplasts, *S. peucetius*, *S. clavuligerus* and *S. lividans* were cultured in R2YE with 0.5% glycine, while NDYE medium (Malla et al., 2009) was used for DXR production, medium composed of 2.0% glycerol, 3% tryptic soy broth, 1% peptone and 1.05% MOPS was used for CA production (Jnawali et al., 2008) and YEME medium was used for ACT production (Maharjan et al., 2009). pGEM-T® Easy (Promega, USA) was used as a cloning vector, whereas pIBR25 (Sthapit et al., 2004) was used as the expression vector. Ampicillin (100 μg ml^{-1}), chloramphenicol (25 μg ml^{-1}), and tetracycline (12.5 μg ml^{-1}) were used for plasmid selection in *E. coli*. All bacterial strains, vectors and recombinant plasmids, including their relevant sources, are listed in Table 1.