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Change in colony morphology and kinetics of tylosin production after UV and gamma irradiation mutagenesis of *Streptomyces fradiae* NRRL-2702

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Summary

Tylosin is a macrolide antibiotic used as veterinary drug and growth promoter. Attempts were made for hyper production of tylosin by a strain of *Streptomyces fradiae* NRRL-2702 through irradiation mutagenesis. Ultraviolet (UV) irradiation of wild-type strain caused development of six morphologically altered colony types on agar plates. After screening using *Bacillus subtilis* bioassay only morphological mutants indicated the production of tylosin. An increase of 2.7 ± 0.22-fold in tylosin production (1500 mg/l) in case of mutant UV-2 in complex medium was achieved as compared to wild-type strain (550 mg/l). Gamma irradiation of mutant UV-2 using ^{60}Co gave one morphologically altered colony type γ-1, which gave 2500 mg/l tylosin yield in complex medium. Chemically defined media promoted tylosin production upto 3800 mg/l. Maximum value of q_p (3.34 mg/gh) was observed by mutant γ-1 as compared to wild strain (0.81 mg/gh). Moreover, UV irradiation associated changes were unstable with loss of tylosin activity whereas mutant γ-1 displayed high stability on subsequent culturing.

Introduction

Tylosin is a 16 membered ring macrolide antibiotic that is produced commercially from the fermentation by *Streptomyces fradiae* (Seno et al., 1977). It is used as veterinary medicine to treat respiratory

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infections, leptospiroses and mycoplasmosis. Moreover, it is used as a feed supplement for growth stimulation of young animals. In general, strain improvement is considered one of the main factors involved in the achievement of higher titers of industrial metabolites (Podojil et al., 1984). Random mutagenesis and fermentation screening have been reported as an effective way to improve the productivity of industrial microbial cultures (Parekh et al., 2000).

The most widely used mutagens are methylmethane sulfonate (MMS), hydroxylamine (HA), ethylmethane sulfonate (EMS), *N*-methyl-*N*-nitro-*N*-Nitrosoguanidine (MNNG), and ultraviolet (UV) irradiation (Baltz, 1999). It is reported that MNNG is the most potent and the most efficient in generating the optimal yield of desired mutants (Baltz, 1986). The major limitation of MNNG is its specificity; it produces GC to AT transition mutations almost exclusively. UV light, on the other hand, induces a broad spectrum of point mutations, for example in *Escherichia coli* (Miller, 1983) but appears to be a weak mutagen in *S. fradiae* (Baltz, 1986). The most effective way to broaden the spectrum of base pair substitutions for yield improvement is to develop a protocol for AT→CG transversions. So far, no chemical mutagen has been reported that can induce AT to CG transversions (Baltz, 2001). However AT→CG transversions were found in cells treated with ^{60}Co-gamma rays (Xie et al., 2004).

Members of genus *Streptomyces* show commonly genetic instability, intra-strain morphological variability and co-relation between colony morphology and antibiotic activity (Schrempf, 1982). Sometimes product regulatory mutants obtained in basic genetic studies are found to be altered in colonial morphology, thus such morphological mutants are very important in the strain improvement activities (Jose and Arnold, 2005). Moreover, the medium constitution together with the metabolic capacity of the producing organism greatly affects antibiotic biosynthesis. Change in the nature and type of carbon, nitrogen sources, and trace elements have been reported to affect antibiotic biosynthesis in Streptomycetes (Abbanat et al., 1999) through the catabolic repression phenomenon (Lopez et al., 2003).

The current study is the very first of its kind to explore the combined effect of UV and gamma irradiation on colonial morphology and activity of tylosin producing *S. fradiae* NRRL-2702. It was also aimed at defining some fermentation conditions necessary to maintain such mutants with higher levels of antibiotic productivity. Therefore, fermentation kinetics of tylosin synthesis by *S. fradiae* NRRL-2702 and hyper producer mutant were studied in a batch system and a simple model, the Luedeking–Piret model was used to calculate important process affecting kinetic parameters.

Material and methods

Microorganisms and media

S. fradiae strain NRRL-2702 was used through out this study. The microbial culture was grown on agar (2.3%) medium having following composition (g/l) glucose 4; yeast extract 5 and peptone 10. Seed culture medium contained (g/l) soluble starch 20; tryptone soy broth 20; yeast extract 3; K_2HPO_4 1; $MgSO_4 \cdot 7H_2O$ 0.25; $CaCO_3$ 3. The pH was adjusted to 7.2 with 2 M HCl before autoclaving and steam-sterilized $CaCO_3$ was added aseptically after sterilization (Seno et al., 1977). The microbial cultures were grown for 44–48 h at 30 °C and 170 rpm on shaking incubator (Kuhner Switzerland). For the production of tylosin, the complex medium contained (g/l) tryptone 40; K_2HPO_4 0.5; $CaCO_3$ 3; KCl 1; KOH 1; linseed oils 60 and 10 ml of trace element solution. The trace element solution was made up of (g/l): $FeCl_2$ 5; $ZnCl_2$ 6; $MnCl_2$ 1, $CoCl_2$ 3. The microbial culture was grown for 168 h at 30 °C and 250 rpm.

Batch culture studies

In batch culture studies, chemically defined medium contained (g/l): K_2HPO_4 5; KH_2PO_4 5; $FeSO_4 \cdot 7H_2O$ 0.2; $MnSO_4 \cdot 4H_2O$ 0.1; $ZnSO_4 \cdot 7H_2O$ 0.2; $MgSO_4 \cdot 7H_2O$ 0.1; $CaCl_2 \cdot 2H_2O$ 0.02; $CuCl_2 \cdot 2H_2O$ 0.005; H_3BO_3 0.011 and $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ 0.005 was used. The effect of different carbon sources (glucose, lactose, sucrose, fructose, glycerol, lactose+glucose, sucrose+glucose, fructose+glucose and glycerol+glucose at a concentration of 45 g/l on microbial growth and tylosin production was evaluated in the presence of tryptone (12.5 g/l) as the sole nitrogen source. However, when NH_4NO_3, L-aspartate, L-alanine, corn steep liquor, $(NH_4)_2SO_4$ and mono sodium-glutamate were used as sole source of nitrogen then only glucose+lactose (22.5+22.5 g/l) combination was used as the sole carbon source. The pH was adjusted to 7.2 with 2 M HCl before autoclaving. All the sugars were separately sterilized and added aseptically to the respective media before inoculation.