Effects of genotoxic agents on his$^+$ revertants and survival of spores of *Streptomyces aureofaciens*

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The effects of u.v. light and of several chemical agents on spores of the tetracycline producer *Streptomyces aureofaciens* MT1 were studied using survival curves and induction of histidine prototrophic revertants (his$^+$). Spores were highly resistant to u.v.; NTG induced most his$^+$ revertants. 4-Nitroquinoline-1-oxide and methyl methanesulphonate also gave good yields of revertants. Whereas ethyl methanesulphonate had the least effect on inducing the revertants.

Key words: Genotoxic agents, spore mutagenesis, *Streptomyces*.

*Streptomyces* spp. have complex life cycles involving the formation of spores and mycelia. They have economic importance as they produce a wide number of useful substances, especially antibiotics (Chater 1984). Genotoxic agents are widely used to obtain mutants with improved abilities for the production of such substances. These agents are applied to mycelia, as for *S. fradiae* (Baltz 1986), or to spores, as for *S. coelicolor* (Delic et al. 1970) and *S. cattleya* (Hromick & Kirby 1989). As the packaging of DNA in spores may be different from that in mycelial cells, the effects of genotoxic agents on spores may give different results from those obtained with mycelia (Chater 1989). The present study is on the effects of u.v. light and several chemical agents on spores of the tetracycline producer, *S. aureofaciens* MT1.

**Materials and Methods**

**Bacterial Strain**

*Streptomyces aureofaciens* MT1 is a histidine auxotroph and tetracycline-producing strain (Torres et al. 1991) derived from *S. aureofaciens* M3031 (ATCC 10764). The manipulation of the strain, preparation and purity control of spore suspensions, harvesting, and media were as described by Hopwood et al. (1985). To prepare spore suspensions, the surface of a sporulating agar culture was scraped and the spores were then suspended in water, filtered through cotton wool, pelleted by centrifugation and stored in 20% (v/v) glycerol.

**Mutagenesis**

Treatment of spores with genotoxic agents was based on the procedures described in Stonesifer & Baltz (1985). For u.v. irradiation, spores (10$^7$ spores/ml; 9 ml total volume) were exposed in Petri dishes (9 cm diam.) to a low pressure germicidal u.v. lamp (254 nm), giving an incident flux of 2.5 J/m². Aliquots were removed at intervals, diluted in water and plated out. All experiments were carried out in semi-dark conditions to avoid photoreactivation. For chemical mutagenesis, spores (10$^7$ spores/ml; 1 ml total volume) were diluted in an appropriate buffer [0.05 M Tris/maleic acid buffer, pH 8.0, for NTG; 0.2 M KH$_2$PO$_4$, pH 7.0, for methylmethane sulphate (MMS) and ethylmethylene sulphate (EMS); 0.05 M KH$_2$PO$_4$, pH 7.0, for 4-nitroquinoline-1-oxide (NQO)] with the desired concentration of the chemical agent. Aliquots were removed at intervals, diluted in 4% (v/v) sodium thiosulphate to stop the reaction, and then plated after appropriate dilution. Each point on the survival curve was obtained as a mean (± 6.7%) of three separate duplicate experiments. The survival rate was measured on Trypticase/soya broth/agar plates. and the induction of his$^+$ revertants was determined on minimal media (Hopwood 1967) after incubation at 30°C for 3 days.

**Results**

*Streptomyces aureofaciens* MT1 spores had a high level of...
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resistance to the lethal effects of u.v. light, with a lower induction of his\(^+\) revertants than when NQO, NTG, MMS or EMS were used. About 50% spore death occurred with 105 J/m\(^2\) and 90% with 210 J/m\(^2\). A significant induction of his\(^+\) revertants only occurred with \(> 50 \) J/m\(^2\), when the spore survival curve was declining (Figure 1A).

The overall induction of his\(^+\) revertants with NQO was higher than that for u.v. (Figure 1B). As survival decreased with NQO treatment, induction of revertants increased sharply and became higher than that obtained for u.v. with any survival percentage (Figure 1A and B).

Of all genotoxic agents used, NTG gave the strongest induction of his\(^+\) revertants, induction occurring with all treatments; on average, \(> 1000\) revertants were obtained under conditions which caused about 85% death (Figure 1C). Although MMS, another alkylating agent, also gave good yields of revertants, the shapes of the survival and revertant induction curves produced using it (Figure 1D) were different from those of NTG (Figure 1C). Among the three alkylating agents used, EMS had the lowest effect on inducing his\(^+\) revertants (Figure 1E).

**Discussion**

NTG was the most effective genotoxic agent for the induction of his\(^+\) revertants in *S. aureofaciens*. This result is probably due to the high content of G + C (the main NTG target) in the *Streptomyces* genome (about 67 to 74%). Ultra-violet light, which has thymidine pairs as the main targets, and alkylating agents EMS and MMS, which have N-7 and O-6 of guanine as the main targets (Friedberg 1985) induced fewer revertants. However, although NQO induces lesions which involve guanine (Ikenaga *et al.* 1975), it did not induce as high level of his\(^+\) revertants as did the alkylating agents (Figures 1B, C and D).

The higher DNA hydration and the presence of binding proteins in the spores of *Bacillus subtilis* decrease hydrolytic de-purination when compared with mycelial cells (Nicholson *et al.* 1991; Setlow 1992). The fact that *Streptomyces* spores are constitutive and functionally different from those of *B. subtilis* should suggest the existence of a different protection system. Most mutagenic events caused by u.v. and NQO involve the induction of error-prone mechanisms. When the lesions become abundant or promote higher distortion of DNA helices, they start to act, as observed in *Escherichia coli* (Walker 1985), *S. fradiae* mycelia (Stonesifer & Baltz 1985) and *S. catalyza* spores (Hromick & Kirby 1989). In *S. aureofaciens* MT1 spores, the induction of his\(^+\) revertants by these two agents became higher when the spore survival was \(< 50\%\) (Figures 1A and B); u.v. and NQO induced mutants only after a treatment long enough to produce a high rate of spore death, probably as a consequence of the activation of repair mechanisms similar to those described above.

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