Strain improvement through chemical mutagenesis for enhanced production of erythromycin using *Saccharopolyspora erythraea* MTCC 1103

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ABSTRACT

Strain improvement is an essential part of process development for fermentation products. Erythromycin is most commercially used antibiotics in day to day basis and is widely subjected to chemical mutagenesis to enhance the production of erythromycin. In this study (EMS) ethyl methane sulphonate was used as mutagen. The efficiency of EMS mutagen treated isolates showed effective rate of erythromycin production on the duration of exposure. The selected mutants of *Saccharopolyspora erythraea* MTCC 1103 isolates were grown in production medium. Erythromycin production was found highest for the mutant *Saccharopolyspora erythraea* MTCC 1103 with 0.48 g/L. The erythromycin production by the mutant strain using chemical mutagenesis showed higher yield than the wild strain. HPLC analysis was performed in order to confirm the presence of erythromycin in the partially purified samples. The preliminary results indicate that erythromycin could be used for the commercial production as antibiotic which has significant industrial application and warrant more consideration as prospective antimicrobials.

**Keywords**: Erythromycin, *Saccharopolyspora erythraea*, bagasse, UV mutagenesis

1. INTRODUCTION

The rapid development of antibiotic resistance among the major respiratory pathogens has created a serious problem for the effective management of respiratory tract infections [1]. There is a great need for new antibiotics that address the problem of antibiotic resistance. Under these circumstances, a substantial amount of study has been carried out on novel macrolides [2].
clinically most important and specific. It is used for the synthesis of next generation azithromycin and clarithromycin. Er-B and Er-C are produced as by-products during its synthesis which have serious side effects hence not in use [4]. Although erythromycin originally developed for its use in human and veterinary medicine, it has also many applications in the animal feeding [5] and the cultivation of marine microalgae [6]. The current success in genetic engineering of S. erythraea relies mainly on chemical mutation and homologous recombination-based manipulations. Hence the study was aimed to mutate *Saccharopolyspora erythraea* MTCC 1103 by chemical mutagenesis.

2. MATERIALS AND METHODS

2.1. Microorganism and inoculum preparation

*Saccharopolyspora erythraea* MTCC 1103 was obtained from the Microbial Type Culture Collection, Chandigarh and maintained in ISP-2 solid medium [7]. pH of the medium was adjusted to 7.2 and kept for sterilization. The organism was then inoculated into the sterile medium. The number of spores was counted by spread plate method. A volume of 1 mL of spore suspension (10^{10} spores/mL) was inoculated into 50 mL of seeding medium and incubated at 28°C for 96 h on a rotary shaker at 200 rpm. 5% seed culture was inoculated into 250 mL of fermentation medium and incubated at 28°C for 10 days on a rotary shaker at 200 rpm [8].

2.2. Morphological observation

A drop of sterile distilled water was transferred onto a glass slide, and a loopful of organism from the ISP-2 plates was then mixed with distilled water to prepare a thin smear. The smear was then air dried and covered with a clean transparent cover slip. The colony morphology was observed under a microscope with 40X magnification.

2.3. Strain improvement by chemical mutagenesis

Spore suspensions of *Saccharopolyspora erythraea* MTCC 1103 (10^{10} spores/mL) were used and subjected to chemical mutagenesis (EMS) ethyl methane sulfonate by the method Khattab [9]. *Saccharopolyspora erythraea* MTCC 1103 (10^{10} spores/mL) were washed once with 0.1 M tris-HCl buffer (pH 7.5) and resuspended in 1 mL of the same buffer. To this 100 μL of EMS was added and the mixture incubated in a shaker water bath for 45 min. The cells were washed 3 times with 0.1 M tris-HCl buffer (pH 7.5) and serial dilutions of chemical mutated spore suspensions were spread on starch casein medium plates and incubated at room temperature for 7 days.

2.4. Erythromycin production and growth rate of mutant *Saccharopolyspora erythraea* MTCC 1103

The organisms were transferred from ISP-2 slants to 100 mL ISP-2 broth. The broth was incubated at room temperature on a rotary shaker at 200 rpm. Then 5% (v/v) inoculum was added to sterile flasks containing production broth and incubated at room temperature on a rotary shaker at 200 rpm. Every day one flask was used for the estimation of erythromycin titre. Data was recorded and the values were plotted to obtain the growth curve of the organism.

2.5. Standard curve of erythromycin

Erythromycin concentration was determined using UV-visible spectroscopy [11]. Erythromycin standard was prepared by varying concentration ranging from 0.1 mg/mL to 0.5 mg/mL. Subsequent amount of erythromycin was weighed and dissolved in 1 mL acetonitrile-water solution (1:1) and equal volume of concentrated sulphuric acid was added. This mixture was kept for incubation at 50°C for 30 min. After incubation period absorbance of various concentrations was determined at 480 nm using UV-visible spectroscopy. From the obtained values, a standard curve for erythromycin was obtained which was used for estimating unknown concentration of erythromycin.

2.6. Determination of erythromycin

After 10 days of incubation, the production broth was centrifuged at 10000 rpm for 20 min at 4°C. Then the supernatant was transferred to sterile conical flask and equal volume of chloroform was added to it. Supernatant and chloroform formed two separate phases. The supernatant-chloroform mixture was incubated overnight at room temperature on a shaker at 100 rpm. Chloroform was allowed to evaporate and erythromycin was separated. The concentration of erythromycin was then estimated using UV/Vis spectroscopy and compared to the standard curve of erythromycin.

2.7. HPLC analysis

Qualitative estimation of erythromycin was done by HPLC system equipped with a UV detector at 205 nm in C18 column. Acetonitrile: methanol: 0.2 M ammonium acetate: water (45:10:10:35) was used as mobile phase at column temperature of 40°C. Sample injection volume was 50 μL [8].

Production of erythromycin using *Saccharopolyspora erythraea* MTCC 1103
3. RESULTS AND DISCUSSION

The mutant *Saccharopolyspora erythraea* MTCC 1103 was subcultured and pure cultures were maintained on solid starch casein medium (Figure 1). Starch casein medium is highly specific for actinomycetes. The relationship between hyphal morphology, size and production of erythromycin has been revealed and it has been reported that long hyphae are the preferred morphology of *S. erythraea* in the fermentation media [12, 13]. While in the fermentation medium, at conditions which support rapid growth, a densely branched mycelium with a large hyphal diameter is observed, a less branched mycelium with small hyphal diameter is observed at poor growth conditions [14]. Results of this investigation showed that the aerial hyphae are clearly visible for mutant *Saccharopolyspora erythraea* MTCC 1103 at 40X indicating star like morphology (Figure 2).

**Figure 1.** Pure colonies of mutant *Saccharopolyspora erythraea* MTCC 1103 on starch casein agar medium

*S. erythraea* strain improvement has been carried out mainly by multiple rounds of random mutagenesis and selection [15]. Previous studies have shown erythromycin production [16] comparably the production from the mutant *Saccharopolyspora erythraea* MTCC 1103 showed better rate of erythromycin production. Growth rate pattern of the mutant type strain was studied in the production medium. Shows the kinetics of dry weight biomass and erythromycin yield over a 7 days growth period. Results suggested that the erythromycin production started from day 3 and it reached maximum on day 5 and remained constant. The maximum erythromycin concentration of 0.48 (g/L) was obtained. (Figure 3). When the *S. erythraea* spore suspension was exposed to chemical mutagenesis using EMS and very few colonies were observed on the plate which indicates possible mutation with the strain. The growth rate of the mutant strain studied in the production broth was determined. When compared to wild type strain, the mutated strain showed maximum growth rate with increased biomass and erythromycin concentration. Specific growth rate ($\mu_w$) and doubling time ($t_{dw}$) of the mutant strain was 0.795 day$^{-1}$ and 0.872 day respectively. This result indicates that the enhancement of antibiotic production by the mutant strain is not due to increase in growth but due to the enhancement in production of the antibiotic. Samples extracted from mutant strain of *Saccharopolyspora erythraea* in standard production medium and mutant type strain in bagasse medium were subjected to HPLC analysis. Which shows the HPLC profile of erythromycin and Retention time was noted. Hence the retention time of all the peaks were found closer to 3.1 min (Figure 4) which corresponds to the standard erythromycin retention time [17].

**Figure 2.** Morphological examination of mutant *Saccharopolyspora erythraea* MTCC 1103 at 40X

**Figure 3.** Erythromycin production and growth rate of Mutant *Saccharopolyspora erythraea* MTCC 1103.
Figure 4. HPLC chromatogram of partially purified erythromycin from crude extract of mutant *Saccharopolyspora erythraea* MTCC 1103.

4. CONCLUSION

Mutation is the primary source of all genetic variation and has been used extensively in industrial improvement of metabolite production. The use of mutation and selection to improve the productivity of cultures has been strongly established for over fifty years and is still recognized as a valuable tool for strain improvement of many bioactive producing organisms. Developed strains can reduce the costs with increased productivity and can possess some specialized desirable characteristics. Such improved strains can be achieved by inducing genetic variation in the natural strain of industrial research in producing less expensive antibiotics.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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