

PRODUCTION OF OXYTETRACYCLINE BY ISOLATED WILD TYPE IRANIAN *STREPTOMYCES* *RIMOSUS*

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ABSTRACT

Production of oxytetracycline by an isolated strain of *Streptomyces rimosus* from Iranian soil was investigated using a special fermentation medium. A comparative study was performed with standard strain PTCC 1144 using the following parameters; optimum growth conditions with respect to time, pH, and different amounts of cornsteep liquor. Surprisingly, the production yield of the wild strain was 20% more than the standard strain.

MJIRI, Vol. 8, No. 3, 187-189, 1994.

Key words: Oxytetracycline, *Streptomyces rimosus*, Production.

INTRODUCTION

Members of the genus streptomycetes yield over 60% of all known antibiotics,¹ including more than 70% of all commercial products.⁸ Tetracyclines are produced by streptomycetals. These antibiotics, with a broad antimicrobial spectrum, are closely related compounds and are widely used in the treatment of infectious diseases.^{3,11}

Oxytetracycline or terramycine, is one of the tetracyclines produced by *Streptomyces rimosus*^{3,8} and other streptomycetes.¹²

In antibiotic production, the nitrogen source of the medium is important.² The economic potential of the production of such antibiotics depends on the availability of cheap substances.

The present work deals with the laboratory production of oxytetracycline using corn steep liquor and a wild type strain of *Streptomyces rimosus* which was isolated from Iranian soil.

MATERIALS AND METHODS

Isolation and identification protocol of the wild strain

Soil samples from different parts of Iran were collected in sterile containers and brought to the laboratory. Standard methods were used for isolating streptomycetes.

The isolated strains of streptomycetes were examined and characterized by T.S.P. method¹⁰ for *Streptomyces rimosus*. For comparison, the standard strain of *Streptomyces rimosus*, PTCC 1144*, was used.

Maintenance of *Streptomyces rimosus*

Isolated strains and the standard strain⁵ (PTCC 1144)* were grown on sporulation medium with the following ingredients: yeast extract, 1g/L; beef extract, 1g/L; tryptose,

*The standard strain is equivalent to ATCC 10790 - NRRI 2234

Production of Oxytetracycline by *S. rimosus*

2g/L; FeSO₄, trace; glucose, 10g/L; agar 15g/L, and distilled water to 1000 mL. The initial pH of the medium was adjusted to 6.8-7.2.

The ingredients were thoroughly digested and portioned into test tubes and sterilized at 121°C for 20 minutes. The inoculated slants with tested organisms were incubated at 30°C for 14 days to obtain luxuriant growth and sporulation. The slants were kept at 5°C in a refrigerator.

Vegetative medium

A suitable suspension of microbial spores or vegetative mycelia was prepared by sporulation broth which contained ingredients similar to sporulation agar except at one-third the concentration of the given quantities and without agar. The initial pH of the vegetative medium was adjusted to 7.0.

This medium was portioned into Erlenmeyer flasks (250 mL capacity) each containing 50 mL, and sterilized at 121°C for 20 minutes.

When the flasks had reached room temperature, they were inoculated with a standard prepared suspension of microbial spores under aseptic conditions. The inoculated flasks were inserted on a rotary shaker (165 r.p.m.) at 27°C for 48 hours. These vegetative media were used for inoculation of the production media.

Production medium

The production medium used for the fermentative production of oxytetracycline by wild standard strains of *Streptomyces rimosus*, contained the following composition:

Citric acid, 12.8g/L; sucrose, 50.0g/L; (NH₄)₂SO₄ 6g/L; MgSO₄ · 7H₂O, 0.25g/L; KH₂PO₄, 0.15g/L; CaCO₃, 1g/L; MnSO₄ · 4H₂O, 0.01g/L; ZnSO₄ · 7H₂O, 0.4g/L.

The initial pH of the medium was adjusted to 7. The nitrogen source of the production medium was replaced by corn steep liquor. On addition of corn liquor to the medium, citric acid, CaCO₃, MnSO₄ · 4H₂O and ZnSO₄ · 7H₂O were depleted from the medium.

The production medium was portioned into Erlenmeyer flasks (500mL capacity) each containing 100 mL. The flasks were sterilized at 121°C for 20 minutes. When the flasks had reached room temperature, they were inoculated with 5% of the vegetative medium, containing growing cells of *Streptomyces rimosus*, under aseptic conditions. The inoculated flasks were inserted on a rotary shaker (165 r.p.m) at 27°C for 120 hours. During the fermentation process, the final pH of the fermented medium and the amount of oxytetracycline produced were determined.

Purification

Purification of oxytetracycline from the fermented medium (standard & wild strain) was done by n-butanol extraction.³

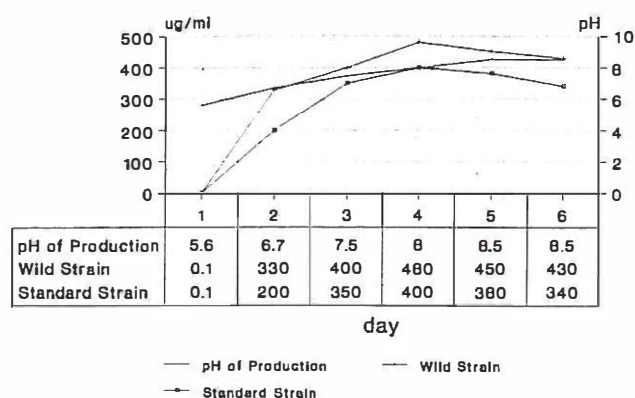


Table I. Production of oxytetracycline by *S. rimosus*. Comparison of wild and standard strains.

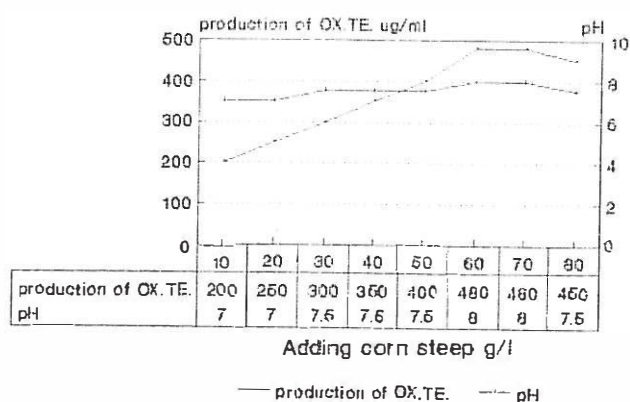


Table II. Evaluation of adding corn steep liquor to the fermented medium.

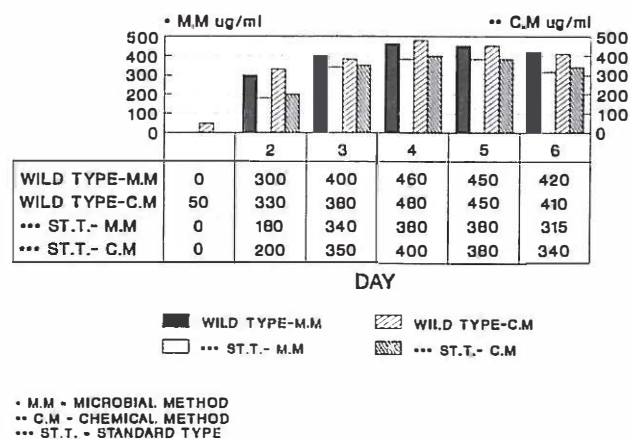


Table III. Comparison of biological and chemical methods for determination of oxytetracycline.

Determination of the oxytetracycline

Extracted material was detected by thin-layer chromatography^{3,7} for oxytetracycline, using pure oxytetracycline as standard.

Antimicrobial activity was measured by the cylinder method on agar⁴ and the spectrophotometry method.⁶

In the cylinder method, the biological activity of oxytetracycline was measured by inhibition zones of *Staphylococcus aureus* (PTCC 1137)⁵ as the test organism.

RESULTS AND DISCUSSION

The results obtained from wild and standard strains showed that the fermentation process of oxytetracycline production increased with an increase in incubation period, reaching its optimum at 96 hours, and decreasing afterwards.

A drop in the pH value of both wild and standard strains was observed during 48 hours of the fermentation process. This may be due to accumulation of organic acids which were further utilized by the microorganisms for their different metabolic processes. At the end of the fermentation process the final pH values were alkaline (pH 8.5). The drop in pH of both strains are given in Table I, which indicates that the wild strain, in comparison with the standard strain, has a higher yield.

The synthetic medium used in the fermentative production of oxytetracycline contained the local ingredient, corn steep liquor, which was used for production of oxytetracycline by the wild and standard strain of *Streptomyces rimosus*. The results obtained showed that when nitrogen sources of the medium were replaced by different concentrations of corn steep liquor without changing the other ingredients of the medium, the amount of oxytetracycline produced increased with the increase of corn steep liquor, reaching its optimum at 6-7 g/L, above which a decline in the yield was observed (Table II). The final pH was variable, depending on the amount of corn steep liquor added.

In this observation, although the yield of the wild strain in comparison with the standard strain was higher, results of both microbiological and spectrophotometric assays of oxytetracycline showed a negligible difference (Table III).

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