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# OPTIMIZED BIOREACTOR TECHNIQUES IN THE PRODUCTION AND PURIFICATION OF TETRACYCLINE FROM "*STREPTOMYCES AUREOFACIENS*"

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## ABSTRACT

The Tetracycline's are a family of antibiotics that inhibit protein synthesis by preventing the attachment of aminoacyl-tRNA to the ribosomal acceptor (A) site. The favourable antimicrobial properties of tetracycline and the absence of major adverse side effects have led to their extensive use in the therapy of human and animal infections. The spectrum of activity of tetracyclines encompasses various protozoan parasites such as *P. falciparum, Entamoeba histolytica, Giardia lamblia, Leishmania major, Trichomonas vaginalis,* and *Toxoplasma gondii.* In the present study *Streptomyces aureofaciens* was isolated on Waksman agar media and Actinomycetes isolation agar from different sources of soil and screened for the production of tetracycline. The isolate and media for production of tetracycline was optimization for different physical and chemical parameters like pH, temperature, carbon source and nitrogen source for maximum production of tetracycline and the mass production was carried out with the parameters that are conformed in the optimization results. The mass production media were prepared with the pH 9, carbon source as dextrose, nitrogen sources as yeast extract and temperature were carried out at $35^0$C with rpm 200 and aeration limit was 131units/min. Olive oil was used as the antifoaming agent. The mass produced broth was collected and the tetracycline was purified and checked for antimicrobial activity against *E.coli* and the zone of inhibition was found to be 30mm in diameter. The zone of inhibition was greater in the optimised media when compared to the normal production media. This indicates the capability of this strain in mass production and commercial use of this antibiotic against drug resistant pathogens.

**KEYWORDS:** Tetracycline, Optimisation, *Streptomyces aureofaciens*, *E.coli*.

## INTRODUCTION

Tetracyclines are broad-spectrum antibiotics with considerable activity against both gram-positive and gram-negative bacteria and exert a bacteriostatic effect by inhibiting protein synthesis. Tetracyclines traverse the outer membrane of gram-negative enteric bacteria through the OmpF and OmpC porin channels, as positively charged cation-tetracycline coordination complexes.[1, 2]

The spectrum of activity of tetracyclines encompasses various protozoan parasites such as *P. falciparum, Entamoeba histolytica, Giardia lamblia, Leishmania major, Trichomonas vaginalis*, and *Toxoplasma gondii.*[3, 4, 5, 6] The antiparasitic activity is explained in some cases by the finding that certain organisms, e.g., *P. falciparum*, contain mitochondria. However, a number of other protozoa which lack mitochondria nevertheless remain susceptible to tetracyclines.

Many investigators have studied that naturally occurring tetracycline resistance in bacterial systems of *Escherichia coli,*[7] *Staphylococcus aureus,*[8] *Bacteroides fragilis,*[9] and *Bacillus subtilis,*[10] has indicated that drug resistance is mediated by decreased accumulation of the drug by resistant cells.

*Streptomyces* spp. is Gram-positive multi cellular Actinomycetes of industrial importance. More than *60%* of the naturally occurring antibiotics are produced by *Streptomyces* species, including many which are of great medical importance such as tetracyclines.[11] Endophytic Streptomyces species, have been used as antagonistic micro-organisms and for controlling plant diseases.[12, 13]

*Streptomyces aureofaciens* are capable of producing two antibiotic substances simultaneously.[14] In the presence of chloride within the chlortetracycline molecule[15] is essential for its production as disclosed by.[16]

## MATERIALS AND METHODOLOGY

### Isolation and Screening of microorganisms for tetracycline production

The microorganisms were isolated by serially diluting different soil samples and plating them on Waksman agar media and Actinomycetes isolation agar plates and incubate at 28ºC for 5 days. The pure culture of the isolates was cultured in the broth for 5 days and the centrifuged

culture was checked for antagonistic effect against *E.coli* by agar diffusion method. The isolates which showed antagonistic effect were selected and identified using basic microbiological techniques[17] like Staining morphological and Biochemical characterizations. The optimisation of the physical and chemical parameters was carried out with the isolate.

**Optimisation of pH**

The optimisation of the pH was carried out by varying the pH of the production media. The media with different pH like 5.0, 6.0, 7.0, 8.0 and 9.0 were inoculated with the isolate. The flasks were then incubated at $28^0$C for 5 days and the tetracycline activity was checked for media with varied pH.

**Optimisation of Temperature**

The production media was prepared and inoculated with the isolate. The media were then incubated at different temperatures for 5 days to check the effect of temperature in the production of tetracycline by the isolate. The optimisation of temperature was carried out at 25, 30, 35, 40, 45 and $50^0$C.

**Optimisation of Carbon Source**

The effect of carbon source on tetracycline production was studied using Dextrose, Glucose, Starch, Fructose and Maltose which were substituted with the carbon source. The production of tetracycline was estimated for each of the carbon source individually after 5 days of incubation.

**Optimisation of Nitrogen source**

The effect of Nitrogen source on lipase production was studied using peptone, yeast extract, soyabean meal and beef extract which were substituted with the nitrogen source. The media with different nitrogen sources were inoculated with the microorganisms and incubated for 5 days and the activity of tetracycline was determined individually.

**Mass Production**

The mass production was carried out with the parameters that were conformed under optimization results. The mass production media were prepared with the pH-9, carbon source as Dextrose, nitrogen source as Yeast extract and the temperature were carried out at 35ºC with rpm 200 and aeration limit was 131 units/min. The antifoaming agent used was olive oil.

The fermentation was carried out for 5 days, the growth of the isolate was determined at regular intervals of time.

**Extraction and purification of Tetracycline**

The fermented Culture was acidified with $H_2SO_4$ to pH 2.0 and the solids were separated by centrifugation. The supernatant solution was concentrated in vacuum at 32ºC till the volume was reduced to about one-third and the filtrate was brought to pH 8.5 with NaOH. The filtrate was then extracted three times with equal volumes of butanol. The organic phase was washed twice with one-tenth of its volume of distilled water at pH 8.5, and then concentrated at 32ºC. Tetracycline was recovered from the butanolic solution with 0.01 M HCl. The aqueous extract was concentrated at 32ºC to a volume that contained at least 7 mg of the antibiotic per ml. The free base was precipitated carefully raising the pH of the concentrate to 5.0 in an ice bath, then reconverted to the hydrochloride by crystallization. The product was recovered and checked for its antagonistic effect against *E.coli*.

**RESULTS**

The microorganisms were isolated on Waksman agar media and Actinomycetes isolation agar, different isolates were screened for its antibactgerial activity. The isolate showing the highest zone of inhibition against *E.coli* was identified as *Streptomyces aureofaciens* by its morphology, staining and standard biochemical characterization (Figure-1).

**Figure 1: *Streptomyces aureofaciens***

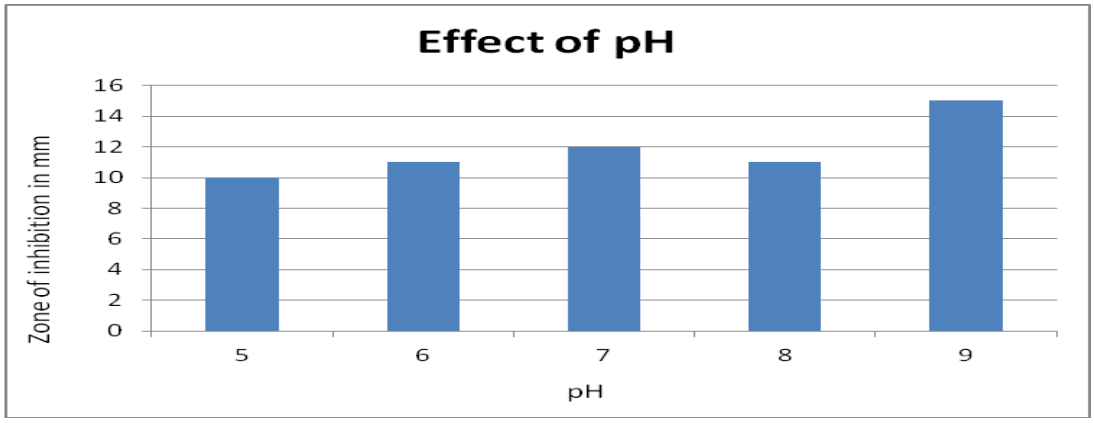


**Figure 2: Optimisation of pH**

The optimisation of the pH was carried out by varying the pH of the production media. It was found that the isolate showed a maximum tetracycline production at pH 9.0 (Figure 2).

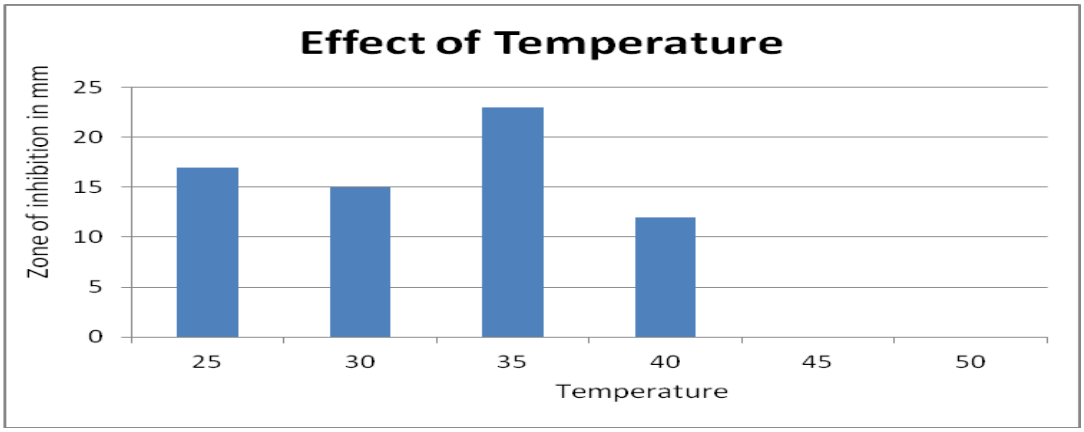


**Figure 3: Optimisation of Temperature**

The production media inoculated with the microorganism was incubated at different temperature to check the effect of temperature and it was found that the isolate produced high amount of tetracycline at $35^0$C when compared to other temperatures (Figure 3).

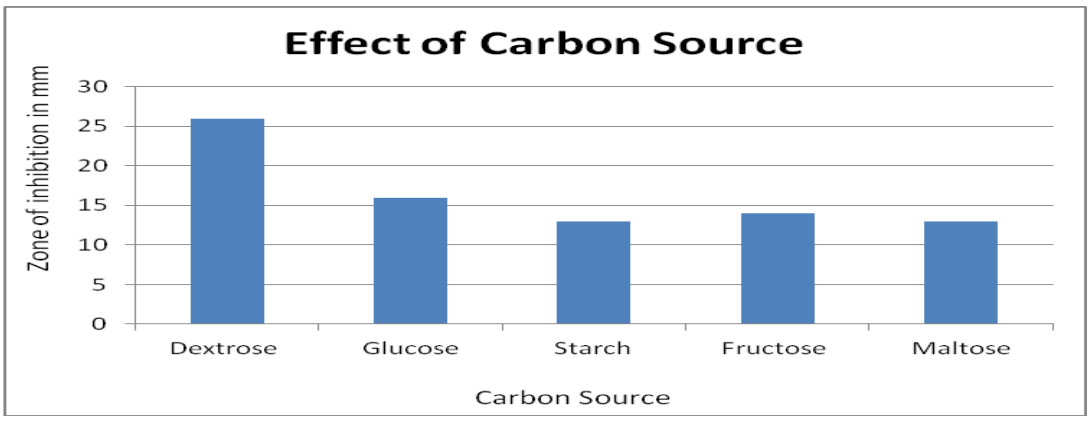


**Figure 4: Optimisation of Carbon Source**

The effect of different carbon sources was checked to analyse the production of tetracycline by the microorganisms. The production media was substituted with different carbon sources and the activity was checked after 5 days of incubation and it was found that the isolate had the ability to grow in the media containing Dextrose the isolate produced the maximum tetracycline (Figure 4).

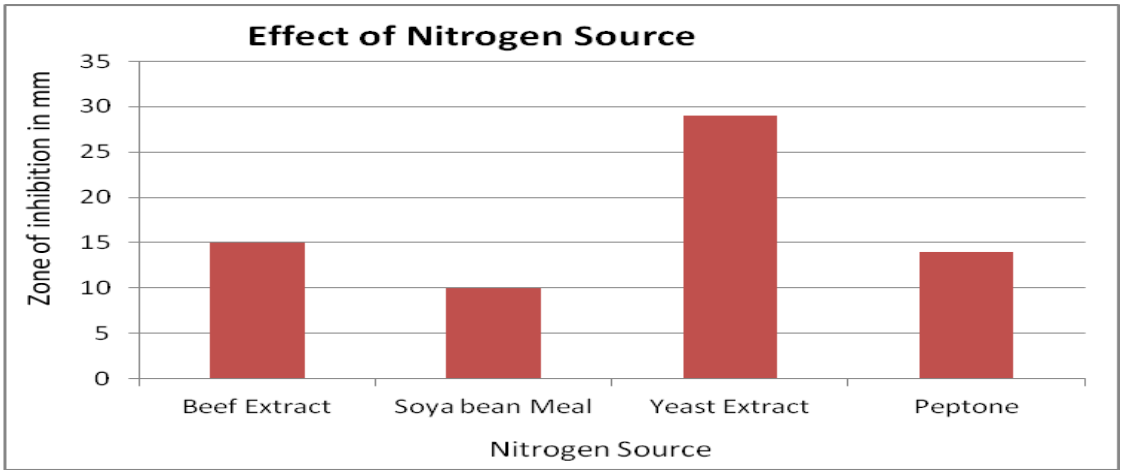


**Figure 5: Optimisation of Nitrogen Source**

The effect of different Nitrogen sources was checked to analyse the production of tetracycline by the microorganisms. The production media was substituted with different Nitrogen sources like peptone, yeast extract, soya bean meal and beef extract. The activity was checked after 5 days of incubation and it was found that the isolate had the ability to grow in the media containing yeast extract as the source of nitrogen (Figure 5).

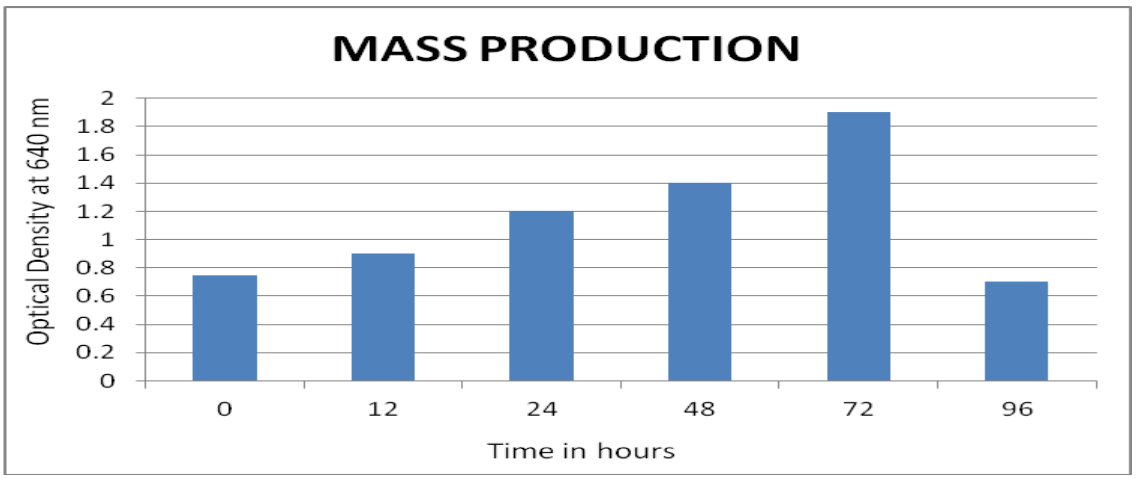


**Figure 6: Optical Density at regular interval of Time.**

The optical density was checked at regular interval of time during fermentation and it was found that the optical density was maximum in 72 hours from the time of inoculation (Figure

6). The broth was collected and purified for the tetracycline. The purified sample was checked for antimicrobial activity against *E.coli* and the zone of inhibition was 30mm in diameter (Figure 7).

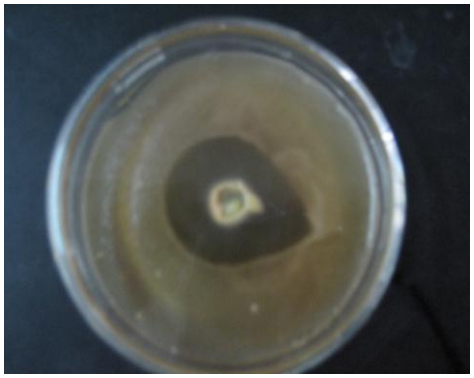

**Figure 7: Antimicrobial Activity of the purified Tetracycline against *E.coli.***

## DISCUSSION

[18] Studied broth stabilities at pH 9.0 and 100 C for 15 min and showed clear differentiation between chlortetracycline and tetracycline they found out that chlortetracycline was completely inactivated and tetracycline gave 40 to 50 per cent inactivation.

The Streptomyces strains could adapt initial pH range from 5 to 7 and optimum level at pH 6.5. as the metabolic activities of the Streptomyces are very much sensitive to the initial pH change.[19, 20] [21], investigated the effect of medium ingredients such as carbon, inorganic and organic nitrogen sources, inorganic salts on tetracycline production by various strains of Streptomyces [*S. aureofaciens* NCIM (2417, 2614, 2615), *S. Rimosus* NCIM 2213 and S. viridifaciens NCIM 2506] in solid-state fermentations (SSF) and concluded that at 65% moisture level, $35^0$C temperature, pH 5-0-6.5, 6 x 4 mm particle size and (1.0 x 108 spores/ ml) inoculum size was suited for maximal tetracycline production. Some authors studied the effects of less expensive organic nitrogen source such as peanut meal.[22] Reported that peanut meal affects the tetracycline yield. A considerable increase in tetracycline production by the addition of soluble starch was already reported in the case of *S.aureofaciens.*[23, 24]

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