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Influence of nutrition and culturing conditions on antimicrobial metabolite production by *Streptomyces mesioensis* isolated from Egyptian soil

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Abstract: The present study reports the effect of critical medium components on antimicrobial compound produced from *Streptomyces mesioensis* isolated from Qalubia soil, Egypt. Culture filtrate of the strain showed good activity against Gram positive bacteria and yeasts. The cell free culture showed 19, 21, 23, 18 and 23 mm inhibition against *Staphylococcus aureus*, Bacillus *subtils*, *Bacillus cereus*, *Candida albicans* and *Saccharomyces cervisiae* respectively. Effect of medium components such as carbon, nitrogen, phosphate sources, incubation period and pH on antimicrobial compound production was tested using *Bacillus cereus* as test organism. Of various variable tested, 20gm mannose, 2.5gm potassium nitrate, 0.85gm $(NH_4)_3$ PO_4 after four days at pH 8 showed significant effect on the antibiotic production.
Key words: *Streptomyces mesioensis*, antimicrobial activity, culture medium.

Introduction

Soil is a natural reservoir for microorganisms and their antimicrobial products. Filamentous soil bacteria belonging to the genus *Streptomyces* is represented in nature by the largest number and variants, producing the majority of known antibiotics among the family Actinomycetaceae [1]. Members of the genus *Streptomyces* are well known as sources of antibiotics and other important noval metabolites including antibacterial, antifungal, antitumor agents, antihelminthic agents and herbicides [2]. Discovery of new antibiotics isolated from *Streptomyces* still continues, for example mediomycins from *Streptomyces mediocidicus* [3], bleomycin from *Streptomyces vericillus* [4] and caboxamycin (new benzoxazole antibiotic) from *Streptomyces* sp. [5] and carriomycin from *Streptomyces hygroscopicus* [6].

The ability of *Streptomyces* cultures to form antibiotics is not a fixed property but can be greatly increased or completely lost under different conditions of nutrition and cultivation [7]. Therefore, the medium constitution together with the metabolic capacity of the producing organism greatly influences antibiotic biosynthesis. Changes in the nature and type of carbon, nitrogen or phosphate sources and trace elements have been reported to influence antibiotic biosynthesis in *Streptomyces* [3]. In addition, antibiotic productivity had a tendency to decrease when metal ion deficient media were used and the incubation for long period at high temperature [8]. To achieve maximum production of antibiotic by any producer strain, it is necessary to optimize nutrient and environmental conditions and this was contributed by researchers [9 and 10].

In our continuous search for noval microbial metabolites having unique activity, a number of *Streptomyces* strains were screened. This was resulted in isolation of strain identified as *Streptomyces mesioensis* which produced in the culture broth a bioactive compound with antibacterial and antiyeast activity.

In this study, effort has been made to understand the effect of various carbon, nitrogen and phosphate sources, incubation period and pH on bioactive metabolite production by *Streptomyces mesioensis* under laboratory scale fermentation.

Materials and methods

Microbial strains and maintenance

Streptomyces mesioensis was isolated from the soil at Qalubia Governorate in Egypt by Ali [11]. The species was characterized morphologically and physiologically following the directions given for the International *Streptomyces* Project [2]. The strain maintained as slant culture using starch casein agar medium [7].

Antimicrobial compound production and activity testing

Production of antimicrobial compound from *Streptomyces mesioensis* was carried out adopting submerged fermentation. The components of the production medium are composed in g/L of: 20 starch, 2.0 KNO_3, 1.0 K_2HPO_4, 0.5 $MgSO_4$ $2H_2O$, 0.5 Na Cl, 3.0 $CaCO_3$ and 0.01 $FeSO_4$ $7H_2O$ at pH 7.4. About 10% inoculum prepared in starch casein broth was transferred into 50 ml production medium in 250 ml Erlenmeyer flask and kept in rotary shaker at 28^oC with 150 rpm. Culture supernatant was tested for antimicrobial activity against four bacteria including three Gram positive bacteria (*Staphylococcus aureus, Bacillus subtils* and *Bacillus cereus*), one Gram negative bacteria (*Escherichia coli*) and two yeasts (*Candida albicans* and *Saccharomyces cervisiae*) by agar well diffusion method [13]. The test microorganisms were obtained from MIRCIN culture collection of the Faculty of Agriculture, Ain Shams University and American Type Culture Collection (ATCC). The above mentioned bacteria were cultured in a Nutrient broth at 37 ±0.1 ^oC for 24 hour and malt extract for yeast at 28 ±0.1^oC for 48 hour and the diameter of inhibition zone were recorded.

Optimization of nutrition and culturing conditions

To determine the optimum nutritional and culturing conditions for antibiotic production, the previous mentioned production medium with or without carbon, nitrogen and phosphate sources were used as a basal medium. One milliliter of homogenous spore suspension (0.2 O.D) in 0.5% Tween 20 solution of *Streptomyces mesioensis* was inoculated from a six-day old slant culture in 25 ml culture broth. Flasks were incubated in shaking condition 150 rpm at 28^oC. Antimicrobial bioassay was carried out at the end of the incubation period using *Bacillus cereus* as test organism.

Different variables of carbon, nitrogen and phosphate supplement were supplemented separately into the basal medium at equimolar concentration. Different concentration from the best source was examined. To avoid denaturation and caramalization of some sources, they were steralized as described by Shrilling and Gottleib [12].

To study the effect of pH of the culture medium, the basal medium was adjusted with different levels of acidic and alkaline pH. The effect of prolongation of incubation for maximum antibiotic production was observed up to 7 days of incubation.

Result and Discussion

The genus *Streptomyces* are especially prolific, producing around 80% of total antibiotic products. The present study focused on antimicrobial production from *Streptomyces mesioensis* isolated from Egyptian soil. The strain showed broad spectrum activity against all tested microorganisms except *Escherichia coli* (Table1).

Table 1: Antimicrobial spectrum of *Streptomyces mesioensis*

Test organisms	*Escherichia coli*	*Staphylococcus aureus*	*Bacillus subtils*	*Bacillus cereus*	*Sarcina lutea*	*Candida albicans*	*Saccharomyces cerevisiae*
Diameter of inhibition zone (mm)	0.0	19.0	21.0	23.0	15.0	18.0	23.0

Effect of carbon, nitrogen and phosphate sources

Many microorganisms have been evaluated for the production of antimicrobial substance. However the high cost and low yields have been the main problem for its industrial production [14]. The isolated strain was able to produce antibiotic in all tested carbon sources except for L-arabinose (Table 2). However, maximum antibiotic production was obtained in medium supplemented with mannose as a sole carbon source followed by galactose. Different concentrations of mannose were examined to study its effect on the antibiotic production. Mannose at concentration of 20 g/L was found to be optimum for the antibiotic production (Fig 1). The obtained result is in agreement with the findings of Kreig and Holt [15], who reported that mannose was one of the most fermentable carbon sources by Streptomycetes for antibiotic production.

Table 2 : Effect of different carbon sources on antibiotic production by *Streptomyces mesioensis*

Carbon source (g/L)	Starch	Lactose	Mannose	D-Fructose	D-Glucose	D-galactose	L-rhamnose	L-arabinose
Diameter of inhibition zone (mm)	23	23	29	23	21	26	18	0.0

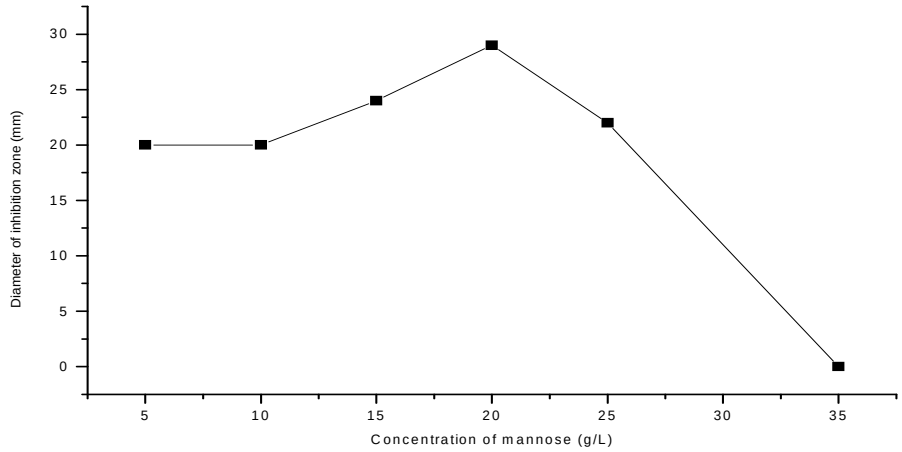

Fig 1: Effect of different concentration of mannose on antibiotic production by *Streptomyces mesioensis*

Different types of nitrogen sources were found to have significant effect on secondary metabolites produced by *Streptomyces mesioensis* (Table 3). Maximum antimicrobial activity was obtained with potassium nitrate followed by triammonium phosphate, while ammonium nitrate does not showing production. The optimum concentration of potassium nitrate for antibiotic production was found to be 2.5 g/L (Fig 2). Similar observation reported by Mansour *et al*. [16] who detected that potassium nitrate at 2.0 g/L produced maximum antibiotic production isolated from *Streptomyces* sp.

The result revealed that the level of antibiotic production from *Streptomyces mesioensis* was influenced by the type of phosphate sources in the culture medium (Table 4). Maximum antibiotic production was detected with triammonium phosphate followed by sodium dihydrogen phosphate. On the other hand, no antibiotic production detected with disodium dihydrogen phosphate and trisodium phosphate. Triammonium phosphate at 0.85 g/l was found to be the optimum concentration for the antibiotic production (Fig 3). Abd El Nasser *et al* [17] reported that tribammonium phosphate was more favorable for the antibiotic production by the *Streptomyces viridodiastaticus.*

Effect of pH

The results recorded in Figure (4) reveal that the antibiotic production from *Streptomyces mesioensis* was low when the initial reaction of the medium was adjusted to be pH 4-6 and 9-10. Maximum antibiotic production was detected at pH 8.0. This result suggests the inclusion of this strain in the alkaliphilic actinomycetes group. The results are compatible with some *Streptomyces* species recorded to produce antibiotic against bacteria, yeast and fungi at alkaline pH [18 and 2].

Table 3: Effect of different nitrogen sources on antibiotic production by *Streptomyces mesioensis*

Nitrogen sources (equimolar)	Diameter of inhibition zone (mm)
Potassium nitrate	29
Sodium nitrate	22
Ammonium nitrate	0.0
Ammonium oxalate	26
Ammonium sulphate	24
Ammonium dihydrogen phosphate	21
Diammonium hydrogen phosphate	21
Triammonium phosphate	28

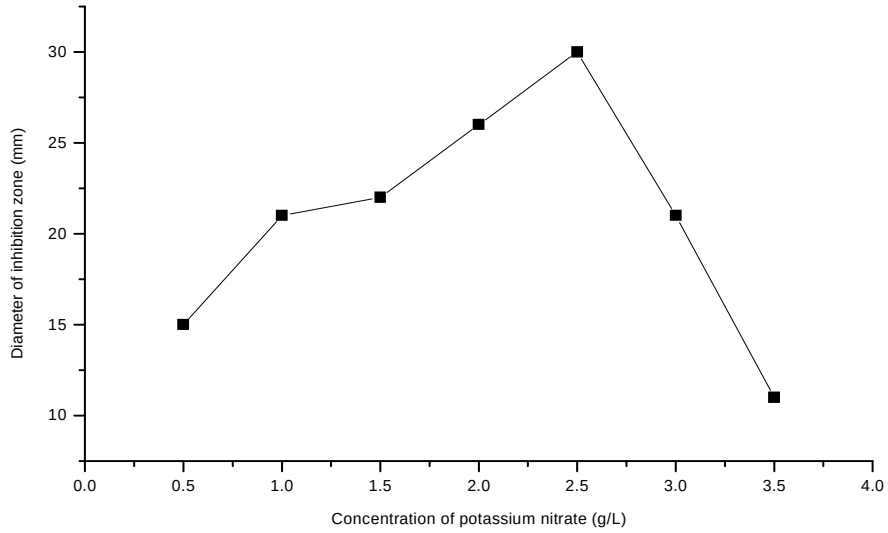

Fig 2: Effect of different concentration of potassium nitrate on antibiotic production by *Streptomyces mesioensis*

Table 4.: Effect of different phosphate sources on antibiotic production by *Streptomyces mesioensis*

Phosphate sources (equimolar)	Diameter of inhibition zone (mm)
Dipotassium hydrogen phosphate	19
Potassium dihydrogen phosphate	22
Ammonium dihydrogen phosphate	21
Triammonium phosphate	29
Sodium dihydrogen phosphate	24
Disodium dihydrogen phosphate	0.0
Trisodium phosphate	0.0

Effect of incubation period

The maximum incubation period required for optimum antibiotic yield by the strain was four days under shaking condition (Fig 5). The incubation period was found to have profound influence on antibiotic production by many species of the genus *Streptomyces* [19 and 2].

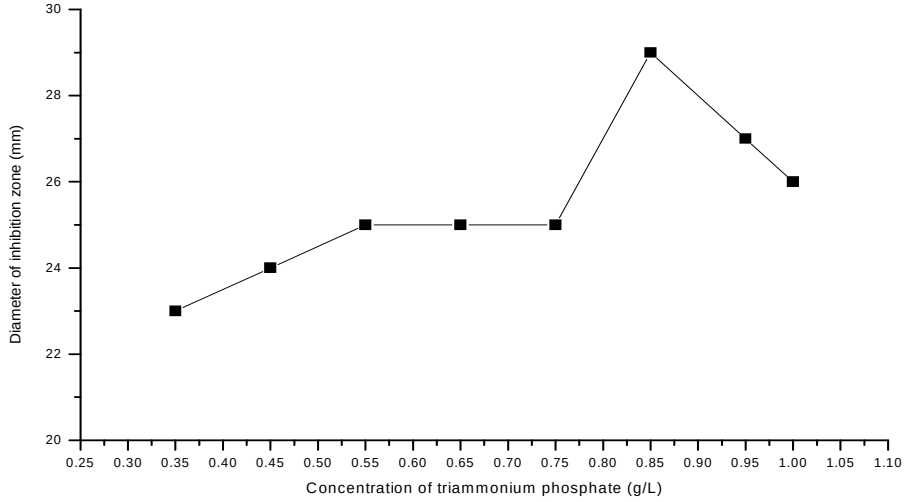

Fig 3: Effect of different concentration of triammonium phosphate on antibiotic production by *Streptomyces mesioensis*

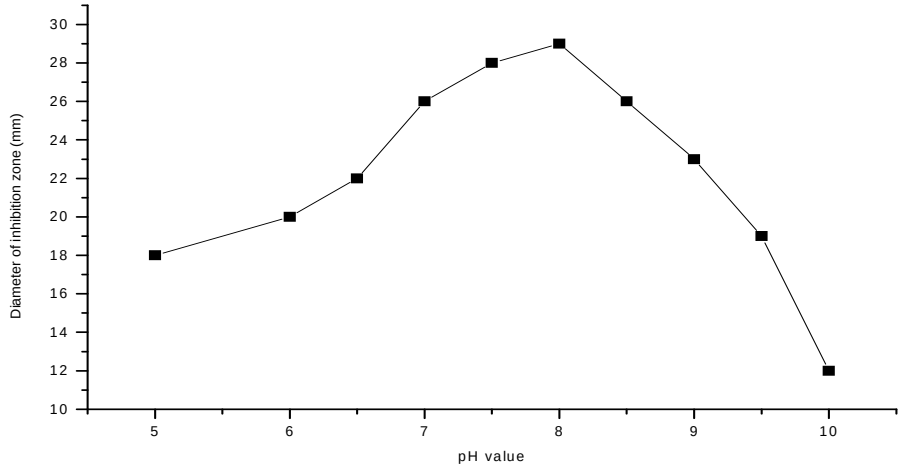

Fig 4 : Effect of pH on antibiotic production by *Streptomyces mesioensis*

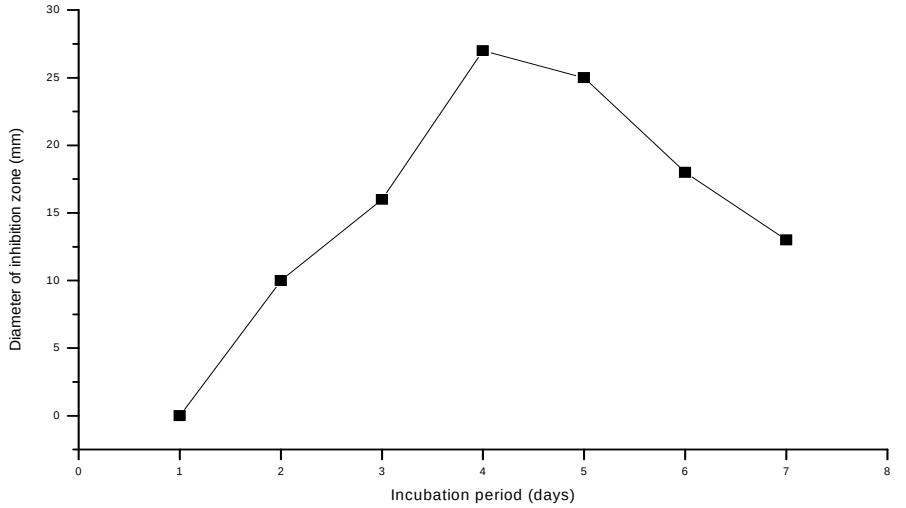

Fig.5 : Effect of incubation period on antibiotic production by *Streptomyces mesioensis*

Conclusion

It is evident from the results of this investigation that the production of bioactive metabolite from *Streptomyces mesioensis* are mainly influenced by the regime of the appropriate nutrient supplements and proper dose in the culture media. The optimum production of the antimicrobial agent in laboratory scale fermentation could be achieved in a medium supplemented with mannose at 20 g/L as carbon, potassium nitrate 2.5 g/L as nitrogen and triammonium phosphate at 0.85 g/L as phosphate sources and further process parameters such as initial pH 8.0 of the medium and incubation period of four days under shaking condition.

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