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# Physiological factors affecting the production of an antimicrobial substance by *Streptomyces violatus* in batch cultures

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**ABSTRACT**

A survey was carried out to select an antibiotic producing *Streptomyces* strain isolated from garden soil in Alexandria, Egypt. *Streptomyces violatus* showed the highest antimicrobial activity in static cultures after 7 days incubation at 30°C. The antibacterial substance was more active against *Bacillus subtilis* and *Staphyllococcus aureus* than *Escherichia coli* or *Sarcina lutea*. Growth of *S. violatus* and production of antibiotic in a starch-nitrate medium were monitored over a period of 14 days. The organism produced a blue pigment associated with the antibiotic appearance in the cultures. Optimization of antibiotic production in batch cultures has been carried out. Substitution of starch by glycerol at a concentration of 12.5 g/l showed 1.32-fold increase of antibiotic production. Cultures containing sodium nitrate (2.5g/l) showed the highest antibiotic production followed by peptone, alanine, monosodium glutamate or phenylalanine. A mixture (w/w) of $K_2HPO_4$ and $KH_2PO_4$ (1g/l) yielded 1.9-fold and 6.1-fold increase in antibiotic production compared to cultures individually supplied with $K_2HPO_4$ or $KH_2PO_4$, respectively. The presence of ferrous sulphate and manganese chloride improved the production of the antibiotic. An inoculum size of $4x10^6$ spores/ml and initial pH 7.0 at 30°C were optimum for a maximum antibiotic production of 268μg/ml in the culture filtrates of *S. violatus*.

**KEYWORDS:** *Streptomyces violatus*, physiological factors, antibiotic MSW2000, batch culture

## INTRODUCTION

Streptomycetes are the source of several useful antibiotics that are used not only in the treatment of various human and animal diseases but also in agriculture and biochemistry as metabolic poisons (Demain 1981; Martin 1982; Ubukata *et al*. 1995; Hayakawa *et al*. 1996; Xue *et al*. 1998; Jones 2000). At least 70 of the approximately 100 marketed antibitics used for the treatment of infections in humans are derived from substances produced by *Streptomyces* spp., for example *Streptomyces aureofaciens* is an important industrial microorganism as a producer of chlortetracycline and tetracycline (Yang & Ling 1989). Discovery of new antibiotics produced by streptomycetes still continues, such as noboritomicins A and B produced by *S. noboritoensis* (Juslen *et al*. 1978), actinomycins $X_2$ produced by *S. nasri* (El-Naggar *et al*. 1998), tetrodecamycin produced by *S. nashvillensis* MJ885-mF8 (Tsuchida *et al*. 1995), demethyltetracycline produced by *S. aureofaciens* (Mansour *et al*. 1996) and pyrroindomycins produced by *S. rugosporus* (Abbanat *et al*. 1999).

The ability of streptomycete cultures to form antibiotics is not a fixed property but can be greatly increased or completely lost under different conditions of nutrition and cultivation (Waksman 1961). Therefore, the medium constitution together with the metabolic capacity of the producing organism greatly affects antibiotic biosynthesis. Changes in the nature and type of carbon, nitrogen or phosphate sources and trace elements have been reported to affect antibiotic biosynthesis in streptomycetes (Barratt

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& Oliver 1994; Loun'es *et al*. 1996; Abbanat *et al*. 1999). In addition, antibiotic productivity tendes to decrease when metal ion deficient media are used and when the inocula are incubated for long periods and at high temperatures (Higashide 1984).

The present study describes the production of an antimicrobial substance MSW2000 (Said 2001) produced by a local isolate of *Streptomyces violatus.* Improvement of antibiotic production was acheived by optimization of the cultural conditions and by developing of a defined medium for the biosynthesis of the antibiotic.

## MATERIALS AND METHODS

*Streptomyces orientalis*, *S. violatus*, *S.craterifer* and *S. astreogriseus* were isolated from garden soil, Faculty of Science (El-Shatby), Alexandria, Egypt. Soil samples were collected at a depth of 5-10 cm. These strains were identified according to the International Streptomyces Project (ISP) Scheme as described by Shirling & Gottlieb (1966) and the diagnostic key of Szabo *et al.* (1975). They were maintained on starch-casein agar slants and kept in a refrigerator at 4$^{o}$C until further use. *S. violatus* proved to be a producer of an antimicrobial substance identified as an aromatic ring with 2 aliphatic side chains (molecular formula $C_{15}H_{23}NO_3$; molecular weight=267.338) and named MSW2000 (Said 2001).

**Target organisms**: The following test organisms were used for the bioassay of the antibiotic during the screening experiment: S*taphylococcus aureus* (209 P FDA), *Sarcina leutea* (NCIB 495), *Bacillus subtilis* (ATCC 6051), *Escherichia coli* (NCIB 1186) and *Klepsiella pneumonia* (Local isolate). *S. aureus* was used as a target organism in all other experiments.

**Cultivation of *Streptomyces violatus* for antibiotic production**: For studies of antibiotic production, starch-nitrate medium was used as a basal medium. It was composed of (g/l): Starch, 10.0, $NaNO_3$ , 2.5, $K_2HPO_4$, 1.0, $KH_2PO_4$,1.0 , $MgSO_4.7H_2O$, 0.5, KCl, 0.5, trace salt solution 1.0 ml ($CuSO_4.5H_2O$ (0.64 g/l), $FeSO_4.7H_2O$ (0.11 g/l), $MnCl_2.4H_2O$ (0.79 g/l) and $ZnSO_4.7H_2O$ (0.15 g/l), distilled water,1.0 litre. Medium pH was adjusted to 7.0 before autoclaving using 0.1N NaOH or 0.1 N HCl solution.

Fifty-ml aliquots of this medium were dispensed in 250 ml Erlenmeyer flasks. The medium was adjusted to pH 7.0 and sterilised at 121$^{o}$C for 20 min. Each flask was inoculated with 1.0 ml *S. violatus* spore suspension obtained from a 6-day-old slant culture. The flasks were then incubated under static conditions at 30$^{o}$C for 7 days. The antibiotic bioassay was carried out at the end of the incubation period.

**Determination of dry weight**: The cells were separated from the culture filtrate by centrifugation at 5,000 rpm for 15 minutes, washed twice with distilled water and then dried at 70$^{o}$C until reaching a constant weight.

**Preparation of the crude antibiotic**: Following 7 day incubation period, *S. violatus* cells were separated from the culture by centrifugation at 5,000 rpm for 15 minutes in a cooling centrifuge at 4$^{o}$C (Chilspin centrifuge MSE Fisons). The blue-coloured clear supernatant was then tested for its antibiotic activity.

**Antibiotic bioassay**: This was carried out using the paper-disc diffusion method, Mueller-Hinton agar as an assay medium and *S. aureus* as a test organism. The Mueller-Hinton agar (45$^{o}$C) was poured into sterile Petri-dishes (9 cm diameter) and allowed to solidify. 0.1 ml bacterial suspension (3 x $10^6$ cells) of the test organism was inoculated into the agar surface. Sterile paper discs (6.0 mm diameter, Whatman antibiotic assay discs) were placed on the dried surface of the medium using alcohol-flame-sterilised forceps. Each disc received 20 µl of the culture filtrate. Petri-dishes were kept in a refrigerator for 2 hours to allow for the diffusion of the antibiotic. Petri-dishes were then

incubated inverted for 18-24 hours at 37°C. The inhibition zone diameter was measured in mm (Amade *et al*. 1994). The antibiotic concentration (μg/ml) was determined using a standard calibration curve using the purified antimicrobial substance (MSW2000) produced by *S. violatus* (Said 2001).

**Pigment estimation:** The blue pigment concentration in the culture broth was estimated colorimetrically at 566 nm. This wave length was selected since it showed a maximum absorption of the coloured supernatant measured in UV VIS 4B Spectrophotometer. Each experiment in this work was repeated three times and the average of the three replicates was taken.

## RESULTS AND DISCUSSION

**Survey of some locally isolated actinomycetes for the production of antibiotic(s).**

A survey of four locally isolated *Streptomyces* strains for antibiotic production was carried out in static and shaken cultures (Table 1). It was generally observed that the growth and antibacterial activity obtained in static cultutres were higher than shaken cultures. *Streptomyces astreogriseus* showed the longest incubation time (12 days) needed to obtain maximum antibacterial activity, while *Streptomyces violatus* showed a relatively short time (7-days) and produced the highest activity among the tested strains. *Streptomyces violatus* was also characterised by its broader antibacterial activity, because it affected the growth of all the tested bacteria, showing a stronger activity on *S. aureus* and *B. subtilis*. Accordingly, *S. violatus* was selected for further investigation.

**Table (1): Screening for the antibacterial activity of *Streptomyces* strains in static (St) and shaken (Sh) cultures.**

| Organism | Incubation period (day) | Final pH | | Dry weight (mg/ml) | | The average of the inhibition zone diameter (mm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | *B. subtilis* | | *Sarcina* | | *E. coli* | | *S. aureus* | |
| | | St | Sh | St | Sh | St | Sh | St | Sh | St | Sh | St | Sh |
| *Streptomyces orientalis* | 4 | 7.5 | 7.3 | 1.8 | 0.4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 7 | 8.2 | 7.7 | 2.3 | 1.5 | 15 | 12 | 10 | 10 | 10 | 10 | 15 | 12 |
| | 10 | 8.3 | 8.0 | 2.8 | 2.0 | 18 | 12 | 15 | 12 | 15 | 12 | 18 | 12 |
| | 12 | 8.3 | 8.0 | 3.0 | 2.0 | 18 | 10 | 13 | 10 | 13 | 10 | 18 | 10 |
| *Streptomyces violatus* | 4 | 7.7 | 7.5 | 2.6 | 0.9 | 15 | 10 | 13 | 8 | 13 | 8 | 18 | 12 |
| | 7 | 8.3 | 7.9 | 3.4 | 2.6 | 24 | 20 | 15 | 12 | 12 | 12 | 24 | 20 |
| | 10 | 8.3 | 8.2 | 3.5 | 3.1 | 24 | 20 | 15 | 12 | 15 | 12 | 24 | 20 |
| | 12 | 8.5 | 8.2 | 3.5 | 3.1 | 24 | 20 | 15 | 12 | 15 | 12 | 24 | 20 |
| *Streptomyces craterifer* | 4 | 7.4 | 7.2 | 1.3 | 0.3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 7 | 8.0 | 7.6 | 2.4 | 1.8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 10 | 8.0 | 7.8 | 2.7 | 2.0 | 13 | 10 | 0 | 0 | 0 | 0 | 13 | 10 |
| | 12 | 8.0 | 7.8 | 2.9 | 2.0 | 13 | 10 | 0 | 0 | 0 | 0 | 13 | 10 |
| *Streptomyces astreogriseus* | 4 | 7.5 | 7.4 | 1.5 | 0.5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 7 | 8.3 | 7.7 | 2.5 | 1.6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 10 | 8.3 | 7.9 | 3.0 | 2.3 | 12 | 10 | 10 | 0 | 10 | 0 | 15 | 10 |
| | 12 | 8.3 | 8.0 | 3.0 | 2.5 | 15 | 10 | 10 | 0 | 10 | 0 | 16 | 10 |

The growth of *S. violatus* and the production of antibiotic in a starch-nitrate medium were monitored over a period of 14 days (Fig. 1). The antibiotic production by *S. violatus* occurred in a growth-phase dependent manner and the highest antibiotic yield was obtained in the late exponential phase and the stationary phase, indicating that it is mainly a product of secondary metabolism (Rose 1979, Demain & Fang 1995; Bibb 1996). Similar results were observed for streptomycin production in batch cultures of *S. griseus* ATCC 12475 when grown in a mineral medium (Fazeli *et al*. 1995) and for the production of candicidin in liquid grown cultures of *S. griseus* (Martin & McDaniel 1975). The results also showed that *S. violatus* produced a blue pigment associated with the antibiotic appearance in the culture. It was noticed that a direct tight relationship occurred between the antibiotic production and the intensity of the blue colour formed in the culture (r=0.95). These results may suggest the production of a pigmented antibiotic in *S. violatus* cultures. The production of the blue-pigmented antibiotic actinorhodin and its physiology are known in *S. coelicolor* cultures (Hobbs *et al*. 1990).

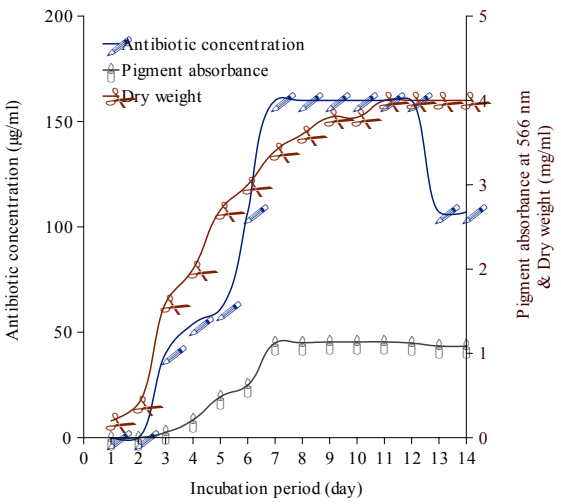


**Figure 1: Effect of different incubation periods on the production of antibiotic by *Streptomyces violatus*.**

## Influence of some cultivation factors on the production of antibiotic

Optimisation of antibiotic production in batch cultures of *S. violatus* was carried out. This strain was able to grow in all the tested carbon sources (Table 2). However, maximum antibiotic production was obtained in cultures supplemented with glycerol as a sole carbon source followed by cultures containing starch. Cultures containing fructose, maltose, xylose or cellulose did not yield any detectable amounts of the antibiotic. The results also showed that the increase of glycerol level in the culture from 10g/l to 12.5 g/l led to 1.32-fold increase in antibiotic production (Fig 2). The utilisation of glycerol and starch by *S. violatus* for growth and production of the antibiotic indicates the presence of an active uptake system for these substrates. Glycerol was also found to be used as a sole carbon source by other *Streptomyces* species (Minambres *et al*. 1992; Sengupta & Paul 1992).

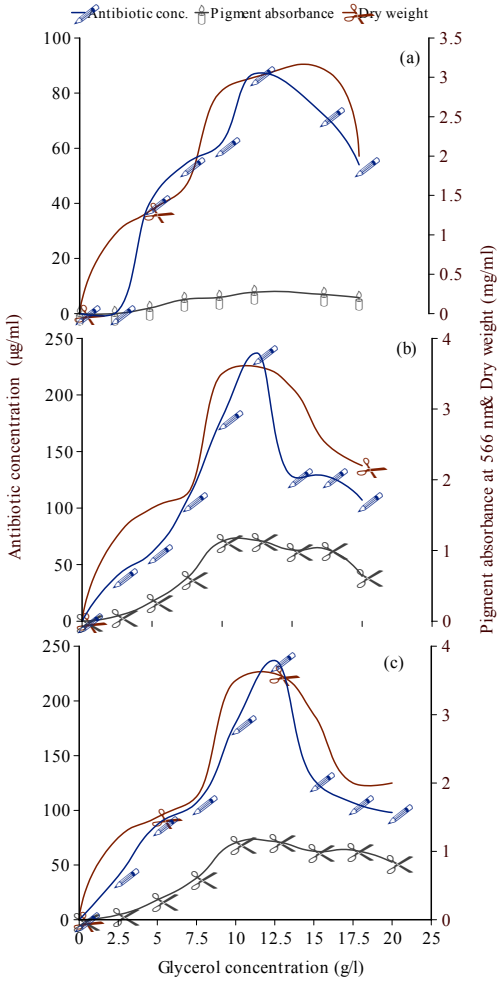


**Figure 2: Effect of glycerol concentration on the production of antibiotic by *Streptomyces violatus* at different incubation periods: a) 4 days, b) 7 days and c) 10 days.**

Table 2. Effect of different carbon sources on the production of antibiotic by *S. violatus*.

| Carbon source | Final pH | Dry weight (mg/ml) | Antibiotic concentration (µg/ml) | Inhibition zone diameter (mm) | Pigment absorbance (566nm) |
|---|---|---|---|---|---|
| Mannitol | 7.8 | 2.0 | 92 | 18 | 0.398 |
| Glycerol | 8.3 | 3.6 | 180 | 25 | 1.130 |
| Glucose | 5.3 | 1.8 | 107 | 20 | 0.516 |
| Fructose | 5.5 | 1.6 | 0 | 0 | 0.000 |
| Galactose | 7.1 | 1.8 | 40 | 10 | 0.078 |
| Xylose | 5.5 | 1.0 | 0 | 0 | 0.000 |
| Rhamnose | 7.1 | 0.9 | 49 | 12 | 0.142 |
| Maltose | 6.4 | 2.0 | 0 | 0 | 0.000 |
| Sucrose | 6.6 | 1.8 | 61 | 15 | 0.181 |
| Lactose | 6.2 | 1.0 | 49 | 12 | 0.161 |
| Starch | 8.3 | 3.4 | 160 | 24 | 1.126 |
| Cellulose | 7.2 | 0.8 | 0 | 0 | 0.000 |
| Raffinose | 7.2 | 1.6 | 107 | 20 | 0.390 |

## Influence of nitrogen source

The results revealed that the level of antibiotic production may be greatly influenced by the nature, type and concentration of the nitrogen source supplied in the culture medoium (Table 3). Similar observations have been reported by many investigators (Khaoua *et al*. 1991; Mansour *et al.* 1996). The highest antibiotic production was obtained in cultures of *S. violatus* containing sodium nitrate or potassium nitrate as a nitrogen source, followed by cultures containing peptone, alanine, monosodium glutamate or phenylalanine. However, cultures containing asparagine or ammonium citrate did not yield any antibiotic activity and showed lowest growth. The results also showed that the concentration of $NaNO_3$ (Fig. 3) greatly influenced the production of the antibiotic by *S. violatus* cultures, while the maximum antibiotic yield was obtained in cultures suplemented with 2.5 g/l $NaNO_3$. These results are in partial agreement with those of other investigators (Hobbs *et al*. 1990; Mansour *et al*. 1996). A negative effect of asparagine on the production of cephamycin C was also observed on cultures of *S. cattleya, S. latamdurans* and *Cephalosporium acremonium* (Castro *et al*. 1985; Khaoua *et al*. 1991).

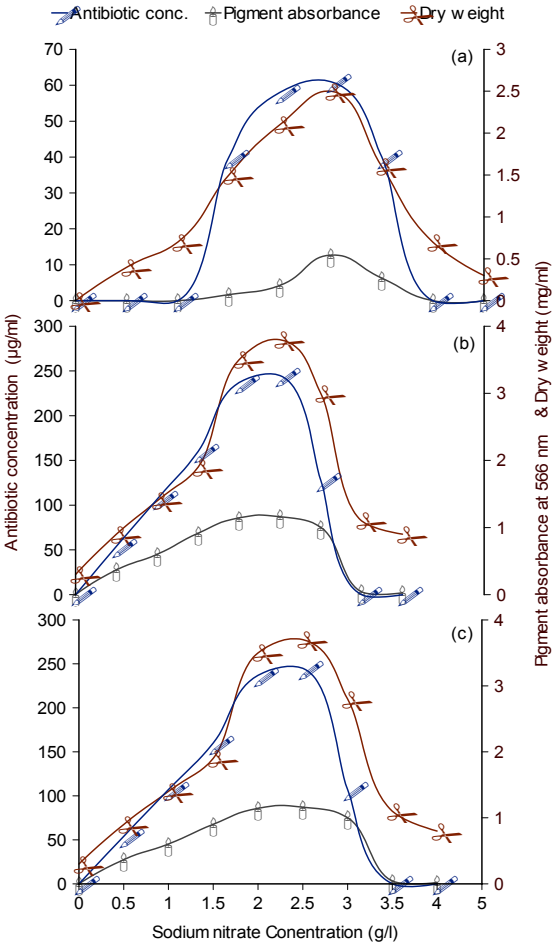


**Figure 3: Effect of sodium nitrate ($NaNO_3$) concentration on the production of antibiotic by *Streptomyces violatus* at different incubation periods: a) 4 days, b) 7 days and c) 10 days.**

Table 3. Effect of different nitrogen sources on the production of antibiotic by *S. violatus*.

| Nitrogen source | Final pH | Dry weight (mg/ml) | Antibiotic concentration (µg/ml) | Inhibition zone diameter (mm) | Pigment absorbance (566nm) |
|---|---|---|---|---|---|
| **I. Organic source** | | | | | |
| Casein | 6.2 | 1.2 | 49 | 12 | 0.172 |
| Peptone | 6.3 | 3.0 | 140 | 23 | 0.404 |
| Peptone+Yeast extract | 6.6 | 3.5 | 107 | 20 | 0.362 |
| Yeast extract | 6.8 | 2.8 | 92 | 18 | 0.302 |
| Tryptone | 6.2 | 2.6 | 54 | 13 | 0.202 |
| Soyabean meal | 6.7 | 2.5 | 72 | 16 | 0.222 |
| **II. Inorganic source** | | | | | |
| Ammonium nitrate | 5.3 | 1.3 | 40 | 10 | 0.018 |
| Ammonium citrate | 5.5 | 0.9 | 0 | 0 | 0.000 |
| Ammonium sulphate | 5.0 | 2.1 | 92 | 18 | 0.250 |
| $NaNO_3$ | 8.3 | 3.6 | 237 | 27 | 1.144 |
| $KNO_3$ | 8.0 | 2.9 | 180 | 25 | 1.126 |
| Monosodium glutamate | 8.1 | 2.4 | 128 | 22 | 0.738 |
| **III. Amino acids** | | | | | |
| Alanine | 6.7 | 2.8 | 140 | 23 | 0.414 |
| Glutamic acid | 6.5 | 2.4 | 92 | 18 | 0.298 |
| Leucine | 6.3 | 3.0 | 107 | 20 | 0.372 |
| Tyrosine | 6.5 | 2.0 | 87 | 17 | 0.262 |
| Tryptophan | 6.5 | 3.5 | 72 | 16 | 0.204 |
| Phenylalanine | 6.1 | 2.3 | 128 | 22 | 0.642 |
| Asparagine | 6.7 | 0.2 | 0 | 0 | 0.000 |
| Aspartic acid | 6.4 | 1.9 | 107 | 20 | 0.370 |

## Influence of potassium phosphate and magnesium sulphate salts

Phosphate is a major factor in the synthesis of a wide range of antibiotics (Martin & Demain 1980). However, an excessive amount of inorganic phosphate suppresses the production of antibiotics such as tetracycline, actinomycin and candicidin (Kishimoto *et al*. 1996). The results of the present work (Fig 4) showed that $KH_2PO_4$ was not favourable for the production of antibiotic by *S. violatus*, while $K_2HPO_4$ at a concentration of 1g/l yieldes an inhibition zone of 22 mm, equivalent to an antibiotic concentration of 128 µg/ml. It was also observed that addition of a mixture of both phosphate salts ($KH_2PO_4$ and $K_2HPO_4$) showed the most positive effect on the production of antibiotic by *S. violatus*. The antibiotic concentration reached its maximum value (245µg/ml) when using a phosphate salt mixture of 1g/l, showing a 1.9-fold and 6.1-fold increase when compared to the highest values obtained when $K_2HPO_4$ and $KH_2PO_4$ were individually supplied to the medium, respectively. These results are in agreement with those reported by other investigators (Harold 1966; Kishimoto *et al*. 1996).

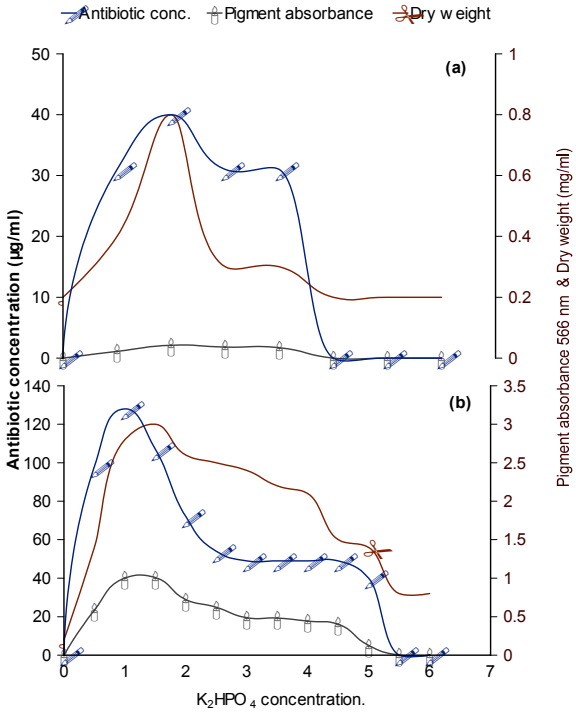


**Figure 4: Effect of different (a) $KH_2PO_4$ and (b) $K_2HPO_4$ concentrations on the production of antibiotic by *Streptomyces violatus***

The results also showed that addition of 0.5g/l magnesium sulphate to the culture medium was optimal for the production of a maximum yield of antibiotic by *S. violatus* (Fig 5). At this $MgSO_4.7H_2O$ concentration, the antibiotic yield was 4.2-fold than that in cultures devoid of magnesium sulphate. The importance of magnesium sulphate for antibiotic production by other *Streptomyces* species has been reported by several investigators (Khaoua *et al*. 1991; Chen & Wilde 1991; Kang *et al*. 1998). The effects of magnesium availability are presumably due to requirements of this cation for protein synthesis, and its depletion may restrict enzyme synthesis and activity (Aasen *et al*. 1992; Natsume *et al*. 1994).

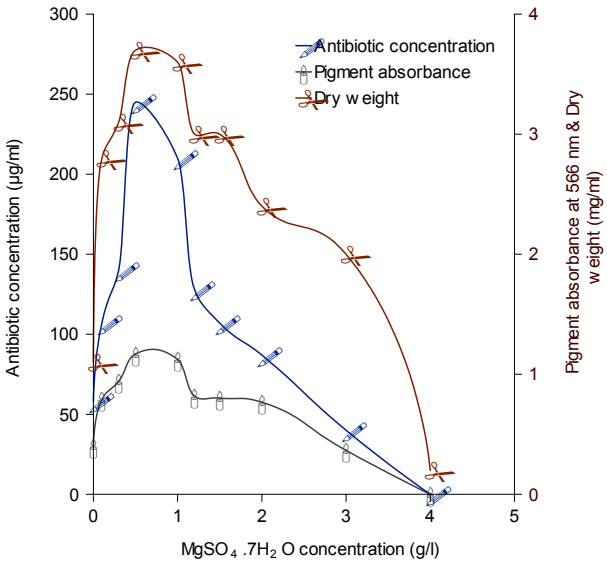


**Figure 5: Effect of ($MgSO_4.7H_2O$) concentration on the production of antibiotic by *Streptomyces violatus***

## Influence of trace elements

The results given in Table 4 showed that iron and manganese could play an important role in the promotion of antibiotic production, the highest dry weight (3.8 mg/ml) was also recorded for manganese. A slight increase in the antibiotic concentration was recorded for Cu, whereas Zn addition lowered the antibiotic concentration compared to the control. The highest antibiotic concentration was achieved in the presence of all elements in the culture medium, yielding a 2.1-fold increase compared to the control. Kishimoto *et al*. (1996 and 1997) reported on the importance of ferrous ions for the growth and antibiotic production by *Streptoverticillium rimofaciens*. Mansour *et al.* (1996) showed that manganese ions enhanced growth and granaticin production in *S. violaceolatus*.

Table 4. The role of trace elements on the production of antibiotic by *S. violatus*.

| Trace element | Final pH | Dry weight (mg/ml) | Antibiotic concentration (µg/ml) | Inhibition zone diameter (mm) | Pigment absorbance (566nm) |
|---|---|---|---|---|---|
| Control | 8.0 | 3.0 | 128 | 22 | 1.006 |
| $CuSO_4.5H_2O$ | 8.3 | 3.1 | 140 | 23 | 1.024 |
| $FeSO_4.7H_2O$ | 8.3 | 3.6 | 245 | 28 | 1.170 |
| $MnCl_2.4H_2O$ | 8.2 | 3.8 | 160 | 24 | 1.164 |
| $ZnSO_4.7H_2O$ | 8.1 | 1.4 | 107 | 20 | 0.932 |
| Total (T) | 8.5 | 3.8 | 268 | 29 | 1.182 |
| T- $CuSO_4.5H_2O$ | 8.1 | 3.2 | 107 | 20 | 0.970 |
| T- $FeSO_4.7H_2O$ | 8.2 | 2.2 | 72 | 16 | 0.788 |
| T- $MnCl_2.4H_2O$ | 8.2 | 2.5 | 92 | 18 | 0.806 |
| T- $ZnSO_4.7H_2O$ | 8.1 | 2.3 | 128 | 22 | 1.068 |

**Influence of incubation temperature, inoculum size and initial pH value**

*S. violatus* showed a narrow range of incubation temperature for relatively good growth and antibiotic production (Fig 6).The increase of the incubation temperature from 20°C to 30°C, increased the growth of the cells and the production of the antibiotic by 3.45-fold and 4.39-fold, respectively. Maximum antibiotic production was obtained at 30°C. A higher incubation temperature (35°C) had an adverse effect on growth and antibiotic production.

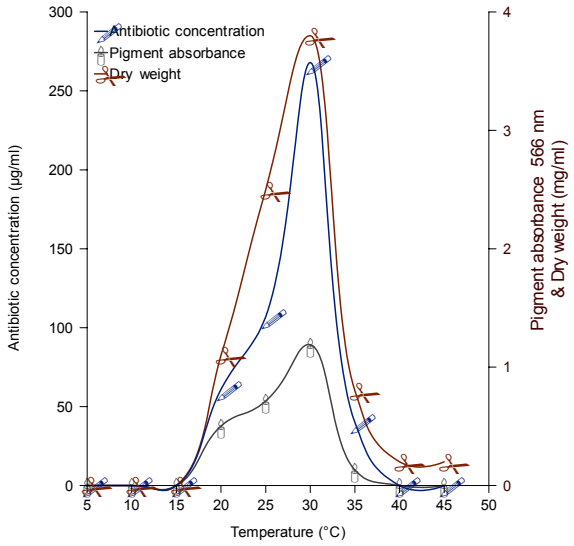


**Figure 6: Effect of incubation temperature on the production of antibiotic by *Streptomyces violatus* .**

The size of inoculum affected the ability of *S. violatus* to produce the antibiotic in the tested cultures. An increase of the inoculum size from 0.5 ml to 2 ml /50 ml medium, enhanced the production of the antibiotic by approximately 4.6-fold. These results are in agreement with those of Grag & Neelakantan (1981) who proved that the size of inoculum may be an important factor in microbial fermentations.

The initial pH value of the culture showed a significant influence on the maximum productivity of the antibiotic as well as on the growth of the test organism. *S. violatus* cells showed no growth when the initial reaction of the medium was adjusted to pH 5, while higher pH values yielded better growth and antibiotic activity. The maximum antibiotic activity was obtained at an initial pH of 7.0. Similarly, actinorhodin, a blue pigment-antibiotic, was produced extracellularly in *S. coelicolor* cultures at pH values around 7 (Bystrykh *et al*. 1996).

The present study determined the optimal culture conditions for antibiotic production by *S. violatus*. Further studies were carried out and will be reported later concerning the isolation and characterisation of the antimicrobial substance produced by this strain.

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## الملخص العربى

## العوامل الفسيولوجية المؤثرة علي إنتاج مادة مضادة لنمو البكتريا من مزارع ستربتوميسس فيولاتس

مها علي حسن[1]-مصطفي يوسف النجار[2]-وفاء يوسف سعيد[2]

1. قسم التاريخ الطبيعي-كلية التربية-جامعة الإسكندرية-جمهورية مصر العربية
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تم إجراء مسح لانتقاء سلالة من الستربتوميسيتات المعزولة محليا قادرة علي إنتاج مضادات حيوية. أظهر ستربتوميسس فيولاتس أعلي إنتاجية للمضادات الحيوية في المزارع السائلة خلال فترة تحضين 7 أيام و عند درجة حرارة 30°م. كان النشاط المضاد لنمو البكتريا "باسيلاس ستلاس"و "ستافيللوكوكاس أورياس"أعلي من بكتريا " إشيريشيا كولاي" أو "سارسينا لوتيا". تمت متابعة نمو ستربتوميسس فيولاتس و إنتاج المضاد الحيوي خلال فترةتحضين تبلغ 14 يوما في وسط غذائي يحتوي علي النشا و النترات. و قد ظهر أن الكائن الدقيق المختبر ينتج صبغ أزرق مرادف لظهور المضاد الحيوي في المزرعة. أجريت دراسة للتوصل لأفضل الظروف الفسيولوجية لإنتاج المضاد الحيوي في المزارع المختبرة. أسفر استخدام الجيليسرول بتركيز 12.5 جم/لتر إلي زيادة إنتاج المضاد الحيوي بمقدار 32و1-ضعف عنه في حالة استخدام النشا كمصدر للكربون. كانت نترات الصوديوم بتركيز 2و5جم/لتر هي أفضل المصادر النيتروجينية المستخدمة في المنبت الغذائي حيث سجلت أعلي إنتاج للمضاد الحيوي يليه علي الترتيب البيتون ,الألانين, جلوتامات أحادي الصوديوم و الفينيل ألانين. أظهرت الدراسة أيضا أن استخدام مزيج من فوسفات البوتاسيوم أحادي الهيدروجين و فوسفات البوتاسيوم ثنائي الهيدروجين (بنسبة 1:1) في المنبت الغذائي و بتركيز 1جم/لتر قد أدي إلي زيادة في إنتاج المضاد الحيوي بمعدل 9و1-ضعف و 1و6ضعف من ذلك المتحصل عليه في حالة استخدام فوسفات البوتاسيوم أحادي الهيدروجين أو فوسفات البوتاسيوم ثنائي الهيدروجين كل علي حده. عند دراسة تأثير إضافة بعض العناصر المعدنية الدقيقة للمنبت الغذائي أظهرت النتائج أن كبريتات الحديدوز و كلوريد المنجنيز كان لها أفضل تأثير علي زيادة إنتاج للمضاد الحيوي في المزرعة. و قد وجد أيضا ان أفضل تركيز للاقحة المستخدمة في المنبت الغذائي هو $4\times10^6$جرثومة/مل كما كان أفضل رقم أس هيدروجيني ابتدائي للمزرعة هو رقم 7 عند درجة حرارة تحضين30°م حيث تم الحصول علي أقصي إنتاج للمضاد الحيوي (268 ميكروجرام/مل) في رشيح مزارع ستربتوميسس فيولاتس.