Qatar Univ. Sci. J. (1994), 14 (2): 281-288

STUDIES ON PLANT GROWTH REGULATORS AND ENZYMES PRODUCTION BY SOME BACTERIA

By

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دراسات على الأنشطة الهرمونية والانزيمية لبعض البكتريا

فتحي عواد منصور و حشمت سليمان الدسوقي و هند عبد الحميد حميدو

يهدف هذا البحث نحو دراسة الأنشطة الهرمونية لأربع وعشرين سلالة بكتيرية تابعة لجنس ستربتوميسيس وقدرتها على إنتاج أنزيمي الألفا أميليز والبروتينيز . وقد أسفرت هذه الدراسة عن النتائج التالية :

١ ـ وجـــد أن Streptomyces olivaceoviridis, S. rimosus, S. rochei هـي أكـثـر الكائنات نشاطا من حيث قدرتها على إنتاج منظمات النمو (الأوكسينات والجبريلينات والسيتوكينينات) وأنزيمات الألفا أميليز والبروتينيز يليها في ذلك :
S. alboviridis, S. griseoviridis, S. phaeochromogenes, Streptomyces sp. No. 20.

٢ ـ أظهرت الكائنات :
S. albidoflavus, S. albus, S. caesius, S. citreus, S. griseinus, S. scabies, Streptomyces sp. No. 21, S. tetranusemus, S. violaceus and S. viridosporus

نشاطا متوسطا عند مقارنتها بالكائنات المذكورة أعلاه من حيث قدرتها على إنتاج منظمات النمو والانزيمات موضوع الدراسة ، في حين كانت بقية الكائنات غير ذي نشاط يذكر من حيث انتاج منظمات النمو أو الانزيمات .

وقـد وجـد أن S. rimosus, S. olivaceoviridis تعطي أعلى قدر من منظمات النمو والنشاط الأنزيمـي (أنزيمي الفـا أميليـز والبروتينيـز بعد فترة تحصين ٨٤ ساعـة أما بالنسبة S. rochei فكانت بعد ٦٠ ساعـة) .

Key Words: α-Amylase, Auxins, Cytokinin, Gibberellins, *Streptomyces*

ABSTRACT

Twenty four bacterial strains of thallobacteria belonging to genus *Streptomyces* were tested for their potentialities to produce plant growth regulators and some hydrolases in shake cultures. Seven strains namely; *Streptomyces alboviridis, S. griseoviridis, S. olivaceoviridis, S. rimosus, S. phaeochromogenes, S. rochei* and *Streptomyces* sp. No. 20, proved to possess comparatively high capacities for production of auxins, gibberellins and cytokinin-like substances together with substantial levels of α- amylase and protease. Moderate capacities were exhibited by *S. albidoflavus, S. albus, S. casesius, S. citreus, S. griseinus, S. scabies,*

Streptomyces sp. No. 21, *S. tetranusemus, S. violaceus* and *S. viridosporus*. The remaining seven strains produced comparatively low levels of growth regulators and enzymes.

The maximum production of growth regulating substances and enzymes by *S. olivaceoviridis* and *S. rimosus* was attained after 84 hrs, but at 60 hrs for *S. rochei.*

INTRODUCTION

Various *Streptomyces* species are capable of producing extremely valuable biologically active substances; of which the most important are growth promoting substances, enzymes, antibiotics, pigments, amino acids and vitamins [1].

There is much evidence that bacteria produce indolyl-3-acetic acid (IAA) in culture media [2, 3]. Katuznelson and Cole [4] and Sobieszczanki [5] found gibberellin-like substances in culture liquids of several micro-organisms such as *Azotobacter chroococcum* [6] and *Pseudomonas species* [7]. Also, Kampert and Strzelczyk, [8] found that several soil bacteria produced substantial amounts of cytokinin - like substances.

The ability of many *Streptomyces* to produce extracellular enzymes has been demonstrated by many investigators. Amylases and proteases are among the most intensively investigated enzymes [9-18]. However, there is no available report (as the authors are aware) concerning the relationship between the capacity of *Streptomyces* to produce growth-promoting substances and their amylases and proteases activities.

The present investigation was undertaken to study the growth promoters, α-amylase and protease producing potential of some bacterial strains isolated from Egyptian soil.

MATERIALS AND METHODS

Microorganisms and cultural conditions:

Twenty four *Streptomyces* strains, formerly isolated and identified by Mansour [19] were used in this experiment.

Each of the experimental strains was cultured in 250 ml Erlenmyer flasks, each containing 50 ml of starch - casein medium composed of (gl^{-1} tap water): starch, 10.0; Casein, 0.3; NaCl, 2.0; K_2HPO_4, 2.0; KNO_3, 2.0; $MgSO_4$. $7H_2O$, 0.5; $CaCO_3$, 0.2 and $FeSO_4$. $7H_2O$, 0.1. The pH was adjusted to 7-7.5. Dense spore suspensions (5 ml each), prepared from 5-day-old cultures, grown on starch casein agar at 28°C, were used for inoculation. Inoculated flasks (3 replicates) were kept on a gyratory shaker (200 rpm) at 28°C for 4 days. At the end of the incubation period, the whole cultures were filtered through Whatman number 50 filter papers, The obtained filtrates were then filtered through millipore (0.450 μm) filter and the cell-free filtrates were used for determination of growth regulating activity as well as for α-amylase and proteinase producing potential.

The most active strains (i.e. *S. olivaceovirids, S. rimosus* and *S. rochei*) were selected and cultured as above mentioned and left to grow for 96 hrs, meanwhile their growth-regulating production as well as their amylase and proteinase producing-potential of their culture filtrates were assayed every 12 hrs intervals; so that the suitable time for harvesting and the most active organism(s) can be determined.

Hormone extraction, purification and bioassay

The method of extraction was that originally described by Shindy and Smith [20]. The amount of either acidic or neutral auxins were estimated according to straight growth test of barley coleoptile adopted by Foda and Radwan [21]. Gibberellic acid in extracts was determined by the lettuce hypocotyl bioassay developed and adopted by Frankland and Wareing [22]. Cytokinin content of the cell free extracts was estimated according to the method described by Esashi and Leopold [23].

α-amylase assay:

The reaction mixture composed of: 0.1 ml cell-free filtrate plus 5 ml of 0.5% starch plus 0.1 ml acetate buffer at pH 4.5. Incubation was at 37°C for 30 minutes. The direct reducing value (D.R.V) which was considered to be equivalent to reducing sugars was determined in the filtrates following the procedure of Nelson [24]. One unit of α-amylase was chosen to equal the amount of enzymes which produce 10 μg glucose from starch hydrolysis.

Proteinase assay

Anson's casein assay method [25], with slight modification [26], was used.

RESULTS

Production of growth regulators

The results presented in Table 1 showed that the experimental organisms possess variable potentialities for production of plant growth regulators.

Total auxins

The total auxin content was measured as μg IAA equivalent ml^{-1} of culture filtrate. It was obvious from Table 1 that *S. alboviridis, S. griseoviridis, S. olivaceoviridis, S. phaeschromogenes, S. rimosus, S. rochei* and *Streptomyces* sp. No. 20 had the ability to produce substantial amounts of auxins. Lesser amounts of IAA-like substances were detected in the culture filtrate of *S. albus, S. albidoflavus, S. citreus, S. scabies. Streptomyces* sp. No. 21, *S. tetranusemus, S. viridosporus* and *S. violaceus*. The remaining other species of *Streptomyces* seemed to produce very slight amount of auxins.

Gibberellins

The gibberellin content was measured as μg GA_3 equivalent ml^{-1} of culture filtrate. The data presented in table 1 showed that, 6 species, namely *S. albviridis, S. citreus, S. griseoviridis, S. prasinus, S. rimosus* and *S. tetranusemus*

proved to be the most active in production of gibberellins. These were followed by *S. albus, S. minoensis, S. olivaceovirids, S. olivaceus, S. rochei* and *S.* sp. which produced detectable amounts of gibberellins. On the other hand, *S. albidoflavus, S. atroolivaceus, S. caesius, S. coelicolor, S. griseinus, S. gougerotii, S. matensis* and *S. viridosporus* showed mush lesser capacity for production of gibberellins.

S. olivaceoviridis, S. violaceus and *S. viridosporus* showed comparatively the highest biomass growth. These are followed by: *S. albus, S. alboviridis, S. caesius, S. coelicolor, S. matensis, S. olivaceus, S. phaeochromogenes, S. prasinus, S. rochei, S. rimosus* and *Streptomyces* spp. No. 20 & 21, whereas, the remaining species, namely *S. citreus, S. griseinus, S. minoensis, S. scabies* and *S. tetranusemus* produced slight

Table 1

Preliminary screening for growth-regulating substances produced by some thallobacteria grown in starch-casein shaken cultures for 4 days at 28°C. Each value is the mean of duplicate determinations ± standard errors.

Organisms Names	Strain Nos.	Total Auxins Equivalent To IAA μg ml^{-1}	Gibberellins Equivalent To GA_3 μg ml^{-1}	Cytokinins Equivalent To Kinetin μg ml^{-1}
Streptomyces albidoflavus	1	24.01±1.23	2.76≅0.22	3.93±0.13
Streptomyces alboviridis	2	35.87±1.25	9.74±0.41	8.62±0.29
Streptomyces albus	3	27.79±0.92	5.57±0.99	3.04±0.10
Streptomyces atroolivaceus	4	17.35±0.15	1.21±0.13	2.53±0.22
Streptomyces caesius	5	21.65±0.40	2.56±0.51	2.77±0.09
Streptomyces citreus	6	23.13±0.89	10.08±0.18	6.37±0.21
Streptomyces coelicolor	7	14.36±1.26	4.61±0.85	7.32±0.13
Streptomyces griseinus	8	29.57±0.24	2.03±0.52	2.00±0.07
Streptomyces griseolviridis	9	38.86±0.51	13.26±0.19	2.47±0.08
Streptomyces gougerotii	10	19.67±0.31	3.44±0.12	6.40±0.11
Streptomyces matensis	11	15.75±0.84	2.13±0.39	4.78±0.16
Streptomyces minoensis	12	17.40±0.11	5.81±0.40	6.11±0.11
Streptomyces olivaceoviridis	13	34.19±1.46	5.53±0.16	14.88±0.26
Streptomyces olivaceus	14	15.22±0.25	5.61±0.24	5.68±0.10
Streptomyces phacochromogenes	15	36.57±1.36	6.91±0.46	6.55±0.22
Streptomyces prasinus	16	10.51±0.48	9.56±0.41	3.38±0.06
Streptomyces rimosus	17	39.04±0.61	8.30±0.87	4.70±0.08
Streptomyces rochei	18	32.75±0.23	5.69±0.09	6.73±0.37
Streptomyces scabies	19	28.26±0.67	6.87±0.47	9.16±0.16
Streptomyces sp.	20	30.78±0.79	5.27±0.89	5.55±0.10
Streptomyces sp.	21	22.17±2.29	1.90±0.12	3.94±0.39
Streptomyces tetranusemus	22	24.97±0.49	8.46±1.12	3.95±0.07
Streptomyces viridosporus	23	23.09±1.03	2.92±0.48	8.59±0.15
Streptomyces violaceus	24	27.85±1.31	1.84±0.04	2.74±0.05

Cytokinins

The cytokinin content was measured as μg kinetin equivalent ml^{-1} of culture filtrate. As can be seen from table 1, only 5 species, namely *S. alboviridis, S. coelicolor, S. olivaceoviridis, S. scabis* and *S. viridosporus* proved to be capable of production of substantial amounts of cytokinin-substances. The remaining other strains seemed, however, to have a slight or negligible cytokinin - producing potential.

Biomass growth

As can be seen from Table 2, all of the employed strains were able to grow in starch - casein medium, though with variable growth magnitudes. Thus, 7 species, namely *S. albidoflavus, S. atroolivaceus, S. griseoviridis, S. gougerotii,* biomass weight.

Production of α-amylase

The results presented in table 2 showed that isolates: *S. albovirids, S. griseovirids, S. olivaceoviridis* and *S. violaceus* were of comparatively high α-amylase -producing potential. Moderate α-amylase activity were recorded with *S. albus, S. albidoflavus, S. rimosus, S. scabies* and *S. tetanusemus,* whereas, *S. atroolivaceus, S. griseinus, S. gougerotii, S. olivaceus, S. matensis, S. minoensis, S. phaseochromogenus, S. prasinus, S. rochei* and *S.* sp. were of slight α-amylase production. On the other hand, *S. caesius, S. citreus, S. coelicolor* and *S. viridosporus* had very weak amylolytic activity.

Production of proteinase

The results in table 2 indicate that, *S. albidoflavus, S. albus, S. atroolivaceus, S. caesius, S. gougerotii, S. olivaceus, S. olivaceoviridis, S. phaeochromogenes, S. prasinus, S. rimosus, S.* sp., *S. viridosporus* and *S. violaceus* were very active in proteinase production, whereas *S. alboviridis, S. coelicolor, S. griseinus* and *S. rochei* resulted in moderate activities towards proteinase production. On the other hand, the remaining four species were of negligible activities.

Effect of different incubation periods on growth regulating substances produced by three isolates of *Streptomyces* species.

Total auxins

Streptomyces olivaceoviridis and *Streptomyces rimosus* exhibited a progressive increase in auxins production till 84 hrs incubation, followed by a marked reduction at 96 hrs. On the other hand, *Streptomyces rochei* resulted in high amounts of auxins during the first two periods (24-48 hrs) where upon, auxins production decreased gradually during the subsequent incubation period. *Streptomyces olivaceoviridis* appeared to be the most active producer for auxins (Table 3).

Gibberellins

The data presented in Table 3 indicates that *Streptomyces olivaceoviridis* isolate No. 13 showed a gradual increase in the gibberellin production till 60 hrs, followed by a slight decrease at 72 hrs. Again gibberellin production increased at 24 hrs, followed by a marked decrease at 96 hrs. Both *S. rimosus* and *S. rochei* showed a progressive increase in gibberellin production during all the incubation periods.

Cytokinins

S. olivaceoviridis and *S. rochei* showed a progressive increase in cytokinins production till 84 hrs, where upon a marked decrease in case of former strain was observed at 96 hrs. On the other hand, *S. rimosus* produced a comparatively higher amount of *cytokinins* at 24 hrs, but a gradual decrease in cytokinin production was manifested during the subsequent incubation period. Nevertheless, *S. olivaceoviridis* proved, by far, to be more active for production of cytokinins than *S. rimosus* and *S. rochei.*

Effect of different incubation periods on biomass weight, α-amylase and proteinase production by three isolates of *Streptomyces* species.

The data given in table 4 show that biomass weight of *S. olivaceoviridis* increased gradually till 60 hrs followed by a gradual decrease up to 96 hrs. α-amylase production was very high at the first incubation period, where the production decreased gradually till 60 hrs. The production of α-amylase was stable at 60 and 72 hrs. Again a slight increase or decrease in α-amylase production was observed at 84 and 96 hrs respectively. Proteinase production increased gradually till 60 hrs, remained unchanged up to 84 hrs and decreased drastically at 96 hrs.

Biomass weight of *S. rimosus* was increased progressively until 60 hrs, followed by a gradual decrease till 96 hrs. α-amylase production was high at 24 hrs, then decreased gradually during the subsequent incubation period. On the other hand, proteinase production increased gradually during the first two incubation periods followed by a slight decrease at 60 hrs, where upon a gradual decline was detected during the subsequent incubation period.

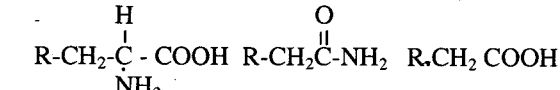
Concerning *S. rochei,* the biomass weight was increased gradually till 72 hrs, then leveled-off during the remaining incubation period. α-Amylase production was very high after 24 hrs then leveled-off till 72 hrs where upon a gradual decline was detected during the subsequent growth period. Proteinase production increased progressively till 84 hrs incubation period followed by a drastic decrease at 96 hrs.

DISCUSSION

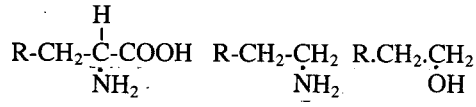
This study conducted to determine the growth regulators producing potential of 24 *Streptomyces* species clearly shows that these organisms exhibit a great deal of variations in their ability to elaborate growth promoters in their culture media. Thus, *Streptomyces olivaceoviridis, S. rimosus* and *S. rochei* were superior to the remaining other species in their capacity to produce growth-promoting substances particularly gibberellin-like ones. This may explain the comparatively higher proteinase and α-amylase activities of the former strains. Gibberellic acid has been suggested to be acting at the gene level as a depressor of those genes which code for hydrolytic enzymes [27, 28]. The relatively high protease activity may have an indirect role in auxin synthesis via the production of amino acids especially tryptophan which incorporates in the biosynthesis of IAA and related substances.

El-Sayed *et al.,* [29] found that the metabolite of *Streptomyces mutabilis* contained tryptophan and anthranilic acid, and that of *S. atroolivaceus* contained tryptamine and indole acetaldehyde. Also, IAA synthesis follows different pathways in the two species: via indole acetamide in *S. mutabilis,* and via tryptamine, tryptophan and indole acetaldehyde in *S. atroolivaceus.*

Tryptophan ------> Indole acetamide -------> Indole acetic acid



Tryptophan ------> Tryptamine -------> Tryptophan



------> Indole acetaldehyde -------> Indole acetic acid

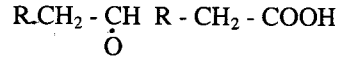


Fig. 1: Pathway of IAA synthesis according to El-Sayed *et al.,* [29].

Gordon *et al.,* [30] found that free cytokinin is released by *Frankia* isolate HFPArI3 in defined culture medium. El-Shanshoury [2] reported that *S. atroolivaceus* produced activity, exogenous IAA, kinetin and gibberellic acid along with other growth regulating substances. Furthermore, plant

Table 2

Preliminary screening for α-amylase and proteinase-producing potential of some isolates of thallobacteria grown in starch-casein shaken cultures for 4 days at 28°C. Each value is the mean of duplicate determinations ± standard errors

Organisms Names	Strain Nos.	Biomass Weight $g.l^{-1}$	α-Amylase Unit ml^{-1}	Proteinase Unit ml^{-1}
Streptomyces albidoflavus	1	3.32±0.20	13.40±0.13	37.50±0.00
Streptomyces alboviridis	2	2.54±0.08	19.04±7.57	27.50±1.06
Streptomyces albus	3	2.62±0.11	11.25±0.37	39.50±0.35
Streptomyces atroolivaceus	4	3.74±0.21	5.17±0.13	34.00±0.71
Streptomyces caesius	5	2.44±0.05	2.14±2.53	30.00±0.35
Streptomyces citreus	6	1.88±0.11	1.07±0.63	27.75±0.18
Streptomyces coelicolor	7	2.74±0.10	2.04±0.00	28.50+0.00
Streptomyces griseinus	8	1.74±0.03	6.64±0.50	24.00±0.00
Streptomyces griseolviridis	9	3.08±0.00	13.75±0.13	19.25±0.18
Streptomyces gougerotii	10	3.48±0.14	7.32±0.38	34.75±0.53
Streptomyces matensis	11	2.74±0.06	7.94±0.63	10.00±0.00
Streptomyces minoensis	12	1.66±0.30	6.00±1.01	25.00±0.00
Streptomyces olivaceoviridis	13	3.1±0.19	16.78±0.25	45.00±0.00
Streptomyces olivaceus	14	2.06±0.04	8.57±0.25	40.00±0.53
Streptomyces phacochromogenes	15	2.36±0.12	8.74±0.13	38.50±0.00
Streptomyces prasinus	16	2.00±0.72	7.07±0.50	36.20±0.53
Streptomyces rimosus	17	2.72±0.51	11.43±0.25	39.75±0.18
Streptomyces rochei	18	2.11±0.02	8.39±0.37	38.00±0.71
Streptomyces scabies	19	1.60±0.29	11.78±0.51	16.50±0.71
Streptomyces sp.	20	2.24±0.17	5.78±0.50	15.00±0.71
Streptomyces sp.	21	2.68±0.15	6.25±0.13	33.50±1.06
Streptomyces tetranusemus	22	1.70±0.22	10.15±0.12	11.54±0.17
Streptomyces viridosporus	23	3.34±1.20	2.48±0.37	41.50±0.35
Streptomyces violaceus	24	3.54±1.01	16.07±0.25	37.25±0.53

Table 3

Effect of different incubation period on growth-regulating substance produced by three isolates of *Streptomyces* species. Value listed are expressed as the mean of duplicate determination ± s.r.

Incubation Period (Hr)		24			48			60		
Organisms Names	Organisms No.	Total Auxins Eqvuivalent To IAA µg ml^{-1}	Gibberellins Equivalent To GA_3 µg ml^{-1}	Cytokinin Equivalent To Kinetin µg ml^{-1}	Total Auxins Equivalent To IAA µg ml^{-1}	Gibberellins Equivalent To GA_3 µg ml^{-1}	Cytokinin Equivalent To Kinetin µg ml^{-1}	Total Auxins Eqvuivalent To IAA µg ml^{-1}	Gibberellins Equivalent To GA_3 µg ml^{-1}	Cytokinin Equivalent To Kinetin µg ml^{-1}
Streptomyces olivaceoviridis	13	14.25±4.53	11.87±3.001	12.01±0.21	85.80±1.42	13.62±0.45	21.04±0.68	22.34±0.28	14.14±2.99	14.70±0.50
Streptomyces rimosus	17	12.73±0.0	4.22±0.52	17.86±0.66	37.96±0.79	14.30±0.73	7.48±0.13	26.46±11.99	6.59±1.50	12.37±0.21
Streptomyces rochei	18	37.65±0.08	3.58±0.71	2.08±0.71	31.23±0.25	6.94±0.24	5.89±1.00	39.33±5.25	5.84±0.13	4.21±1.01

Incubation Period (hr)		72			84			96		
Organisms Names	Organisms No.	Total Auxins Eqvuivalent To IAA µg ml^{-1}	Gibberellins Equivalent To GA_3 µg ml^{-1}	Cytokinin Equivalent To Kinetin µg ml^{-1}	Total Auxins Equivalent To IAA µg ml^{-1}	Gibberellins Equivalent To GA_3 µg ml^{-1}	Cytokinin Equivalent To Kinetin µg ml^{-1}	Total Auxins Eqvuivalent To IAA µg ml^{-1}	Gibberellins Equivalent To GA_3 µg ml^{-1}	Cytokinin Equivalent To Kinetin µg ml^{-1}
Streptomyces olivaceoviridis	13	26.21±0.15	14.22±0.24	16.61±0.40	44.46±9.77	15.41±0.296	28.71±2.03	33.92±0.35	13.63±2.5	19.02±0.72
Streptomyces rimosus	17	30.46±0.64	13.92±0.30	6.67±0.21	39.8±0.69	16.30±0.02	6.73±0.17	33.75±0.01	19.87±2.27	6.31±0.21
Streptomyces rochei	18	38.84±1.13	6.21±0.71	4.82±0.41	29.68±0.22	8.58±0.22	8.58±0.0	7.19±0.22	17.64±0.0	8.11±0.29

Table 4

Effect of different incubation periods on biomass weight, α-amylase and proteinase production by three isolates of *Streptomyces* species

	Incubation Periods Hr.	24			48			60			72			84			96		
Organisms Names	Parameters Organisms No.	Biomass Weight g/L	α- Amylase Unit ml^{-1}	Proteinase Unit ml^{-1}	Biomass Weight g/L	α-Amylase Unit ml^{-1}	Proteinase Unit ml^{-1}	Biomass Weight g/L	α-Amylase Unit ml^{-1}	Proteinase Unit ml^{-1}	Biomass Weight g/L	α-Amylase Unit ml^{-1}	Proteinase Unit ml^{-1}	Biomass Weight g/L	α-Amylase Unit ml^{-1}	Proteinase Unit ml^{-1}	Biomass Weight g/L	α-Amylase Unit ml^{-1}	Proteinase Unit ml^{-1}
Streptomyces olivaceoviridis	13	2.76±0.07	42.86±0.0	50.75±0.18	3.87±-.07	18.93±1.01	51.40±0.0	4.39±0.11	16.43±0.50	56.25±0.17	3.61±0.14	16.43±1.01	56.25±0.18	2.94±0.11	16.78±0.25	65.0±0.70	2.78±0.0	15.71±0.50	28.0±0.0
Streptomyces rimosus	17	2.61±0.0	38.87±0.0	55.00±1.77	3.91±0.01	12.67±0.12	65.50±0.35	3.95±0.03	9.46±0.38	64.50±0.72	2.90±0.1	9.83±0.12	58.50±0.35	2.43±0.08	9.10±0.13	33.00±1.06	2.34±0.05	8.02±0.12	35.0±0.35
Streptomyces rochei	18	2.69±0.15	25.00±0.0	25.50±0.35	3.98±1.20	12.42±0.51	25.25±0.18	4.03±2.01	12.30±0.00	27.75±0.18	4.40±0.02	12.32±1.27	29.25±0.18	3.41±0.50	11.74±0.90	38.00±0.00	3.92±0.00	10.00±0.00	17.00±0.01

growth regulators produced by *A. brasilense* have included auxins, gibberellin-like substances, cytokinins and other unidentified substances [31, 32]. El-Shanshoury [2] carried out a survey of a number of azotobacters from Egyptian soil and demonstrated that some of these micro-organisms contained growth-regulating compounds.

The production of auxins, gibberellins and cytokinins was influenced by the biomass and incubation time [26]. Thus the present investigation clearly showed that the maximum production of enzymes (α-amylase and proteinase) and growth-regulating substances was attained at 84 hrs for *Streptomyces olivaceovirids* and *Streptomyces rimosus*, but at 60 hrs for *Streptomyces rochei.*

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