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Factors Affecting the Biological Activity of *Streptomyces aureofaciens* MY18 and *Str. roseviolaceus* MR13

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ABSTRACT. The biological activity of *Streptomyces aureofaciens* MY18 and *Streptomyces roseviolaceus* MR13 (local isolates) as influenced by temperature, aeration, different media and carbon and nitrogen sources were investigated. It was found that biomass, growth parameters and antibiotic activity of both strains were highly affected by these factors. The first strain showed higher temperature range than that observed for second strain. The optimum temperature for growth and antibiotic production of both strains was 30°C. Shaken cultures supported growth and antibiotic activity than static cultures. Starch and nitrate stimulated antibiotic production in the culture filtrate of both organisms. The optical density of golden yellow soluble pigment of *Streptomyces* MY18 (gray series) cultures increased with the increase of antibiotic activity, which can be used as an indication for antibiotic concentration and its activity.

Introduction

Production of antibiotics by micro-organisms is highly affected by many factors. Prescott and Dunn[1] stated that the propagation medium contains, in addition to the usual sources of carbon, nitrogen, minerals and buffers, precursors, (these are known to be of value for increasing the total or the yield of a given type of antibiotic). Salle[2] mentioned that all antibiotic producing organisms which have been studied must have free oxygen for normal metabolic activity.

With respect to the effect of different carbon sources, Orlova and Andrianova[3] mentioned that *Actinomyces rimosus* LST grows well in media containing starch, maltose, glucose, galactose, or ribose and poorly in a medium containing arabinose, whereas sucrose, lactose, xylose and rhamnose are not utilized. They also added that

the biosynthesis of antibiotic was intense in media containing maltose, starch or ribose. On the contrary, galactose showed less oxytetracycline productivity. Abou-Zeid and Mostafa[4] pointed out that the best medium for tetracycline production by *Streptomyces aureofaciens* NRRLB 2209 contained sucrose as a sole carbon source.

Hydrolyzed casein, soybean meal, cotton meal, peanut meal, beef extract, cornsteep liquor are considered to be a suitable nitrogen source for propagation of *Streptomyces aureofaciens*. Zygmunt[5] found that organic nitrogen is an important source of nitrogen for antibiotic production from *Streptomyces rimosus*. Inorganic nitrogen source was also recommended by Darken *et al.* who reported that various ammonium salts, ammonium hydroxide and liquid ammonia gave satisfactory yields of tetracycline by *Streptomyces aureofaciens*[6] Osman added that potassium nitrate was the best nitrogen source for production of antibiotic by *Str. aureofaciens*.[7]

The aim of this work was to study the effect of temperature, aeration, different media and carbon and nitrogen sources on the growth parameters and antibiotic excretion by *Streptomyces aureofaciens* MY18 and *Str. roseviolaceus* MR13. The relationship between the optical density of yellow pigment produced by *Str. aureofaciens* MY18 and antibiotic activity was also observed.

Material and Methods

Microorganism Used

Two strains of *Streptomyces sp. (Streptomyces aureofaciens* MY18 and *Streptomyces* MR13) were used throughout this investigation. *Staphylococcus aureus* 209p was used as a sensitive organism for determination of antibiotic activity in the culture filtrate of *Streptomyces*. Both *Streptomyces* strains are local isolates from western region, Saudi Arabia[8] These organisms were obtained from the Department of Biological Sciences, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia.

Effect of Temperature

In this experiment, 250 ml Erlenmeyer flasks containing 100 ml glycerol casein medium[9] were inoculated with 10 ml of standard inoculum of tested organism. The inoculated flasks were incubated on rotary shaker (180 rpm) at different temperatures being 15, 20, 25, 30, 35, 40 and 45°C for 11 days. Five ml of the culture were taken aseptically daily and filtered. The dry weight of pellets and effectiveness of excrete antibiotic were done. Growth parameters as influenced by growth temperature were also calculated.

Effect of Aeration

One hundred ml glycerol casein medium were inoculated with tested organisms (as mentioned before). A group of these flasks was shaken on rotary shaker (180 rpm) whereas the other group was incubated without shaking (static cultures). The incubation temperature was 30°C for 11 days. The biomass and effectiveness of culture filtrate in shake and static flasks were observed.

Effect of Different Media

Malt extract[10], starch nitrate[11], glycerol-nitrate[11], and glycerol casein[9] media were used in this investigation. Erlenmeyer flasks (250 ml vol.) containing 100 ml medium were inoculated and were shaken on rotary shaker (180 rpm) for 11 days at 30°C. Biomass, growth parameters and effectiveness of excrete antibiotic were determined.

Effect of Carbon and Nitrogen Sources

In this experiment, glucose, fructose, sucrose, maltose, starch and glycerol were used as carbon sources. Peptone, casein, urea, potassium nitrate and ammonium sulfate were used as nitrogen sources. Carbon source of starch nitrate medium[11] was substituted with other carbon sources (containing the same quantity of carbon). Erlenmeyer flasks containing medium were inoculated and incubated as mentioned before. Growth and effectiveness of excrete antibiotics were determined. Nitrogen source of this medium was also substituted with other nitrogen sources containing the same quantity of nitrogen. The efficiency of tested organism to grow and excrete antibiotics on different nitrogen sources was also studied in conical flasks as usual manner.

Maintenance of microbial cultures

Bacterial cultures were maintained by lyophilization at –50°C using freeze drier (Labconco). They were also maintained at 4-6°C after their propagation on the specific medium for each organism.

Standard inoculum

It was carried out by the propagation of *Streptomyces* strains in glycerol casein medium[9] for 10 days on rotary shaker (180 rpm) at 30°C. The growth (pellets) was washed twice with sterile tap water. Pellets were again suspended in sterile tap water to from 5-7 pellets per 20 ml and were used as a standard inoculum for batch cultures (shake and static cultures).

Growth Parameters

Specific growth rate (the logarithmic increase of growth per unit of time), doubling time and effective yield (amount of dried biomass per unit of initial substrate concentration) were calculated according to Painter and Marr[12] and Doelle[13].

Antibiotic Activity

It was determined in the culture filtrates of *Streptomyces* strains using culture filtrate technique[14]. Sensitive organism was *Staphylococcus aureus* 209P which was grown on *Staphylococcus* medium[10] at 37°C.

Results and Discussion

Effect of Temperature

Results in Fig. (1) show the growth intensity during 11 days of incubation period as influenced by different degrees of temperature. *Streptomyces aureofaciens* MY18 did not show any growth at 15°C, and 45°C whereas no growth was detected at 15°C, 20°C, 35°C, and 45°C for *Streptomyces* MR13. The highest growth was observed at 25°C and 30°C for *Streptomyces* MY18 where 511 and 543 mg dried biomass/100 ml culture were obtained at the end of exponential phase respectively. These figures were significantly higher than that observed at 20°C and 35°C (L.S.D. = 88.96 at 5% and 122.57 at 1%). The biomass produced by *Streptomyces* MR13 was also significantly higher at 30°C as compared with other temperature levels being 234 mg/ 100 ml (L.S.D. = 15.75 at 5% and 22.93 at 1%).

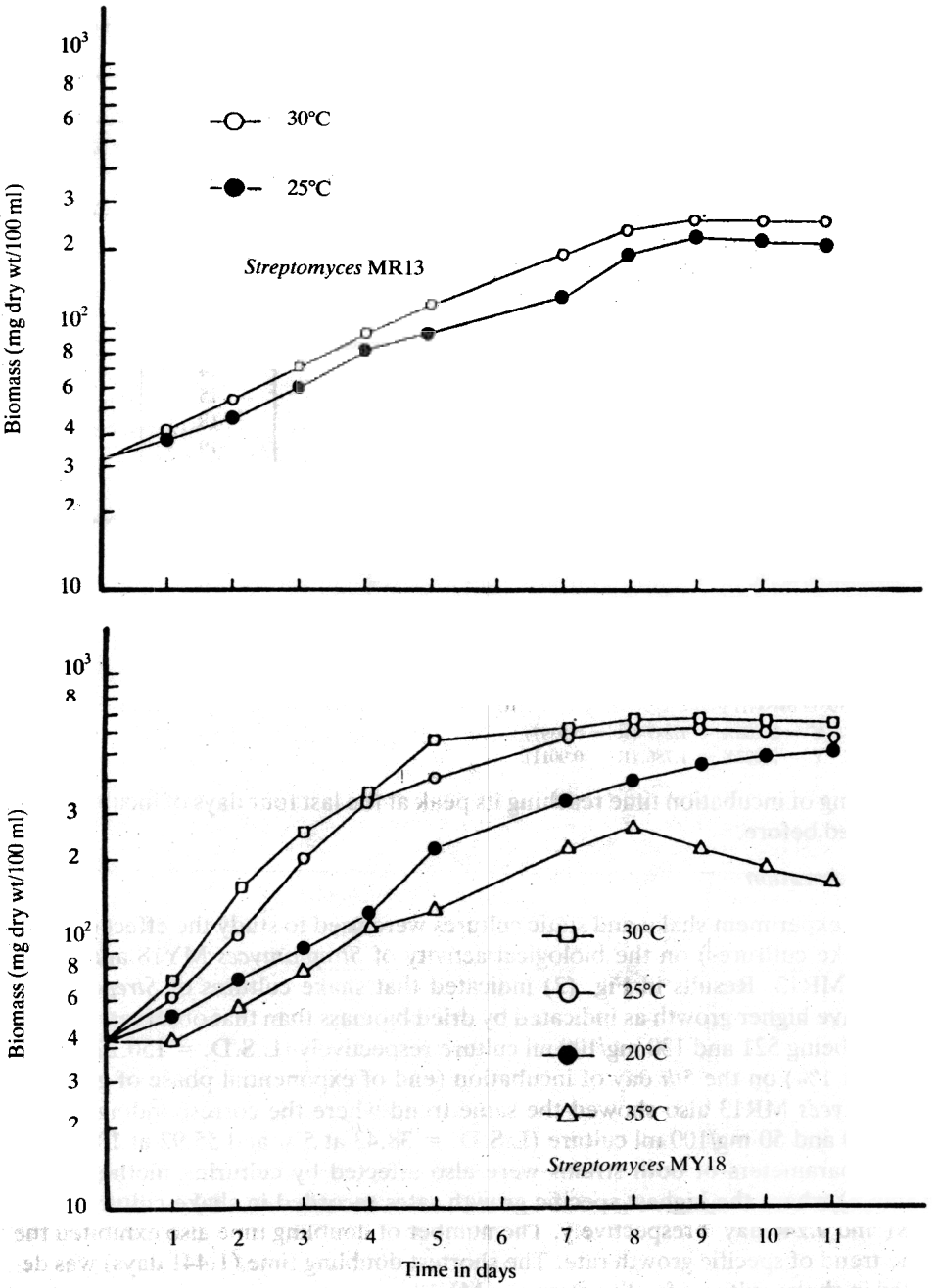
The highest specific growth rate and number of doubling time were also detected at 30°C for both strains as compared with other degrees of temperature being 0.507 and 0.249 day^{-1} specific growth rate for number of doubling time were 3.659 and 2.870 (Table 1).

With respect to the antibiotic activity in the cultural filtrate of both strains as influenced by temperature, results in Table (2) clearly showed that the highest antibiotic activity excreted in the culture was detected at 25 and 30°C where 28 mm inhibition zone was noticed for *Streptomyces* MY18 at 25 and 30°C for 11 days incubation. Other tested strain gave 20 mm inhibition zone at the same temperature and incubation time of MY18. On the other hand, the antibiotic activities were highly affected

TABLE 1. Some growth parameters of *Streptomyces* MY18 and *Streptomyces* MR13 as influenced by different degrees of temperature.

Strains	Growth parameters	Temperature						
		15°C	20°C	25°C	30°C	35°C	40°C	45°C
MY18	U	–	0.282	0.495	0.507	0.250	–	–
	t_d	–	2.458	1.400	1.367	2.773	–	–
	N	–	3.257	3.571	3.659	1.419	–	–
MR13	U	–	–	0.225	0.249	–	–	–
	t_d	–	–	3.081	2.784	–	–	–
	N	–	–	2.592	2.870	–	–	–

u = specific growth rate
t_d = doubling time
N = number of doubling times



L.S.D.	*5%*	*1%*
1. *5th* day for *Streptomyces* MY18	88.96	122.57
2. *8th* day for *Streptomyces* MR13	15.75	22.93

FIG. 1. Dried biomass of *Streptomyces* MY18 and *Streptomyces* MR13 as influenced by temperature.

TABLE 2. Antibiotic activity of *Streptomyces* MY18 and MR13 as indicated by zone of inhibition (mm) against *Staph. aureus* at different degrees of temperature.

Time in days	Zone of inhibition (mm)					
	Streptomyces MY18				*Streptomyces* MR13	
	20°C	25°C	30°C	35°C	25°C	30°C
0	0	0	0	0	0	0
1	0	0	0	0	0	0
2	0	13	14	12	0	0
3	12	18	17	14	0	12
4	14	20	19	15	12	14
5	16	21	20	16	14	15
7	16	24	23	17	15	17
8	17	25	24	17	18	20
9	18	27	27	17	19	20
10	18	27	28	17	20	21
11	20	28	28	16	20	20

Regression analysis of data :

A – *Streptomyces* MY18

1. 20°C $Y = 1.873X + 1.688$ ($R = 0.8927$).
2. 25°C $Y = 2.429X + 5.204$ ($R = 0.9040$).
3. 30°C $Y = 2.439X + 4.879$ ($R = 0.9161$).
4. 35°C $Y = 1.338X + 5.522$ ($R = 0.7746$).

B – *Streptomyces* MR13

1. 25°C $Y = 2.203X + 1.287$ ($R = 0.9397$).
2. 30°C $Y = 2.023X + 1.786$ ($R = 0.9011$).

by elapsing of incubation time reaching its peak at the last four days of incubation as mentioned before.

Effect of Aeration

In this experiment shake and static cultures were used to study the effect of aeration (shake cultrures) on the biological activity of *Streptomyces* MY18 and *Streptomyces* MR13. Results in Fig. (2) indicated that shake cultures of *Streptomyces* MY18 gave higher growth as indicated by dried biomass than that observed in static cultures being 521 and 130 mg/100 ml culture respectively (L.S.D. = 150.27 at 5%, 218.63 at 1%) on the 5*th* day of incubation (end of exponential phase of growth). *Streptomyces* MR13 also showed the same trend where the corresponding figures were 150 and 50 mg/100 ml culture (L.S.D. = 38.43 at 5% and 55.92 at 1%). The growth parameters of both strains were also affected by culturing methods used (Table 3) where the highest specific growth rates recorded in shake cultures were 0.481 and 0.246 day^{-1} respectively. The number of doubling time also exhibited the same trend of specific growth rate. The shortest doubling time (1.441 days) was detected in shake cultures for *Streptomyces* MY18.

The antagonistic effect of the culture filtrate of *Streptomyces* MY18 using *Staphylococcus aureus* as a test organism was highly affected by culturing methods.

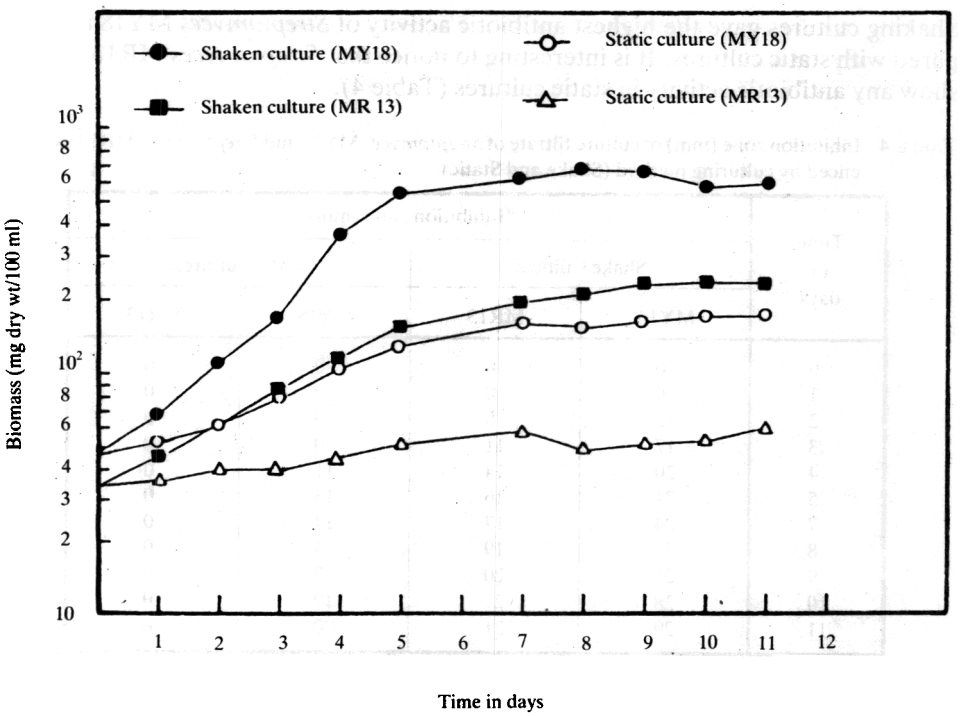


FIG. 2. Dried biomass of *Streptomyces* MY18 and *Streptomyces* MR13 as influenced by culturing methods.

L.S.D. on 5th day	*5%*	*1%*
1. *Streptomyces* MY18	150.27	218.63
2. *Streptomyces* MR13	38.43	55.92

TABLE 3. Growth parameters of *Streptomyces* MY18 and *Streptomyces* MR13 as influenced by culturing methods.

Growth Parameters	Shake cultures		Static cultures	
	Streptomyces 18	*Streptomyces* 13	*Streptomyces* 18	*Streptomyces* 13
Specific growth rate/day	0.481	0.246	0.203	0.071
Doubling time in days	1.441	2.818	3.415	9.763
Number of doubling times	3.471	2.485	1.468	0.515

Shaking cultures gave the highest antibiotic activity of *Streptomyces* MY18 as compared with static cultures. It is interesting to notice that *Streptomyces* MR13 did not show any antibiotic activity in static cultures (Table 4).

TABLE 4. Inhibition zone (mm) of culture filtrate of *Streptomyces* MY18 and *Streptomyces* MR13 as influenced by culturing method (Shake and Static).

Time in days	*Inhibition zone (mm)			
	Shake Culture		Static Cultures	
	MY18	MR13	MY18	MR13
0	0	0	0	0
1	0	0	0	0
2	15	0	0	0
3	17	11	0	0
4	20	14	11	0
5	21	16	13	0
7	24	17	14	0
8	23	19	14	0
9	27	20	17	0
10	28	21	19	0
11	29	21	20	0

Test organism : *Staphylococcus aureus*

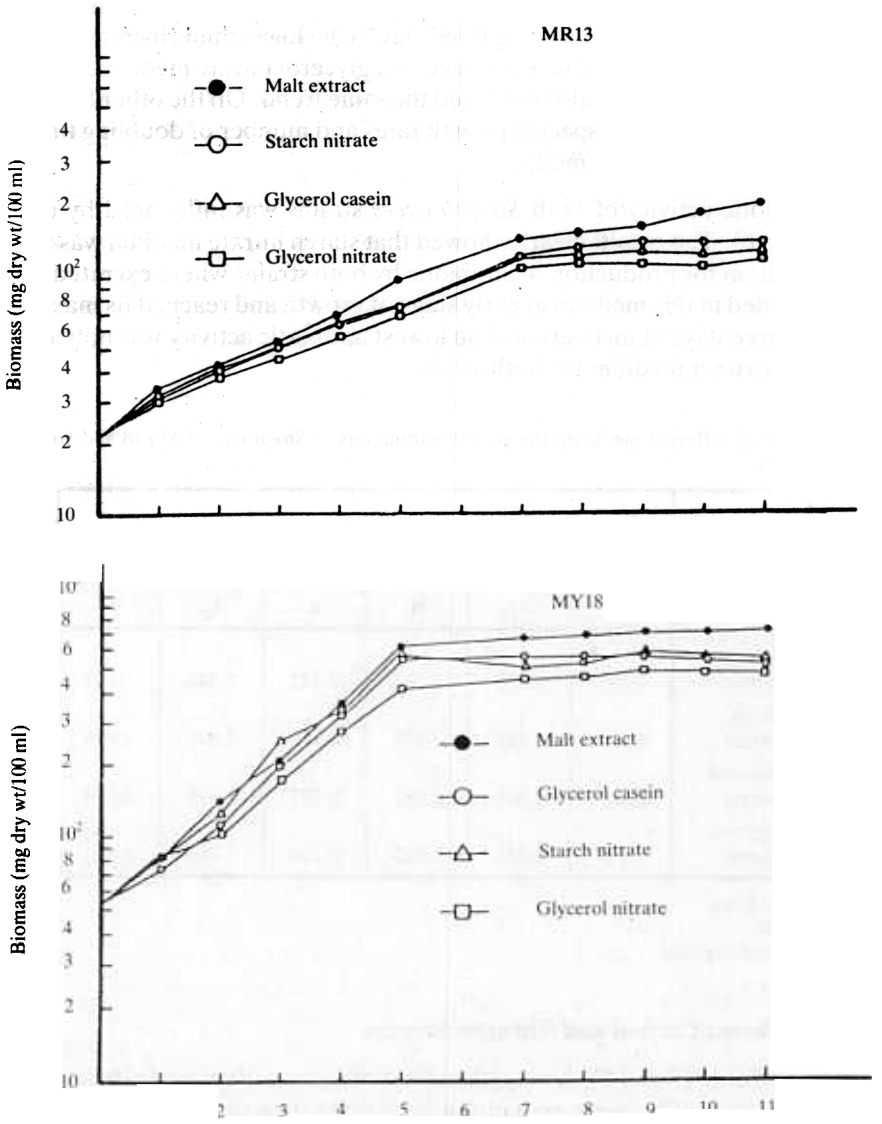
Regression analysis :

1. Shake Cultures :
   MY18 : Y = 2.433X + 5.27 (R = 0.9054)
   MR13 : Y = 2.123X + 1.06 (R = 0.9260)
2. Static Cultures :
   MY18 : Y = 2.052X – 1.38 (R = 0.9460)

Effect of Media

In this experiment, malt extract, starch nitrate, glycerol nitrate and glycerol media were used to cultivate *Streptomyces* MY18 and *Streptomyces* MR13. Biomass as indicated by dry weight of growth during time course of incubation was determined. Growth parameters and antibiotic activity of the cultures as influenced by different media were also observed. Figure 3 shows that the growth increased gradually with the increase of incubation period reaching its peak on the 5*th* day of incubation (end of exponential phase) for all tested media. The highest biomass productivity was obtained in malt extract medium where 720 mg dried growth/100 ml culture was recorded on the 11*th* day of incubation. Starch nitrate and glycerol casein media showed approximately the same quantity of growth, whereas the biomass was slightly lower in the case of glycerol nitrate medium as compared with other media.

The biomass of *Streptomyces* MR13 as influenced by different media also exhibited the same trend of *Streptomyces* MY18 except the growth of MR13 was lower than the other strain.



L.S.D.	*5%*	*1%*
1. *Streptomyces* MY18	30.74	42.20
2. *Streptomyces* MR13	5.49	7.56

FIG. 3. Growth curves of *Streptomyces* MY18 and *Streptomyces* MR13 as influenced by different media

Comparing the growth parameters of both strains, Table (5) shows that the maximum specific growth rate of *Streptomyces* MY18 was recorded during its propagation on malt extract medium being 0.497 day^{-1} (the logarithmic increase of growth per day), while the lowest value was noticed on glycerol nitrate medium. Number of doubling times of this strain also exhibited the same trend. On the other hand, *Streptomyces* MY18 gave higher specific growth rates and number of doubling times than the other strain on different media.

The antibiotic activity of both *Streptomyces* strains was influenced by different media (Table 6). The results clearly showed that starch nitrate medium was the most suitable medium for production of antibiotic by both strains where excreted antibiotic was recorded in this medium at early stage of growth and reached its maximum at the end of three days of incubation. The lowest antibiotic activity was noticed in the case of malt extract medium for both strains.

TABLE 5. Effect of different media on the growth parameters of *Streptomyces* MY18 and *Streptomyces* MR13.

Media	Growth Parameters					
	Streptomyces 18			*Streptomyces* 13		
	u	t_d	N	u	t_d	N
Malt Extract	0.497	1.395	3.581	0.272	2.548	2.747
Starch Nitrate	0.468	1.481	3.375	0.257	2.697	2.596
Glycerol Nitrate	0.429	1.616	3.092	0.237	2.925	2.389
Glycerol Casein	0.477	1.453	3.043	0.249	2.783	2.515

u = specific growth rate
t_d = doubling time
N = number of doubling times

Effect of Different Carbon and Nitrogen Sources

Results in Table (7 and 8) showed the effect of some carbon and nitrogen sources on the production of biomass and antibiotic activity. Starch is considered to be the best carbon sources for growth and excretion of antibiotic by both strains. The amount of growth was 601 and 308 mg/100 ml culture for *Streptomyces* MY18 and MR13 respectively. The corresponding figure for zone of inhibition were 30 and 20 mm. Peptone as a nitrogen source gave the highest growth for both strains whereas the maximum antibiotic activity was noticed in the case of nitrate being 28 and 20 mm respectively.

TABLE 6. Antibiotic activity of *Streptomyces* MY18 and *Streptomyces* MR13 as influenced by different media.

Time in days	*Inhibition zone (mm)							
	Malt extract		Starch nitrate		Glycerol nitrate		Glycerol casein	
	Streptomyces		*Streptomyces*		*Streptomyces*		*Streptomyces*	
	MY18	MR13	MY18	MR13	MY18	MR13	MY18	MR13
0	0	0	0	0	0	0	0	0
1	0	0	12	0	0	0	0	0
2	0	0	14	12	0	0	13	0
3	0	0	15	13	11	0	17	13
4	11	0	16	16	17	0	18	15
5	16	11	20	17	20	11	23	16
7	17	13	22	18	21	14	24	17
8	21	15	28	22	25	16	30	19
9	21	15	28	22	25	16	30	19
10	22	15	30	23	24	17	30	20
11	22	16	27	22	25	17	28	19
R	0.944	0.928	0.937	0.906	0.932	0.934	0.920	0.898

Test Organism : *Staphylococcus aureus*

TABLE 7. Effect of different carbon sources on the biomass and antibiotic activity of *Streptomyces* MY18 and *Streptomyces* MR13 after 11 days of incubation as batch cultures in shake flasks.

Carbon Sources	Growth (mg dry wt/100 ml culture)		Inhibition zone (mm)	
	Streptomyces MY18	*Streptomyces* MR13	*Streptomyces* MY18	*Streptomyces* MR13
Glucose	402	217	22	18
Fructose	259	163	16	11
Sucrose	172	95	15	12
Maltose	450	123	20	13
Starch	601	308	30	20
Glycerol	505	291	28	15

L.S.D.		*5%*	*1%*
MY18	1. Growth	97.67	133.20
	2. Inh. Zone	2.37	3.24
MR13	1. Growth	64.56	88.05
	2. Inh. Zone	2.90	3.95

Generally, it could be concluded that the growth of *Streptomyces* MY18 and *Streptomyces* MR13, growth parameters and antibiotic activity were highly affected by temperature, aeration and different media. *Streptomyces* MY18 showed higher

TABLE 8. Effect of different nitrogen sources on the biomass and antibiotic activity of *Streptomyces* MY18 and *Streptomyces* MR13 after 11 days of incubation as batch cultures in shake flasks.

Nitrogen Sources	Growth (mg dry wt/100 ml culture)		Inhibition zone (mm)	
	Streptomyces MY18	*Streptomyces* MR13	*Streptomyces* MY18	*Streptomyces* MR13
Peptone	711	451	20	16
Casein	619	405	25	15
Urea	120	25	12	0
KHO_3	517	301	28	20
$(NH_4)_2SO_4$	218	212	18	12

L.S.D.		*5%*	*1%*
MY18	1. Growth	55.49	75.20
	2. Inh. Zone	2.15	2.91
MR13	1. Growth	26.57	36.01
	2. Inh. Zone	1.24	1.68

temperature range (20 to 35°C) than that observed in the other strain (25 and 30°C). The optimum temperature for growth and antibiotic activity was 30°C. Minieri *et al.*[14] came to the same conclusion where they stated that the optimum range of temperature for growth of different strains of *Streptomyces aureofaciens* (ATCC 11652, 11653 and 11654) and *Streptomyces viridifaciens* was 25-30°C. They also reported that the optimum temperature for tetracycline production by these strains was 28°C. Mostafa *et al*[15] also reported that 25-30°C was the optimum range of temperature for production of antibiotics from *Streptomyces aureofaciens*.

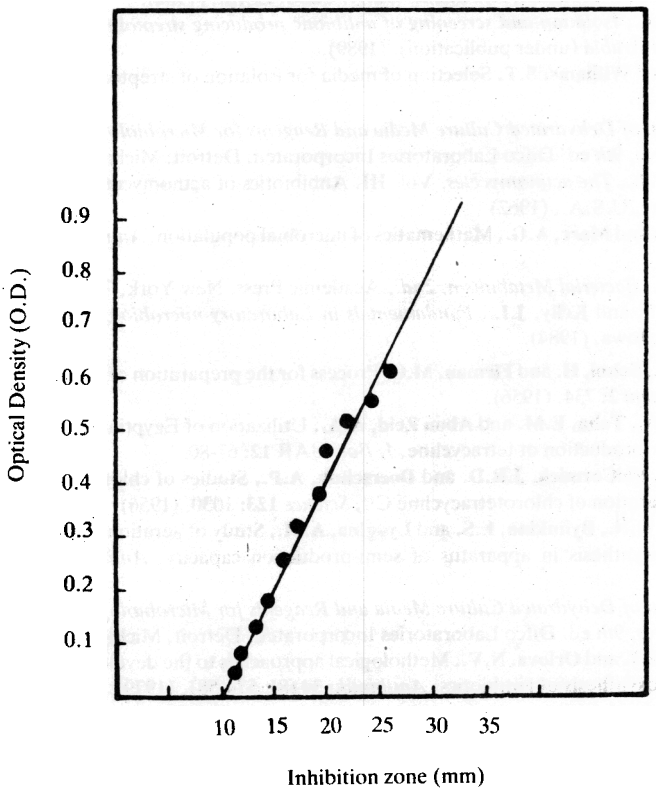
Results also revealed that the aeration (shake culture) supported growth and antibiotic activily of both tested strains than static cultures. It means that aeration is very important for metabolic activity of these strains. This confirms results obtained by Cherkasova *et al*[16] who reported that the accumulation of antibiotic depends on volume of the air supplied for aeration.

Supporting growth and antibiotic activity by starch was also reported by Miller *et al*[17] and Bryzgalova and Orlova[18] who stated that the starch is the best carbon source for antibiotic production by some strains of actinomycetes. Stimulatory effect was also recorded in the media containing KNO_3 as sole source of nitrogen. This result is in agreement with those obtained by Mostafa *et al*[15] and Mamonova and Orlova[19]. Osman[20] reported that the slow conversion of starch may be led to protect the mycelium from the depressive effect of carbon metabolite repression.

Relationship between the optical density of yellow pigment produced by Streptomyces MY18 and antibiotic activity

It was noticed that the golden yellow pigment produced by *Streptomyces* MY18 increased with the increase of incubation period. So, it was found valuable to study the optical density of this color at 920 nm (the highest peak was recorded at this wave

length) during incubation time course and its relation with antibiotic activity as indicated by inhibition zone (mm). Results in Fig. 4 showed that the increase of optical density of color was accompanied with the increase of inhibition zone, forming a straight line when the diameter of inhibition zone was plotted against the corresponding figures of optical density. It means that the optical density of color can be used as an indication of antibiotic activity for strain under investigation.



Regression analysis :

$Y = 32.60X + 6.09$
$R = 0.9252$

FIG. 4. Relationship between the optical density of yellow pigment produced by *Streptomyces* MY18 and antibiotic activity (Inhibition zone).

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العوامل المؤثرة على النشاط الحيوي لستربتوميسس أورفاشينس إم. واي-١٨ ، ستربتوميسس روزيفيولاشيس إم. آر-١٣

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قسم علوم الأحياء ، كلية العلوم ، جامعة الملك عبد العزيز
جـــدة ، المملكة العربية السعودية

المستخلص . درس في هذا البحث تأثير درجات الحرارة والتهوية والأوساط الغذائية المختلفة ومصادر الكربون والنيتروجين على النشاط الحيوي لسلالة ستربتوميسس أورفاشينس إم. واي-١٨ ، ستربتوميسس روزيفيولاشيس أم. آر-١٣ . وقد وجد أن الكتلة الحيوية ومعايير النمو ونشاط المضادات الحيوية قد تأثرات كثيرا بهذه العوامل . وأظهرت السلالة الأولى نطاقًا حراريًا أعلى من السلالة الثانية ، وكانت درجة الحرارة المثلى للنمو وإنتاج المضاد الحيوي للسلالتين هي ٣٠°م . وأدت المزارع المهتزة أيضًا إلى زيادة النمو والمضاد الحيوي عن المزارع الساكنة ، كما كان لكل من النشا والنترات تأثير مشجع لإنتاج المضادات الحيوية في راشح مزرعة كل من السلالتين . ووجد أيضا أن زيادة الكثافة الضوئية للصبغة الصفراء الذهبية التي تنتجها السلالة الأولى (السلسلة الرمادية) في المزرعة تؤدي إلى زيادة نشاط المضاد الحيوي والتي يمكن استخدامها كدليل على تركيز المضاد الحيوي ونشاطه .