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Effect of Some Physical Factors on Natural Biosynthesis by *Streptomyces* spp

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Author's contribution

This whole work was carried out by the author NEN.

Original Research Article

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ABSTRACT

Aims: This study was performed to detect the effects of certain environmental conditions mostly physical ones on the biosynthesis of secondary metabolites produced by *Streptomyces* spp.
Study Design: Interventional study.
Place and Duration of the Study: Department of biology, College of science, Al-Mustansiriya University, Baghdad ,Iraq between February 2012 and December 2012.
Methodology: eight pure *Streptomyces* isolates, that produce antibiotics ,were exposed to different ranges of temperature, pH, aeration and radiation to elicit the optimum physical conditions for the biosynthesis of their secondary metabolites by estimating the diameter of inhibition zone of the growth on an isolate of *E. coli* on the nutrient broth and nutrient agar media after it`s inoculation with these products compared to control ones.
Results: The optimum growth temperature, pH, and aeration for the growth of *Streptomyces* were (28-32)ºC, (6.5-7.5) and 250 RPM respectively. Exposure to radiation for a period of 10 minutes had a lethal effect on *Streptomyces* but various responses were noticed upon exposure to radiant for less than 5 minutes depending on the species kind.
Conclusion: The production of antibiotics as a secondary metabolites by *Streptomyces* species could be enhanced by optimizing the physical factors affecting their growth like Temperature, pH, aeration and radiation.

Keywords: Streptomyces; temperature; pH; aeration; radiation.

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1. INTRODUCTION

Streptomyces are considered the largest genus of Streptomycetaceae family. More than 500 species of *Streptomyces* were discovered [1,2]. They are Gram positive bacteria, found in the soil and plant wastes [3]. It has earth smell, that produces volatile metabolites, [4,5,6]. These bacteria characterized by their complex metabolites [7]. Several species of *Streptomyces* are among the most important industrial microorganism because of their capacity to produce numerous molecules particularly antibiotics and two third of the total antibiotics used for medical application are produced by them [8,9,10,11,12]. As other bacteria, *Streptomyces* are very sensitive to physical factors, such as temperature, pH, aeration, ultrasonic vibrations, ultra violate and ionizing radiation which damage the ionizing components of the microbial cell structure, or reduce the potential of microbial activity and eventually may lead to cell death, [12,13]. The yield of antibiotic has been increased tremendously by media and physical factor optimization [3]. The aim of the present study is to evaluate the effects of some environmental physical factors on the natural biosynthesis activities of *Streptomyces*.

2. MATERIALS AND METHODS

2.1 Study Design and Setting

An experimental study had been conducted at 5 governorates of the northern region of Iraq Erbil, Sulymania, Kurkuk, Mosule and Duhoke for the period from the 1st of February to the 30th of December 2012.

2.2 Sampling

Eight pure cultures of *Streptomyces* spp. that isolated from soil in Iraq were applied by Nazar & Hadi [14], and numbered as: A, B, C, D, E, F, G and H. These strains were chosen according to their inhibition activity of pathogenic *E. coli*, that isolated from hospitals and diagnosed according to the characteristics of their colonies, growth behavior and preliminary identification, that depended on the following:

- Physiological and biochemical characteristics by using API-20E (bioMerienx, Marcy I' Etoile, Paris, France)
- Using related reference [15]

2.3 Culture Media

Gauze no.1 broth medium was used for refreshing the *Streptomyces* isolates at 28ºC [14]. These strains were then used to study the effect of physical factors on their ability for the production of antibiotics. Nutrient agar and nutrient broth media (Oxoid) were used for cultivation of pathogenic *E. coli*. The nutrient media were sterilized by autoclave at 121ºC, 15min., 15 P/in^2, subsequently *E. coli* cultures were prepared. 500ml of Gauze no.1 were poured in eight 1L conical flasks. These flasks were then inoculated with *Streptomyces* isolates by inoculation loop [16] and kept in an orbital shaker previously adjusted at 250 RPM and 28ºC for 10 days.

Control sample included 20ml of Gauze no.1 but without *Streptomyces* inoculums was added to the Petri dish that previously contain nutrient medium and inoculated with *E. coli* using agar diffusion method.

All replica of *E. coli* cultures; those with *Streptomyces* isolates and control sample were kept in an incubator at 37ºC for 24hr.

2.4 Effect of Physical Factors

2.4.1 Temperature

From each culture of study isolates , 0.5ml of inoculums was taken and added to 25ml of Gauze no.1 medium, these constituents were poured in 8 (150ml size) conical flasks and kept in an orbital shaker, previously adjusted at (18 , 27 , 28 , 32 , 36 and 40)ºC, for 10 days at 250 RPM, then 0,25ml were taken from each flask and added to Petri dish, that contained freshly prepared bacterial lawn of *E. coli* on nutrient agar. The Petri dish were kept in an incubator, adjusted at 37ºC, for 24h. In the next day the diffusion on agar was investigated .

2.4.2 pH

Twenty five ml aliquots of Gauze no.1 medium were poured in ten conical flasks (150ml in size) and adjusted to the following initial pH 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8 and 8.5. These flasks were inoculated with 0.5ml of *Streptomyces* cultures and were placed in a shaker incubator which was adjusted at 250 RPM and optimal temperature for the growth of each isolate (as illustrated in Table 1), then incubated for 10 days . After incubation period, 0.25ml aliquots were taken from each flask and added to the *E*. coli lawn as illustrated in section 2.4.1.

2.4.3 Aeration

Twenty five ml aliquots of Gauze no.1 broth were dispensed in 48 conical flasks of 150ml size and inoculated with 0.5ml of *Streptomyces* culture and kept in orbital shaker that adjusted to the following RPM: zero, 50, 100, 150, 200 and 250 respectively for ten days using the optimal pH and temperature of growth for each isolate according to Tables 2 and 3. 0.25ml of each isolate was added to test tube that already contained 10ml of nutrient broth and inoculated with *E. coli*, then these test tubes were incubated at 37ºC for 24h. On the other hand the control samples were contained nutrient broth and inoculated with *E. coli* only.

Spectrophotometer was used to detect the optical density (O.D.) that reflects the intensity of bacterial growth. Low O.D. values compared to control samples (i.e. *E. coli* without *Streptomyces*) indicate reduction in the production of *E. coli* biomasses due to *Streptomyces* metabolites that interfere with the growth of *E. coli*.

2.4.4 Ultra violet radiation

Twenty five ml aliquots Gauze no.1 medium were added to 72 (150ml in size) flasks, eight for each isolate and eight for control sample. These flasks were inoculated with 0.25ml of *Streptomyces* culture and then exposed to ultraviolet light (except control sample) of 260nm at 5cm distance and for the following times (in minutes): 1, 2, 3, 4, 5, 10, 20

and 30 respectively. The flasks were then kept in the incubator at optimal pH, temperature, aeration for each isolate and according to the parameters mentioned in Tables 2, 3 and 4 and for 10 days. 0.25ml sample was taken from each flask and transferred to Petri dish that contain *E. coli* lawn on nutrient agar and kept in incubator at 37ºC for 24h.

3. RESULTS

Some of the physiological and morphological characteristics of *Streptomyces* isolates used in this study are shown in Table 1, beside it`s inhibitory effect on the growth of *E. coli* on nutrient agar had been assessed by measuring the diameter of inhibition zone to confirm their production for antibiotics.

Table 1. Some physical and morphological characteristics of *Streptomyces* isolates of this study

No. of isolate	Growth on gauze no. 1 medium*	Color of aerial mycelium	Color of substrate mycelium	Inhibition zone (mm) in *E. coli* growth on nutrient agar**
A	++++	PINK	BROWN	12.5
B	++++	PINK	BROWN	12
C	++++	PINK	BROWN	11.5
D	++++	WHITE	BROWN	11
E	++++	GRAY-GREEN	CREAM	11.5
F	++++	WHITE PALE	BROWN	11
G	++++	WHITE	BROWN	13
H	++++	WHITE	BROWN	11.5

** ++++: excellence bacterial growth*

*** Growth conditions were 37^{0}C and pH 7.2 for 24 hr. incubation*

3.1 Temperature

It was noticed that there were variable diameter of inhibition zones of *E. coli* due to various concentration of antibiotics produced by *Streptomyces*. The results indicated temperature had played a role in the biosynthesis of antibiotics compared to the control, the sizes of diameters of inhibition zones varied between the isolates. The optimum temperature for the production of antibiotics for the isolates A, B, C, D, E, G and H was 28ºC. While 32ºC was the optimum temperature for isolate F (Table 2), that indicated the effects of temperature on the enzymes that are contributed in the metabolic pathways for antibiotics synthesis.

3.2 pH

The value of pH of cultivating media found to have a tremendous effects on the process of natural biosynthesis of antibiotics produced by *Streptomyces* (Table 3). The results revealed that the diameter of inhibition zone of *E. coli* have increased gradually and then begun to decrease in relation to increasing the pH value (from 4 to 8.5) .The optimum pH, that expressed as the biggest inhibition zone, found to be 7 for the isolates A, B and C, while it was 6.5 for the isolates G and H and 7.5 for the isolate D. On the other hand, the optimum pH for the isolates E and F was between 6.5 to 7. Accordingly the optimum pH is fluctuating between 6.5 and 7.5 depending on the type of *Streptomyces* isolate.

Table 2. The effects of incubation temperature on the quantities of the antibiotics formed by the studied *Streptomyces*

No. of the isolates	Diameter of inhibition zone (mm) of *E. coli* growth on nutrient agar					
	18^0C	22^0C	28^0C	32^0C	36^0C	40^0C
A	9	12.5	15	8	0	0
B	2	10	14.5	12	9	3
C	0	8	14	11	9	4
D	11	14	16	13	0	0
E	12	14	15.5	12.5	8	4
F	10	14	14.5	15	5	0
G	0	9	15.5	11	9	2
H	8.5	14	16	8	0	0

Table. 3 The influence of graduated values of pH on the production of antibiotics by *Streptomyces* studied isolates

No. of the isolates	Diameter of inhibition zone (mm) of *E. coli* on nutrient agar pH									
	4	4.5	5	5.5	6	6.5	7	7.5	8	8.5
A	0	0	0	0	9.5	14	18	16	12	8
B	0	0	9	12	15	16	18	14.5	9	0
C	0	0	4	5	11	17	19.5	7	0	0
D	0	0	8	10	14	18	19.5	20	19	8
E	0	0	8	11	12	18	18	12	9	7
F	0	7	10	10	15	20.5	20.5	16	9	0
G	0	0	9	14	17	19.5	16	12	8	0
H	0	0	8	13	16	20	18	11	6	0

3.3 Aeration

It has been reported that large quantities of bioproducts could be obtained by using orbital shaker, particularly enzymes and antibiotics that have inhibitory effects on the bacteriological growth. This would lead to reduction in the biomass production compared to the control samples [5,12].

The results that illustrated in Table 4 indicated that the best growth was obtained in control sample, while it was reduced in the studied sample due to the presence of bioproduct in the studied isolates, that inversely proportioned with the number of revolution per minute of the shaker.

The values of O.D. were nearly similar for all the studied isolates at RPM of zero, but it started to be reduced at a percentage between 25 to 50% when the RPM was 250, subsequently the reported O.D. for isolate A was 0.3, for isolates B, E, G, H was 0.4, for isolate C was 0.5 and for isolates D, F was 0.6 at RPM 250. The overall results revealed that RPM of 250 was the best for the production of antibiotics for all the studied isolates.

3.4 Ultra Violet Radiation

Two replicates were used for the studying the effect of ultra violet radiation on the production of antibiotics, therefore, the mean values were considered. Effects of ultra violet radiation exposure on the studied isolates were changed depending on the time of exposure. At the first 5 minutes (1, 2, 3, 4 & 5)min. were nearly equal to the control samples except isolates D & H, whose zone of inhibition increased at 3 minutes of exposure.

Table 4. The Influence of different rate of oxygen supply for studied isolates and their abilities to production of antibiotics

No. of the isolates	O.D. of *E. coli* with studied isolates in nutrient broth at various RPM						O.D. in control (*E. coli* alone)
	0	50	100	150	200	250	
A	1.2	0.9	0.7	0.5	0.4	0.3	1.4
B	1.0	0.8	0.7	0.6	0.5	0.4	1.4
C	1.1	0.9	0.8	0.7	0.6	0.5	1.5
D	1.2	0.9	0.8	0.8	0.7	0.6	1.4
E	1.4	1.3	0.9	0.7	0.65	0.4	1.5
F	1.1	0.9	0.8	0.8	0.7	0.6	1.5
G	1.4	1.0	0.9	0.8	0.4	0.4	1.4
H	1.1	1.0	0.9	0.7	0.4	0.4	1.4

The result, also illustrated that upon exposure to ultra violet radiation for extended times (i.e. 10, 20 and 30) minutes led to a reduction in the zone of inhibition (at 10 and 20) minutes and sometimes they reached to zero zone of inhibition as in isolates B, C and G, while, for all isolates, the zone of inhibition were zero upon exposure to 30 min. as demonstrated in Table 5.

Table 5. The effect of exposure of ultraviolet radiation on the studied isolates at different interval

No. of the isolate	Control (Diameter of inhibition zone mm, without exposure to U.V.)	Diameter of inhibition zone (mm) after exposure to U.V. in different interval period (Minutes)							
		1	2	3	4	5	10	20	30
A	19	19	19	19	19	18.5	18	18	0
B	18.5	18	18	18	18	18	18	0	0
C	19.5	19.5	19	18.5	18.5	18	17.5	0	0
D	19.5	20	20	25.5	20	19.5	19.5	19.5	0
E	18	18	18	18.5	18	19	18.5	18	0
F	20.5	20	20	20.5	20.5	21	20.5	20	0
G	19.5	19	19	19	19	18.5	18.5	0	0
H	20	20.5	20.5	29.5	22.5	20	20	19.5	0

4. DISCUSSION

In general, it has been noticed that all *Streptomyces* isolates were able to produce many secondary metabolites, including antibiotics with different compounds that are metabolically active. From this point of view, *Streptomyces* considered as a rich source for biopharmaceutical products, e.g. amino acids, sugar, bacteriocines and aminoglycosides etc. that contribute, in the production of more complex compounds in biochemical pathways [3,6].

Physical conditions ;specifically temperature, pH, aeration and ultraviolet radiation affect the natural biosynthesis of antibiotics among the secondary metabolites of the vegetative cells of *Streptomyces*.

4.1 Temperature

There is an optimal temperature for the activity of each enzyme in all forms of life and any change in this temperature leads to a reduction in certain activity for that organism. At optimum temperature there is no possibility for any change in the nature of protein. Therefore, at 37ºC many enzymes can work without any changes in their nature. The kinetic energy of essential molecules photons change to static energy. The change is affected by the surrounding environmental conditions, e.g. change in temperature system that elevates molecules collision with respect to time. This will lead to the elevation of molecules temperature, and result in the increasing of reaction rate , and when the temperature is high enough, i.e. energy of reaction of the reactants higher than the energy of the products, the energy will consumed for reorientation of the chemical state of the products, [17]. Enzymes that are protein in nature , play a role in the acceleration of biodegradation of the reactants to the products through the reduction in reaction energy of the reactants, i.e. by breaking chemical bonds, therefore, chemical reactions that produce heat and have reaction energy (and by this reduction) will lead the chemical compounds to reorientation their structure by the activity of the enzymes. Therefore optimum temperature for enzyme activity is a temperature that make the molecules of the reactants to desolate (due to increase their higher energy) before the beginning of the effect of temperature on the denaturation of the enzymes, therefore, each enzyme have it's optimum temperature that assign it's highest activity, that will reduce upon change of this temperature. Enzymes denaturation by high temperature means that the active site of enzyme has changed irreversibly , this lead to that the reactants become in a position which is unable to continue it's fitting with the enzyme complex that end in reactant dissolution, [17]. The role of enzymes in the cells are for the acceleration of biochemical pathways which include the production of secondary metabolites, particularly antibiotics, therefore there is direct relationship between temperature and the degree of antibiotic concentration on one hand and it's activity of inhibition on bacterial growth. All the isolates in this study showed optimum production of antibiotics at 28-32ºC, yet Some of them continue to have antibiotic effect even at 36-40ºC but of a lower magnitude, while at 41ºC no inhibition activity was reported for any of them [18].

4.2 pH

Hydrogen ions concentration in the media is important factor due to it's role in molecule movement through the cell membrane [19]. These ions choose organic molecules through controlling of the molecules movement in and out of the cell [20]. The direct effect of pH

lead to diffusion across the cell membrane. This may lead to microbial inhibition. The value of pH in the protoplasm is constant, but acidity and alkalinity of the compounds in the medium varied according to it's composition. It should be suitable for protoplasm pH. In general, it is considered as neutral, but when the ions are more or less than the normal condition, it leads to the reduction in the permeability, or it may be stopped and results in cell death [21]. Therefore the cells have it's limit for tolerance of pH values and optimum pH fall within this limit , and consequently the cell is able to produce primary secondary metabolites including enzymes and antibiotics, etc. Therefore, the activity of enzyme, are varied according to the pH value in the growth medium and thereafter pH value for each enzyme [22].

4.3 Aeration

Atmospheric oxygen diffusion depends on the surface area of the liquids. A large surface area and agitation process help in supplying of O_2 to the organism for continuing respiration process in liquid media. The orbital shaker rotate circularity, that help in atmospheric oxygen supply for the culture in the liquid medium. This situation is better than stagnant cultures. Moving medium increase the chance of nutrient molecules collision with the microorganisms, therefore, these organisms grow better and consequently lead to active metabolic activities for build-up the biomass and therefore formation of secondary metabolites [5].

4.4 U.V. Radiation

The energy of radiation wave absorption is variable. It depends on the duration of exposure and the quantity of radiation. This energy are able to be absorbed by some parts of cell constituents e.g. ribosomes, chromosomes etc. [23]. This absorption lead to wrong configuration of the molecules and may results in cell death [24]. Such changes in cells may not always cause cell death , but it may reduce it's activities and sometimes may lead to increment in their ability for the production of certain metabolites. It have been reported that the application of radiation energy on the chromosomes of microorganisms results in the increment in their certain metabolites [23], but sometimes increasing of radiation dose may kill the microorganisms, due to the tremendous ionic change that overcome the tolerance ability of the organism.

5. CONCLUSION

Streptomyces spp. are potential microbes for secondary metabolites production, and various environmental factors have significant effects on their growth and biomass production. In this study the optimum conditions for maximum production of bioactive compounds of *Streptomyces* bacteria found to be at Temperature (28-32) Cº, pH (6.5-7.5), aeration (250) RPM and radiation 260 nm. This optimized cultural conditions might be implemented in large scale for the production of secondary metabolites by *Streptomyces* species

COMPETING INTERESTS

Author has declared that no competing interests exist.

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