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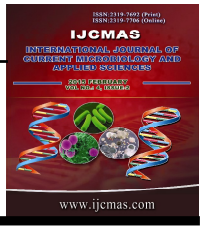

Original Research Article

Enzymatic Activities of some Streptomycetes Isolated from Soil at Taif Region

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A B S T R A C T

This study was aimed at isolating, purifying and identifying some streptomycetes from soil of Taif region having enzymatic activities. Some streptomycete isolates were isolated from soil in Taif and their enzymatic activities and optimum conditions for producing the selected enzyme were studied. A number of 23 of streptomycetes-like isolates (SLI) were isolated from different locations (Al-Qaiem, Al-Qamaryiah, Al-Sail Al-Kabeer, Al-Sail Al-Sagheer, Al-Sawtah, Gabrah (1), Gabrah (2), Tarabah, Qaiah and Wadi-Bowa) at Taif region of KSA. All isolates were found to produce amylase. A number of 17 (73.9%) out of the 23 purified isolates showed excellent growth on starch casein agar medium. In case of cellulase, lipase and gelatinase, the growth was 15 (65.2%), 21(91.3%) and 17 (73.9%), respectively, and were active. A number of three isolates representing the highest active isolates (10, 12 and 13), produce all the tested enzymes, and were selected for identification. The isolate 10 was identified as a strain of *S. lateritius*. The *Streptomyces* isolate 12, was very likely to be a strain of *S. cacaoi* subsp. *asoensis*. The tested isolate 13 was closely related to *S. phaeopurpureus*. Data showed that amylase enzyme production was affected by different culture conditions. It was observed that the optimum zone of hydrolysis in mm was obtained at pH 7.0 and temperature 30°C with the incubation period of 6.0 days.

Keywords

Streptomycetes, Taif, Enzymatic Activities, Identification, Amylase Enzyme Production

Introduction

Streptomyces species were found worldwide in soil and were important in soil ecology (Salahuddin *et al.*, 2011). Much of the characteristic earthy smell of soils arises by *Streptomyces* species (Mohamed Sonya *et al.*, 2001). Enzymes are the most important products for human needs in the area of industrial, environmental and food technology through microbial sources Mohamed Sonya (Boing, 1999; Gurudeeban *et al.*, 2011). Streptomycetes are considered as one of the most important bacteria, due to their ability to develop the soil properties as well as producing several extracellular

substances (enzymes) as secondary products (Gandolfi *et al.*, 2000; Techapun *et al.*, 2002; Brühlmann *et al.*, 1994; Jang and Chang, 2005; de Lima *et al.*, 2005; Soares *et al.*, 2006; Saadoun *et al.*, 2007; Altalhi and Mohamed Sonya, 2010; Gulve and Deshmukh, 2011; Mohamed *et al.*, 2013; Mohamed Sonya *et al.*, 2014). About three-fourths of the *Streptomyces* species may produce antibiotics (Gupta *et al.*, 1995). The possibility of using streptomycetes for enzyme production had recently been investigated (Yang and Wang, 1999). A serine protease from the keratin-degrading *Streptomyces pactum* DSM 40530 was purified by casein agarose affinity chromatography. The enzyme was optimally active in the pH range from 7 to 10 and at temperatures from 40 to 75^{o}C (Böckle *et al.*, 1995).

Sixty different strains of *Streptomyces* were tested for their enzymatic hydrolysis of cellulose and related materials. They found that the most active three strains were *S. mutabilis*, *S. chromofuscus* and *S. cyanoviridis* (El-Nasser and El-Shafei, 1994).

Pectinases are today one of the upcoming enzymes of the commercial sector (Kashyap *et al.*, 2001). It was proved that such species of bacteria produces several enzymes such as: Amylase, cellulase, pectinase, chitinase, and protease, etc. Five Streptomycetes obtained from Sinai sandy soil in Egypt, were identified based on the biological properties of Streptomycetes in the Key proposed by Pridham and Tresner (Pridham and Tresner, 1974). Results of the cultural, morphological and physiological characteristics showed that *Streptomyces* isolate Si-1 was a novel violet species. Four isolates were identified as *S. tuirus* Si-4, *S. lateritius* Si-6, *S. mauvecolor* Si-9 and *S. melanogenes* Si-11.

The present study was designed to isolate and purify some Streptomycetes from soil of Taif region, to determine the enzyme activities of the purified isolates, and identify the selected isolates up to species based on their cultural, morphological and physiological characters. Some factors affecting the activity of an enzyme was also studied.

Materials and Methods

Some soil samples were randomly collected from five sites of different regions of Taif. Plate technique as described by Mohamed Sonya (1998) was applied using starch nitrate agar medium (Waksman and Lechevalier, 1961) for isolation, purification and maintenance of Streptomycete-like colonies (SLC). Colonies were maintained on starch nitrate agar slants at 4-5^{o}C until used.

Determination of Enzymatic Activities of Streptomycetes Isolates

In these experiments, SLI were screened for their potential to produce fiber hydrolytic enzymes, *i.e*., amylases (Shaw and Ou-Lee, 1984; Santos and Martins, 2003), cellulases (Carder, 1986; Sharma *et al.*, 2001), lipases (Tendler and Burkholder, 1961) and gelatinase (Stefka *et al.*, 2004). In case of proteases, plates of skimmed milk casein agar medium were separately inoculated with each pure SLI, and incubated at 30^{o}C±2 for five days. The appearance of clearing zones around colonies indicated the presence of proteolytic activity by these isolates due to hydrolysis of casein.

Identification of selected Streptomycetes up to species

Identification of the highest active Streptomycete isolates in producing hydrolytic enzymes were subjected to

identification up to species according to the proposed key of Pridham and Tresner (1974) in Bergey's Manual of Determinative Bacteriology (1974). Media as well as methods used in these keys were described by Shirling and Gottlieb (1966). Identification based on cultural, morphological and physiological characters, growth on Czapek-Dox agar medium; Sensitivity to streptomycin and antibiosis activities of the selected streptomycetes were carried out as exactly described by Mohamed Sonya (1998).

Factors Affecting Amylase Activity of *Streptomyces* Isolate

To determine the effect of different culture conditions on α-amylase enzyme production, three experiments were conducted using starch nitrate agar medium (Waksman and Lechevalier, 1961). Regarding the effect of incubation temperatures, plates of the normal agar medium were inoculated with growth discs of the selected Streptomycete isolates. The inoculated plates were incubated at each of 25, 30 and 35^{o}C for 2 days. In case of incubation period, inoculated plates were incubated at 30^{o}C for 2, 4 and 6 days. To detect the effect of pH on enzyme production, the same medium was prepared with different pH degrees (5.0, 6.0, 7.0 and 8.0). The inoculated plates were incubated at 30^{o}C for two days. Three plates were used as replicates for each treatment. All plates were flooded with iodine solution and left for 30 min at room temperature (RT) and then washed with distilled H_2O. A clear white-yellowish zone around the growth in blue medium indicated hydrolysis of starch, and confirmed production of α-amylase enzyme (Shaw and Ou-Lee, 1984).

Results and Discussion

Isolation, purification and maintenance of Streptomycetes-like isolates from soil at Taif region

Filamentous actinomycetes which formed distinctive different colored colonies were isolated from different soil samples (Mohamed Sonya, 1998; Duangmal *et al.*, 2005; El-Sherbiny, 2006; Ismail, 2006; Al-Askar *et al.*, 2011; Atta *et al.*, 2011; Mohamed Sonya *et al.*, 2012; Shori Ghadeer *et al.*, 2012).

Data in Table 1 and illustrated by Figure 1 show that a few numbers (23) of SLI were isolated from different locations (AlQaiem, Al-Qamaryiah, Al-Sail Al-Kabeer, Al-Sail Al-Sagheer, Al-Sawtah, Gabrah (1), Gabrah (2), Tarabah, Qaiah and Wadi-Bowa) at Taif region of KSA. Numbers of 1, 1, 1, 1, 1, 3, 2, 4, 6 and 3 isolates were purified from those locations, respectively. It worth to mention that a total number of 40 SLI were isolated, but only, 23 out of them was clearly purified.

Determination of enzymatic activities of the purified SLI

Data in **Table (2**) and illustrated by **Figures (2), (3), (4), (5)** and **(6)** showed that the 23 purified SLI were tested for their abilities to hydrolyze starch, oil, casein, cellulose and gelatin as mentioned in the materials and methods. All isolates were found to produce amylase as 100% of the tested isolated showed a white-yellowish hallo around the growth when the plates of starch nitrate agar medium inoculated with the SLI were flooded with iodine. While, 17 (73.9%) out of them showed excellent growth on starch casein agar medium. In case of cellulase, lipase, gelatinase and 15 (65.2%), 21(91.3%) and 17 (73.9%), respectively, SLI, were found to be able to produce the mentioned enzymes. A number of three isolates representing the highest active isolates (10, 12 and 13), produce all the

tested enzymes, and were selected for identification. Several investigators reported the importance of streptomycetes as one of the most important bacteria, due to their ability to develop the soil properties as well as producing several extracellular substances (enzymes) as secondary products (Mohamed Sonya, 1998; Kluepfel *et al.,* 1986; Sztajer *et al.,* 1988; Bormann *et al.,* 1993).

In addition their abilities to analyze the organic matters *via* producing some enzymes which convert the plant dry matters to easy materials can be metabolized by plant. It was proved that such species of bacteria produces several enzymes such as: amylase (Mohamed Sonya, 1998), cellulose (Techapun *et al.,* 2002; Jang and Chang, 2005; Techapun *et al.,* 2003), proteinase (Böckle *et al.,* 1995), chitinase (Gomes *et al.,* 2000), lipase (Gandolfi *et al.,* 2000; Jaeger and Eggert, 2002).

Identification of the Highest Enzymatic-Effective Streptomycetes up to Species

Streptomyces Isolate 10

Data in Table 3 show that the *Streptomyces* isolate 10 belonged to the red colour series, with white vegetative mycelium. This isolate had spiral spore chain with warty surface (Figure 7). Melanoid pigments were produced, and excellent growth on Cazpek's agar medium was recorded.

It actively utilized 6 out of the 8 carbon sources used (D-glucose, D-xylose, L-Rhamnose, D-fructose, i-inositol and sucrose) as sole carbon sources for growth. The isolate showed antibacterial and antifungal activities. However, no growth was observed in the presence of 4 $\mu g ml^{-1}$ streptomycin antibiotic in the medium. This isolate was tolerant to NaCl up to concentration of 7%. According to the key proposed by Pridham and Tresner (1974), the experimental isolate 10 appeared to be related to *S. lateritius* as demonstrated although there were differences in the use of 3 sugars as sole carbon sources (L-arabinose, i-inositol and Sucrose). Therefore, isolate 10 could be considered a strain of *S. lateritius*.

Streptomyces isolate 12

Results presented in Table (4) clearly indicate that the *Streptomyces* isolate 12 belonged to the gray colour series. Aerial spore chains belonged to section RF (Figure 8); the spores were characterized by smooth surface (Figure 8). Melanoid pigments were detected on the standard media used. This isolate was characterized by excellent growth on Cazpek's agar medium.

The physiological characteristics showed that D-glucose, D-xylose, L-arabinose, L-rhamnose and D-mannitol, D-fructose, i-inositol and sucrose were used as carbon sources for growth. In addition, this isolate showed antibacterial and antifungal activities and sensitivity to streptomycin (4 $\mu g ml^{-1}$) was observed. However, it was able to grow in the presence of 7% NaCl in the medium. Comparing the cultural, morphological and physiological characteristics of the *Streptomyces* spp. in Pridham and Tresner (Pridham and Tresner, 1974) with those of *Streptomyces* isolate 12, this isolate is very likely to be a strain of *S. cacaoi* subsp. *asoensis*.

Streptomyces isolate 13

Results of *Streptomyces* isolate 13 illustrated in Table 5 showed that, this isolate has gray aerial mycelium (gray colour series), while the vegetative mycelium was pigmented with brown colour. It had straight and long spore chains (section RF) (Figure 9) and the spores are characterized by smooth surface

without ornamentations (warty) (Figure 9).

The isolate was also characterized by excellent growth on Cazpek's agar medium, actively utilized all used sugar, tolerant to NaCl concentration up to 7%, no sensitivity to streptomycin (4 μgml^{-1}) and antagonized some of the bacterial and fungal test microorganisms used. Considering the description keys proposed by Pridham and Tresner (Pridham and Tresner, 1974), the tested isolate 13 was closely related to *S. phaeopurpureus*.

Factors affecting α-Amylase activity produced by *Streptomyces* lateritius-Isolate 10

α-Amylase activity was assayed by measuring the increase in reducing sugars formed by the enzymatic hydrolysis of starch (Nahas and Waldermarin, 2002). Data in Table 6 and illustrated by Figures 10 and 11 showed that α-amylase enzyme production was affected by different culture conditions. It was observed that the optimum zone of hydrolysis in mm was obtained at pH 7.0, 30°C and incubation period of 6.0 days.

Table.1 Total number of purified SLI isolated from Taif region

Locations	Code numbers	Total purified SLI	Serial numbers
AlQaiem	3-5	1	1
Al-Qamaryiah	28-4	1	2
Al-Sail Al-Kabeer	6-4	1	3
Al-Sail Al-Sagheer	4-8	1	4
Al-Sawtah	19-6	1	5
	20-6		6
Gabrah (1)	20'-6	3	7
	20''-6		8
Gabrah (2)	21-4	2	9
	21'-4		10
	13-1		11
Tarabah	13'-3	4	12
	13''-3		13
	13'''-3		14
	10-1		15
	10-3		16
Qaiah	10-7	6	17
	10'-7		18
	10''-7		19
	10'''-7		20
	17-2		21
Wadi-Bowa	17A-2	3	22
	17B-2		23

Table.2 Screening for enzymatic activities of purified SLI

Locations	Code numbers	Total purified SLI	Serial numbers
AlQaiem	3-5	1	1
Al-Qamaryiah	28-4	1	2
Al-Sail Al-Kabeer	6-4	1	3
Al-Sail Al-Sagheer	4-8	1	4
Al-Sawtah	19-6	1	5

Gabrah (1)	20-6	3	6
	20'-6		7
	20"-6		8
Gabrah (2)	21-4	2	9
	21'-4		10
Tarabah	13-1	4	11
	13'-3		12
	13"-3		13
	13"'-3		14
Qaiah	10-1	6	15
	10-3		16
	10-7		17
	10'-7		18
	10"-7		19
	10"'-7		20
Wadi-Bowa	17-2	3	21
	17A-2		22
	17B-2		23

+: Positive. -: Negative. ±: In-doubt

Table.3 Biological identification of streptomycetes isolate 10 compared with those of similar species reported in the key proposed by Pridham and Tresner[21]

Characters	**Isolate 10**	***S. lateritius***
Color of aerial mycelium	Red	Red
Spore Chain	RA-S	RA-S
Melanoid pigment	C+	C+
Spore surface	Warty	Warty
Growth on Czapek's medium	Excellent	ND
Color of substrate mycelium	White	Blue vegetative mycelium under alkaline conditions
Diffusable pigment		
Utilization of Carbon:		
No carbon	-	ND
D- Glucose	+	+
D- Xylose	+	+
L- Arabinose	-	+
L- Rhamnose	+	+
D- Fructose	+	+
D- Mannitol	-	-
i-inositol	+	±
Sucrose	+	-
Antagnostic activity	Antibacterial and antifungal	Antibacterial
Sensitivity to streptomycin	Se	ND
NaCl tolerance	0-7 %	≥ 4-< 7%

+: Growth -: No growth ±: In doubt ND: Not detected C+: Produce melanoid pigment SM: Smooth S: Spiral Se: Sensitive RA: Retinaculum Apertum (Spore chains in the form of open loops, hooks or greatly extended coils of wide diameter)

Table.4 Biological identification of streptomycetes isolate 12 compared with those of similar species reported in the key proposed by Pridham and Tresner (1974)

Characters	Isolate 12	*S. cacaoi* subsp. *asoensis*
Color of aerial mycelium	Gray	Gray
Spore Chain	RF	RF
Melanoid pigment	C+	C+
Spore surface	Smooth	Smooth
Growth on Czapek's medium	Excellent	ND
Color of substrate mycelium	Gray	ND
Diffusible pigment	-	ND
Utilization of Carbon:		
No carbon	-	ND
D- Glucose	+	+
D- Xylose	+	+
L- Arabinose	+	+
L- Rhamnose	+	ND
D- Fructose	+	+
D- Mannitol	+	ND
i-inositol	+	+
Sucrose	+	+
Antagonistic activity	Antibacterial and antifungal	Produce polyoxin (anti fungal)
Sensitivity to streptomycin	Se	ND
NaCl tolerance	0-7 %	ND

+, -, C+, ND, Se: Refer to Table 3. RF: Rectus-Flexibilis (spores in straight (R) or flexuous (F) chains)

Table.5 Biological identification of streptomycetes isolate 13 compared with those of similar species reported in the key proposed by Pridham and Tresner (1974)

Characters	Isolate 13	*S. phaeopurpureus*
Color of aerial mycelium	Gray	Gray
Spore chain	RF	RF
Melanoid pigment	C+	C+
Spore surface	Warty/Smooth	Smooth
Growth on Czapek's medium	Excellent	Excellent
Color of substrate mycelium	Brown	Red-brown and purple on some media
Diffusible pigment	-	ND
Utilization of Carbon:		
No carbon	-	-
D- Glucose	+	+
D- Xylose	+	+
L- Arabinose	+	+
L- Rhamnose	+	+
D- Fructose	+	+
D- Mannitol	+	+
i-inositol	+	+
Sucrose	+	-
Antagnostic activity	Antibacterial and antifungal	ND

Sensitivity to streptomycin	NSe	S
NaCl tolerance	0-7 %	ND

+, -, RF, C+, ND, Se: Refer to Table 4. NSe: Not sensitive

Table.6 Factors affecting amylase activity of *S. lateritius* isolate 10

Parameters	Details	Zone of analysis (mm) Replicates	Average (mm)
pH	5.0	1.50, 1.40, 1.60	1.50
	6.0	1.80, 1.80, 1.90	1.83
	7.0	3.01, 3.00, 2.99	3.00
	8.0	1.80, 1.80, 1.80	1.80
Incubation Temperature (^{o}C)	25	2.70, 3.30, 2.80	2.93
	30	3.20, 3.30, 2.80	3.10
	35	2.70, 2.70, 2.60	2.66
Incubation period (Days)	02	2.60, 2.80, 2.60	2.66
	04	3.00, 3.10, 2.70	2.93
	06	3.30, 3.20, 3.10	3.20

Figure.1 Purification of SLI on starch nitrate agar medium

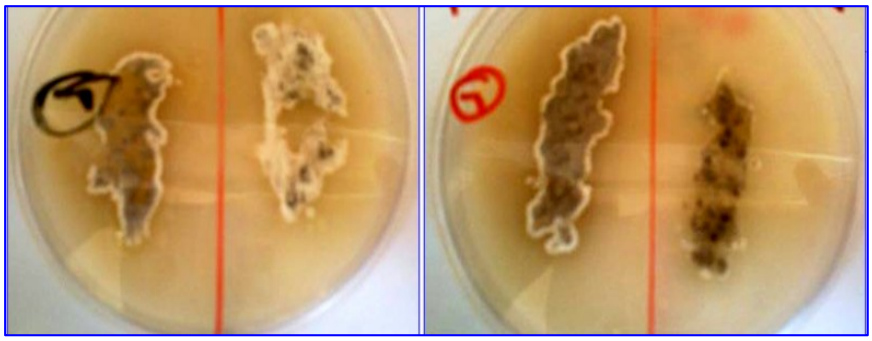


Figure.2 Extracellular amylase enzyme produced by two SLI on starch nitrate agar medium

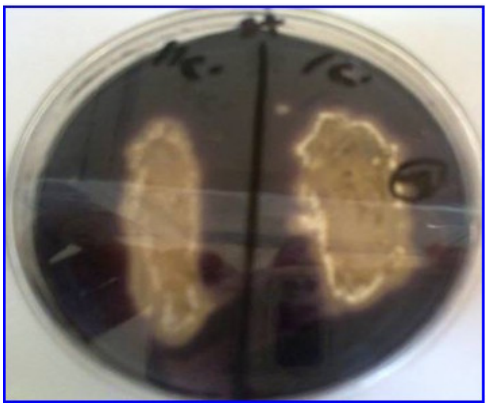


Figure.3 Extracellular caseinase enzyme produced by eight SLI on starch casein agar medium

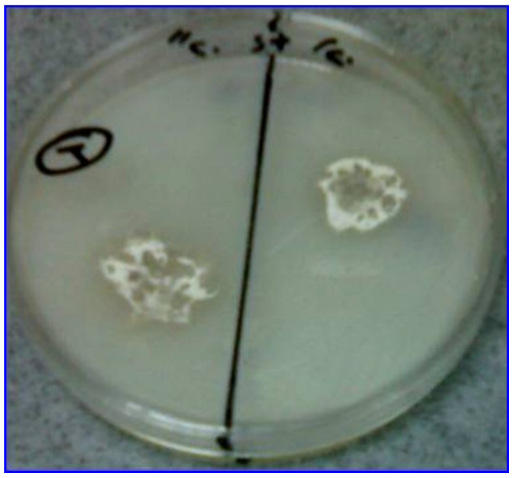


Figure.4 Extracellular cellulase enzyme produced by two SLI on cellulose agar medium

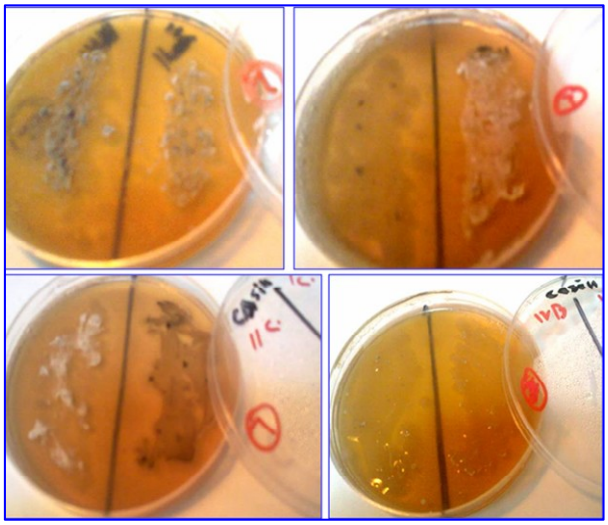


Figure.5 Extracellular lipase enzyme produced by two SLI on cellulose agar medium

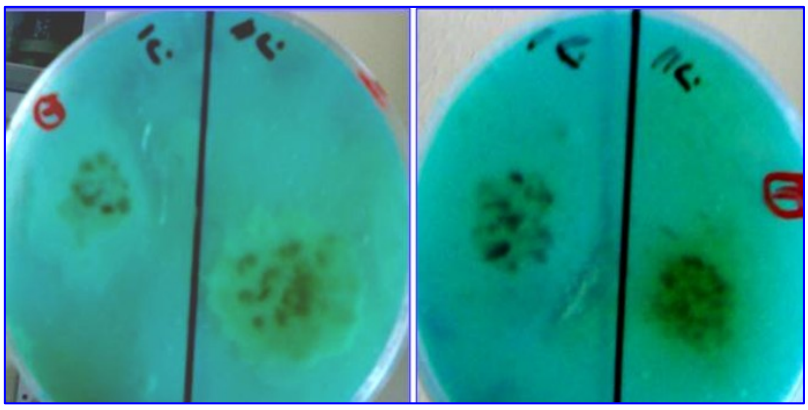


Figure.6 Extracellular gelatinase enzyme produced by 2 SLI on starch nitrate agar medium

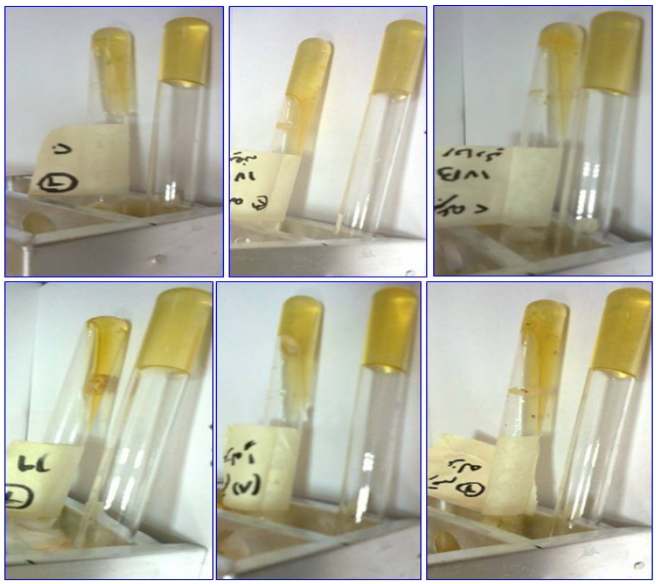


Figure.7 Microphotograph and electron micrograph of streptomycete isolate 10 shows spiral chain (X-1000) and warty spore surface (X-10000)

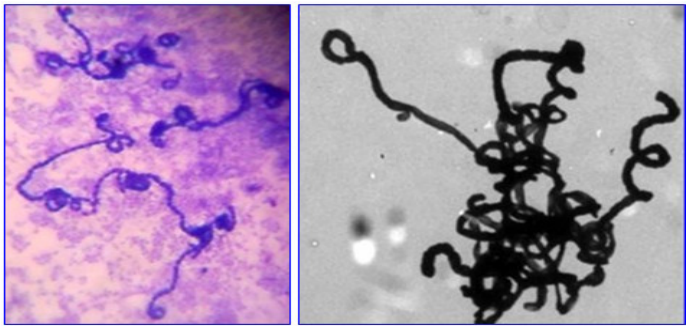


Figure.8 Microphotograph and electron micrograph of streptomycete isolate 12 shows RF chain (X-1000) and smooth spore surface (X-10000)

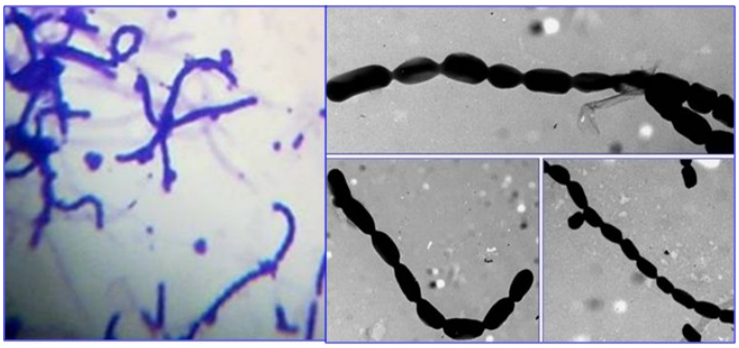


Figure.9 Microphotograph and electron micrograph of streptomycete isolate 13 shows RF chain (X-1000) and warty/smooth spore surface (X-10000)

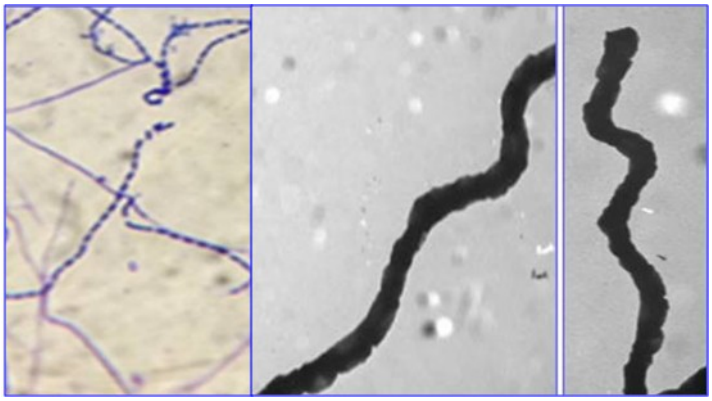


Figure.10 Factors affecting amylase activity of S. lateritius isolate 10, pH (5, 6, 7 and 8), incubation temperature (IT) (25, 30 and 35oC) and incubation period (2, 4 and 6 days)

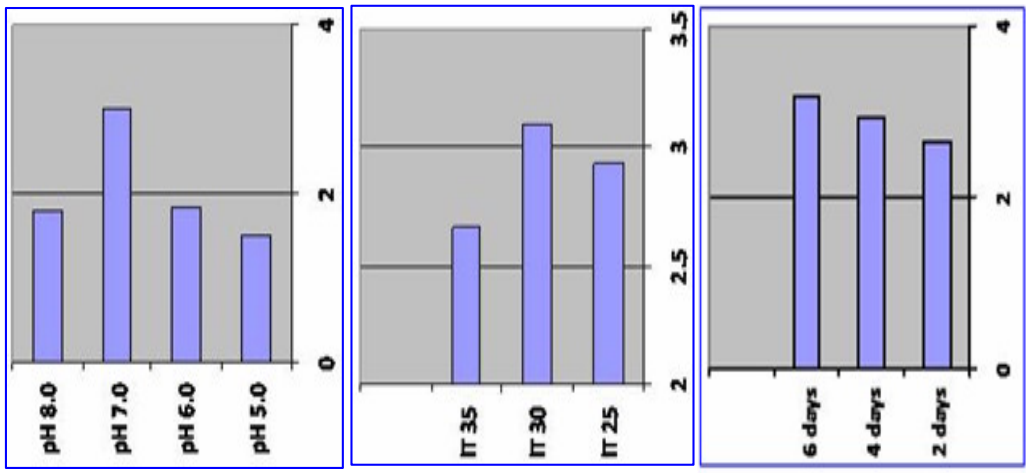
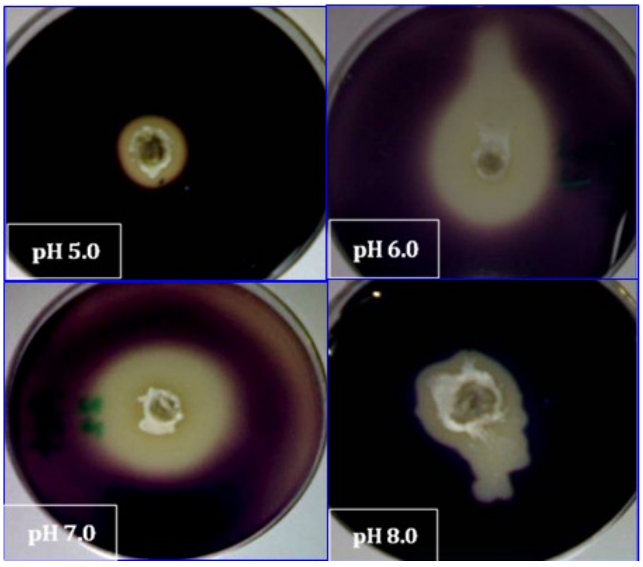


Figure.11 Extracellular amylase enzyme produced by S. lateritius isolate 10 grown on starch nitrate agar medium under different pH (5.0, 6.0 7.0 and 8.0) conditions



These results were in harmony with that found by Ramesh and Lonsane (1989) who reported that the effect of different culture conditions on α-amylase enzyme production was observed, they conducted an experiment using liquid starch medium at varying incubation periods (24, 48, 72, 96 and 120 h), pH (4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0) and temperatures (45, 50, 55, 60 and 70°C). The effect of each factor on enzyme production and reducing sugars was observed. Temperature and pH were the most limiting factors for the activity of α-amylase (Morgan and Prist, 1981).

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