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Screening and Identification of some APPL (Acid Precipitable Polymeric Lignin) Producing Streptomycetes

Nour El-Dein, M. M. ; M. I. Abou-Dobara and Noha E. Abou-Elhamd
Botany and Microbiology Department, Faculty of Science, Damietta University, Damietta El-Gededa, Egypt.

ABSTRACT

Thirty seven actinomycetes isolates were isolated from Damietta soil using starch nitrate agar media. These isolates were screened for APPL (acid precipitable polymeric lignin) by using minimal salts-yeast extract medium with rice and wheat straws. Three actinomycetes isolates showed high APPL production. These isolates also showed production of cellulase and xylanase with different levels. These isolates were identified by using morphological, physiological and chemotaxonomy criteria and were identified as *Streptomyces canus*, *Streptomyces libani* and *Streptomyces cyaneus*.

Keywords: APPL, *Streptomyces canus*, *Streptomyces libani*, *Streptomyces cyaneus*.

INTRODUCTION

Streptomyces is the largest genus of Actinobacteria belonging to the streptomycetaceae family (Kampfer *et al.*, 1991). Euzeby (2008) described over 500 species of *Streptomyces*. Streptomycetes are very oxidative an aerobic gram-positive have high CG-content genomes. It has a high (G+C) ratio of the DNA (>55mol %) (Madigan and Martinko, 2005). Most important streptomycetes character is their ability of large substrates degradation. Crawford *et al*., (1982) reported that streptomycetes can degrade both of cellulose, lignin lignocelluloses, softwoods and hardwoods. *Streptomyces* classification depends on both morphological and biochemical characteristics.

Acid precipitable polymeric lignin (APPL) - a water-soluble lignin polymer- is produced as a major product of degradation of lignin by some Streptomycetes as they can solubilize lignin to get into plant polysaccharides (Crawford *et al*., 1983; Pandey *et al*., 2004). Other many lignin degrading microorganisms were slowly degraded a lignin with using of APPL as intermediate. APPL possess a several number of uses like feed stocks for polyurethanes, emulsifying agents, adhesives, and different chemical processes (Pandey *et al*., 2004; Yamac and Tamer. 2008).

This study aimed to isolate and identify some APPL producing Streptomycetes.

MATERIALS AND METHODS

- Isolation of Actinomycetes isolates:

The actinomycetes isolates that used in this study were isolated from different regions at Damietta governorate (kafr saad, kafr saad elbalad and new Damietta). Sampling was carried out according to the procedure embraced by Johnson, *et al*., (1960) based on starch nitrate agar plates. The pure colonies were obtained by sub-culturing on same medium till become free from any fungal or bacterial contamination.

- Screening for APPL and enzymes production:

Discs of experimental actinomycetes pure colonies from petri dishes were used to inoculate flasks containing wheat or rice straws as carbon source and a medium of minimal salts-yeast extract per 1 litre (distilled water: yeast extract 2 g, (NH4)SO4 0.1 g, NaCl 0.3g 0.1g, CaCO3 0.02g, MgSO4.7H2O and 1 ml trace element solution , ZnSO4.7H2O 0.9 g L-1, (FeSO4.H2O 1 g L-1, MnSO4.7H2O 0.2 g L-1)). Cultures were incubated in shaker at 30ºC up to 14 days at 150 rpm. After incubation and centrifugation, the supernatant used for assaying cellulase and xylanase was carried out according to Miller (1959). For determination of APPL dry weight, supernatant was acidified with HCl to pH 1 to 2 for APPL and protein estimation by Bradford (1976) method.

- Classical identification of Actinomycetes isolates:

The identification of Actinomycetes isolates was carried out according to Bergey's Manual of Systematic Bacteriology (Williams *et al*., 1989 and Nonomura 1974). The micro-morphological properties including the size and shape of Actinomycetes mycelia and spores were performed according to Nallamuthu *et al.*, (2015) using Transmition Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM) at 15 kV (GSM, EM unit, Alexandria University). The occurrence of LL- or DL-hydroxyl diaminopimelic acid (hydroxyl-DAP) and whole cell sugars were determined by Thin Layer Chromatography (TLC) of whole cell hydrolysates according to Schon and Groth (2006).

RESULTS AND DISCUSSION

Isolation of Actinomycetes:

Six Soil samples, their location and their plant cover were listed in Table 1.

From these soils 37 isolates of actinomycetes with different group were isolated and listed also in Table (1).

- Screening and Identification of the Actinomycetes isolates:

About 37 different isolates of actinomycetes were tested for production of APPL. All of the tested isolates could produce APPL with different quantities. Using a medium of minimal salts-yeast extract, in the presence of rice straw and wheat straw respectively.

Results indicated that isolate 5C and isolate 4F showed the highest production in the case of rice straw as mentioned in (Table 2) and isolate 6A showed the highest production in the case of wheat straw as showed in (Table 3). One of the most important natural and renewable sources is lignocelluloses. Many *Streptomyces* species are lignocellulose decomposers (Chamberlain and Crawford, 2000). *Streptomyces* have the ability to produce a wide range of enzymes for insoluble macromolecules decomposition and many other important applications (Saini *et al*., 2015). The main product of solubilization of lignocellulosic residues by actinomycetes is an acid-precipitable polymeric lignin (APPL) (Crawford *et al.*, 1983). In the present study, three isolated strains can degrade different lignocellulytic residues by some enzymes and produce APPL in different quantities, like *Streptomyces viridosporus* T7A and other *Streptomyces* have been reported to have a similar degradative mechanism (Crawford, *et al*., 1983; Zimmermann, 1990; Pasti, *et al*., 1991). Among cellulase

producing actinomycetes, *Microbispora bispora*, *Cellulomonas fimi*, and *Thermobifida fusca* (Wilson, 1992; Lynd *et al.*, 2002). *Streptomyces cyaneus*, *S. tendae*, and *S. caelestis*, were found to be xylanolytic (Ninawe *et al*., 2008). *Streptomyces badius*, *Streptomyces cyaneus* MT813, *Amncolata autotirophica*, *Thermomonospora chromogena*, *Thermomonospora mesophila*, and *Micromonospora* sp. have shown significant activities against lignin related compounds (Ball *et al*., 1989; Godden *et al*., 1992; Saini *et al*., 2015).

Table 1. Soil samples used for isolation of actinomycetes.

No.	Isolates Group	Number of isolates	location	Plant cover
1	A	11	Kaffr saad elbalad	*Mentha spicata* *Nasturtium officinale* *Piper nigrum* *Solanum melongena* *Rosa chinensis*
2	B	4	Kafrr saad	*Citrus X sinensis* *Ocimum basilicum* *Punica granatum* *Mangifera indica* *Carica papaya*
3	C	1	Kafr saad elbalad	Enterobacteria phage phiX174 *Mangifera indica* *Citrus X sinensis officinale* *Piper Phoenix dactylifera*
4	D	9	Kaffr saad elbalad	*Mangifera indica Prunus persica* *Solanum melongena Piper nigrum* *Saccharum officinarum* *Citrus × limon* *Psidium guajava*
5	E	4	New Damietta	*Citrus reticulata* *Mangifera indica* *Ficus carica* *Psidium guajava* *Punica granatum*
6	F	8	Kaffr sad elbalad	*Saccharum officinarum* *Mangifera indica* *Citrus × limon* *Piper nigrum* *Psidium guajava*

Table 2. Survey of APPL producing actinomycetes on rice straw as carbon source.

No.	Isolate code	Cellulase (unit/ml)	Xylanase (unit/ml)	APPL Protein (mg/ml)	APPL dry weight (g)
1	2A	0	0	2.019±0.107	0.0136
2	3A	0	1.371±0.136	1.98±0.691	0.0469
3	4A	0	3.544±1.66	2.91±0.090	0.0668
4	5A	1.1202±0.61	1.601±0.081	2.54±0.168	0.0536
5	6A	0.786±0.0907	1.322±0.007	2.34±0.015	0.0259
6	7A	0	1.187±0.031	2.89±0.371	0.0151
7	8A	0.0151±0.008	3.398±0.566	2.17±0.008	0.0276
8	9A	0	1.372±0.126	2.11±0.135	0.0255
9	10A	0	0.422±0.042	1.378±0.362	0.02525
10	13A	0.0974±0	0	0.270±0.108	0.0486
11	14A	0	0.261±0.031	2.50±0.73	0.027
12	2B	0	8.38±0.853	2.008±0.188	0.0083
13	3B	0.0209±0.0025	5.198±0.429	0.953±0.036	0.0189
14	9B	0.0487±0.009	0.392202±0	1.502±0.336	0.0516
15	12B	1.203±0.094	0	2.265±0.085	0.0298
16	5C	0.1167±0.031	1.347±0.20622	4.644±0.177	0.0728
17	2D	0.154±0.066	0.071±0.0193	2.292±0.041	0.0522
18	4D	0	2.482±0.961	4.679±0.583	0.0474
19	5D	0	1.485±0.064	3.79±0.253	0.0189
20	6D	0	0.710±0.107	3.148±0.45	0.0173
21	7D	0	1.162±0.086	3.297±0.114	0.026
22	8D	0.0886±0.0591	1.346±0.06600	4.1441±0.0541	0.0666
23	9D	0.132±0.0220	1.2457±0.303	2.71±0.011	0.03
24	10D	0	2.814±0.328	0.641±0.005	0.0339
25	11D	0	5.48±0.083	2.318±0.034	0.0366
26	1E	0	4.14±1.8	0.491±0.13	0.0218
27	3E	0	14.06±1.35	1.413±0	0.018
28	4E	0	1.820±0.617	2.81±0.002	0.05035
29	6E	0	0.986±0.024	2.30±0.251	0.0571
30	3F	0.275±0.110	0.213±0.01	3.09±0.080	0.0494
31	4F	0	4.68±0.56	2.42±0.216	0.038
32	5F	0	2.78±0.15	0.977±0.175	0.0366
33	6F	0.058±0	0.73±0.245	2.19±0.237	0.0411
34	8F	0.022±0.0066	0.522±0.136	2.17±0.60	0.0403
35	9F	0	0.745±0.120	1.88±0.083	0.543
36	13F	0.13±0.06	7.212±0.168	1.99±0.355	0.036
37	15F	0	3.55±0	1.034±0.071	0.0362

± represent the standard error of replica on excel. APPL (acid precipitable polymeric lignin)

Table 3. Survey of APPL producing actinomycetes on wheat straw as a carbon source.

No.	Isolate code	Cellulase (unit/ml)	Xylanase (unit/ml)	APPL Protein (mg/ml)	APPL dry weight (g)
1	2A	0.0568±0.0113	1.028±0.0632	1.94±0.14	0.02485
2	3A	0	0.60102±0.158	2.971±0.122	0.05805
3	4A	0	6.204±2.82	4.0805±0.183	0.0544
4	5A	0	7.742±1.38	1.924±0.137	0.0273
5	6A	0.1853±0.0186	5.529±0.420	5.822±0.177	0.14815
6	7A	0	9.26±0.434	2.295±0.162	0.04525
7	8A	0.0591±0.0188	2.310±0.224	5.8310±1.74	0.0666
8	9A	0	0.3084±0.134	1.942±0.097	0.0296
9	10A	0	0.752±0.409	3.147±0.824	0.06425
10	13A	0	3.564±0.765	1.069±0.155	0.04325
11	14A	0	0.829±0.154	2.25±0.635	0.02995
12	2B	0	4.0±0.40072	2.39±0.0445	0.07455
13	3B	0.044±0.014	5.134±0.541	1.873±0.209	0.0283
14	9B	0.118±0.0118	0.886±0.088	2.813±0.103	0.04875
15	12B	0	0.442±0.079	3.864±0.27	0.04355
16	5C	0	1.38536±0.410	4.19±0.09	0.0551
17	2D	0	8.35±1.05	1.677±0.068	0.02795
18	4D	0	6.429±1.60	2.395±0.102	0.0355
19	5D	0	0.2105±0.029	3.132±0.25	0.041
20	6D	0	1.108±0.052	4.33±1.25	0.06465
21	7D	0.024±0.004	0.899±0.05	7.52±0.115	0.896
22	8D	0.0295±0.005	1.346±0.66	3.16±0.36	0.0585
23	9D	0	3.044±1.09	1.1615±0.28	0.02545
24	10D	0	2.715±1.1	1.84±0.09	0.03405
25	11D	0.059±0.029	3.128±1.4	2.29±0.14	0.05035
26	1E	0	6.294±1.8	2.29±0.12	0.0297
27	3E	0.161±0.01	2.83±0.2	4.707±1.3	0.0642
28	4E	0	0.567±0.11	3.35±0.16	0.0492
29	6E	0	0.212±0.029	4.16±0.19	0.0517
30	3F	1.108±0.03	0.442±0.28	2.99±0.04	0.06455
31	4F	0	6.55±0.99	2.801±0.23	0.1122
32	5F	0	0.387±0.033	1.49±0.30	0.06085
33	6F	0	0.316±0.03	4.04±0.02	0.094
34	8F	0.443±0.014	0.99±0.042	2.8±0.45	0.0453
35	9F	0.0622±0.012	2.98±0.36	2.39±0.04	0.0349
36	13F	0	6.2618±0.121	2.79±0.002	0.033
37	15F	0	2.51±0.35	2.34±0.07	0.022

± represent the standard error of replica on excel. APPL (acid precipitable polymeric lignin)

- Morphological and chemotaxonomical identification:

Identification and classification of the best APPL producing actinomycetes are based on morphological, biochemical in addition to physiological characteristics. On the basis of morphological ones, different growth intensity was observed when the Actinomycetes isolates grow on different tested media. With respect to the substrate mycelia and aerial mycelia, different variable colors were observed in Tables 4, 5 and 6. The grey color was the most predominant color.

Table 4. The Cultural properties of 7 days old cultures of the 5C isolate on different media.

Medium	Type of growth	Colour of			Growth intensity	pH sensitivity	
		Aerial mycelium	Substrate mycelium	Medium		NaOH	HCl
Starch-nitrate agar	Powdery, good aerial Mycelium	Grey	Creamy	Non-pigmented	++++	-ve	ve-
Starch-ammonium sulphate agar	Powdery, weak aerial mycelium	Grey	Creamy	Non-pigmented	+	-ve	-ve
Dox agar	Powdery, weak aerial mycelium	Non-pigmented	Brownish	Brownish	+	+ve yellow*	+ve orange*
Glucose-nitrate agar	Powdery, moderate aerial mycelium	Whitish –grey	Creamy	Non-pigmented	++	-ve	-ve
Glycerol-nitrate agar	Powdery, moderate aerial mycelium	Whitish-grey	Creamy	Non-pigmented	++	-ve	-ve
Glycerol- asparagine agar	Powdery, weak aerial mycelium	Whitish-grey	White	Non-pigmented	+	-ve	-ve
Oat meal agar	Powdery, weak aerial mycelium	Whitish-grey	Creamy	Non-pigmented	+	-ve	-ve

***The color of substrate mycelium and medium was pH sensitive when treated with 0.05 N NaOH or 0.05 N HCl. (+, good growth /++, high growth /±, low growth).**

Table 5. Cultural properties of 7 days old cultures of the 4F isolate on different media.

Medium	Type of growth	Colour of			Growth intenisty	pH sensitivity	
		Aerial mycelium	Substrate mycelium	medium		NaOH	HCl
Starch-nitrate agar	Powdery, good aerial mycelium	Grey	Brownish	Pale brownish color	++++	-ve	ve-
Starch-ammonium sulphate agar	Powdery, weak aerial mycelium	Grey	Pale brownish	Brownish color	+	-ve	-ve
Dox agar	Powdery, weak aerial mycelium	Non-pigmented	Creamy	Non-pigmented	+	-ve	-ve
Glucose-nitrate agar	Powdery, weak aerial mycelium	Grey	Brownish	Brownish color	+	-ve	-ve
Glycerol-nitrate agar	Powdery, moderate aerial mycelium	Grey	Brownish	Brownish color	++	-ve	-ve
Glycerol- asparagine agar	Powdery,moderate aerial mycelium	Whitish grey	Brownish	Brownish color	++	-ve	-ve
Oat meal agar	Powdery,moderate aerial mycelium	Grey	brownish	Non-pigmented	++	-ve	-ve

***The color of substrate mycelium and medium was pH sensitive when treated with 0.05 N NaOH or 0.05 N HCl. (+, good growth /++, high growth /±, low growth).**

Table 6. Cultural properties of 7 days old cultures of the 6A isolate on different media.

Medium	Type of growth	Colour of			Growth intenisty	pH sensitivity	
		Aerial mycelium	Substrate mycelium	medium		NaOH	HCl
Starch-nitrate agar	Velvety, good aerial mycelium	Blue to grey	Dark green	Grey	++++	-ve	+ve violet
Starch-ammonium sulphate agar	Velvety, moderate aerial mycelium	Blue to grey	Greenish	Non-pigmented	++	-ve	-ve
Dox agar	Powdery, weak aerial mycelium	Grey	Grey	Pale grey	+	-ve	-ve
Glucose-nitrate Agar	Velvety, good aerial mycelium	Blue to grey	Dark green	Greyish (brownish)color	++++	+ve pale* violet	+ve pale* violet
Glycerol-nitrate agar	Velvety, good aerial mycelium	Whitish grey	Dark green	Greyish (brownish)color	++++	+ve pale* violet	+ve pale* violet
Glycerol- asparagine agar	Powdery, weak aerial mycelium	Whitish	Creamy	Non-pigmented	+	-ve	-ve
Oat meal agar	Velvety, good aerial mycelium	Blue grey	greenish	Grey (brownish)color	++++	-ve	+ve pale* violet

***The color of substrate mycelium and medium was pH sensitive when treated with 0.05 N NaOH or 0.05 N HCl. (+, good growth /++, high growth /±, low growth).**

Results revealed that the spore surface of isolate (5C) and isolate (6A) was spiny and arranged in spiral chain (Fig. 1 and Fig. 3) as in *S. lienomycini* was spirals with spiny spore surface (Labeda *et al*., 2012). On the other hand (Loqman *et al*., 2009) revealed that the spore chain of *S. thinghirensis* S 10 was straight with smooth surface that is similar to isolate (4F) (Fig.2) which it´s spore surface was smooth.

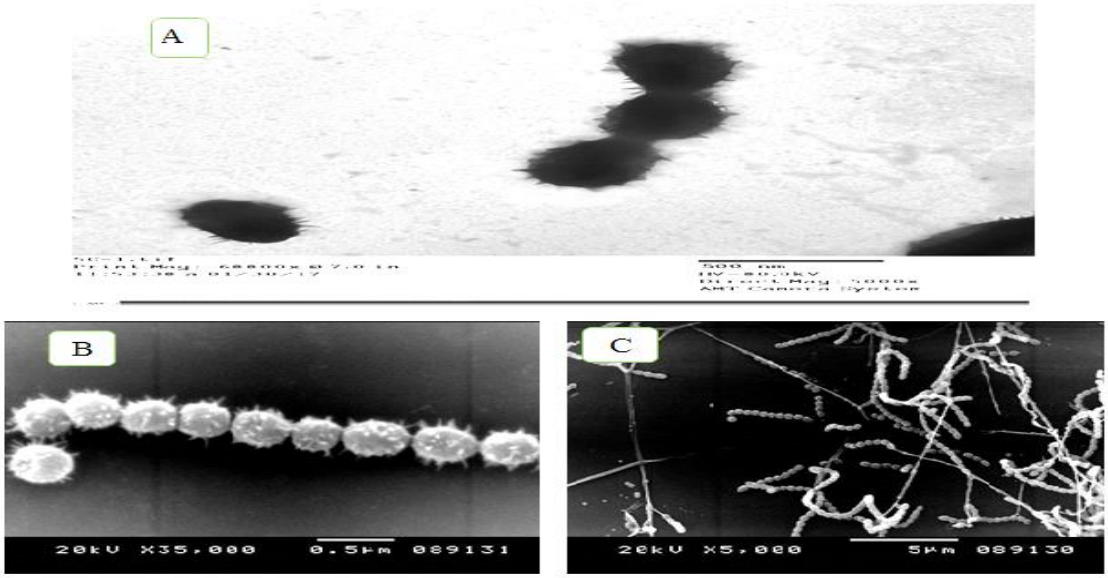

Figure 1. (A)Transmission Electron Microscope (TEM) and Scanning electron micrograph (SEM) of spores (B and C) of isolate 5C.

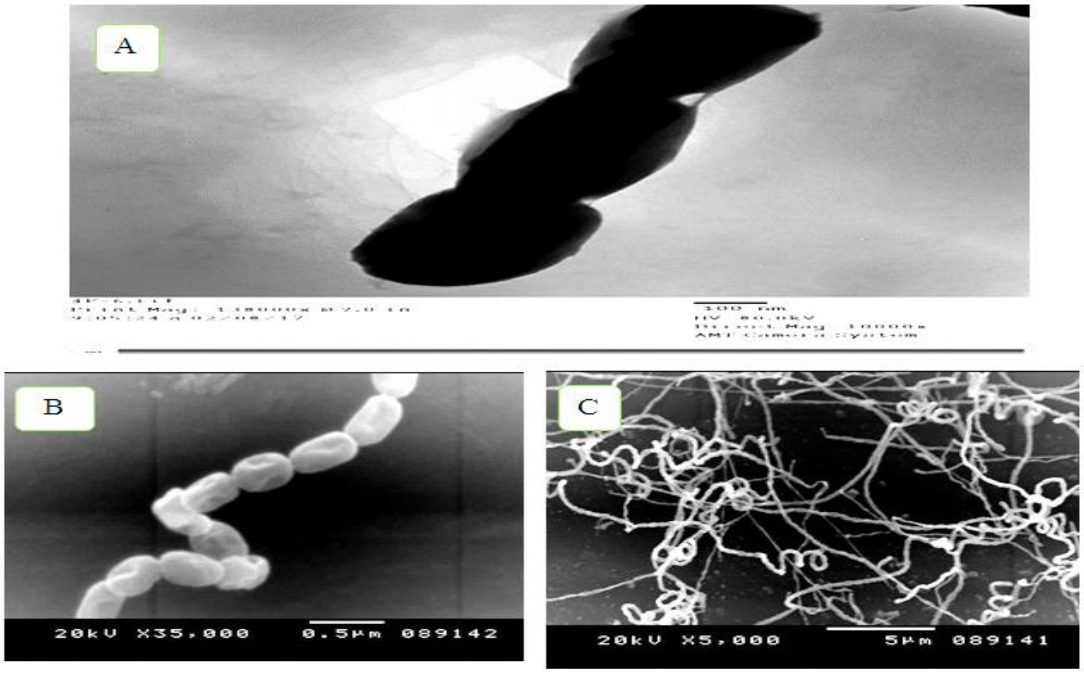

Figure 2. (A) Transmission Electron Microscope and Scanning Electron Microscope of spore chain morphology and spore surface (B and C) of isolate 4F.

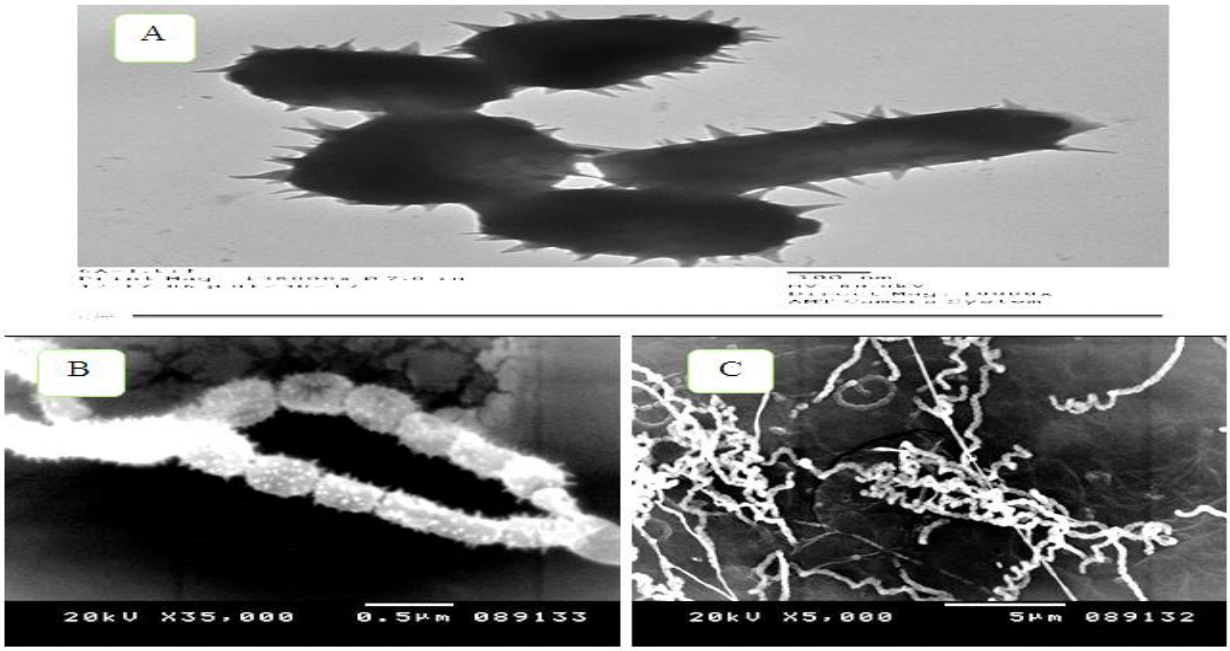

Figure 3. Transmission Electron Micrograph of spore surface (A) and Scanning Electron Micrograph of spores (B and C) of isolate 6A.

The physiological and biochemical characteristics of tested actinomycete isolates are represented in Table 7. They had the ability to utilize various either carbon or nitrogen sources with different variable growth intensity. The most produced growth with respect to carbon source was observed on D-galactose while with respect to nitrogen source was observed on L-proline. The 5C isolate coagulated milk but did not peptonize it, hydrolyzed starch, and did not decompose cellulose. The 5C isolate also has the ability to degrade esculin. Urea could be used by this isolate. The 5C isolate also successed to liquefy gelatin, reduce nitrate, but failed to degrade tyrosine and produce hydrogen sulphide while 4F isolate has the ability to coagulate and peptonize milk, degrade esculin, hydrolyse starch, reduce nitrate, but could not decompose cellulose, this isolate could degrade urea, liquefy gelatin but could not produce hydrogen sulphide. The 6A isolate was not able to coagulate and peptonize milk, but can degrade esculin and hydrolyse starch and urea. This isolate was unable to liquefy gelatin reduce nitrate and can produce hydrogen sulphide. The ability of 6A to decompose cellulose was negligible. Furthermore, they were tested against different wide range of antibiotics including Clavulanic Amoxicillin, Cefepime, Cefotaxime, Cephalexin, Levofloxacine and Tetracycline (Table.7)

Table 7. Physiological and biochemical characteristics of some actinomycetes isolates.

Enzyme detection or Test	Result Isolate code 5C	4F	6A	Enzyme detection or test	Result Isolate code 5C	4F	6A
Gelatinase	+	+	-	Melanin formation on:			
Cellulase	-	-	+	Peptone-yeast iron agar	-	-	-
Nitrate reductase	+	+	-	Tyrosine agar	-	-	+
Urease	+	+	+	Tryptone yeast extract	-	-	-
H_2S production	-	+	+	Carbon sources utilization:			
Milk peptonization	-	+	-	L-arabinose	++++	++++	+
Milk coagulation	+	+	-	D-fructose	+	++	+
Esculin hydrolysis	+	+	+	D-glucose	++++	++++	++++
Tyrosinase	-	-	+	Starch	++	++	++
Sensitivity to antibiotics (µg):				Sucrose	+	++	+
Clavulanic & Amoxicillin(30)	+	+	+	D-xylose	++++	++++	++
Cephalexin (30)	+	+	+	Meso-inositol	+	++	++
Tetracycline (30)	-	+	-	Sodium acetate	+	+	+
Cefotaxime (30)	+	+	+	Cellulose	±	±	±
Levofloxacine (5)	-	-	-	D-galactose	+++++	++++	+++
Cefepime (30)	+	+	+	D-maltose	++++	+	+
Growth temperature (°C):				Nitrogen source utilization:			
10	-	-	-	Proline	++++	++++	++++
15	-	-	-	Threonine	+	++	+++
25	+	+	+	methonine	+	+	+++
37	+	+	+	Hydroxy proline	+++	+++	+++
35	+	+	+	Serine	+	++	+++
40	+	+	+	KNO_3	+++	+	+++
45	+	+	+	Heat resistance (100° C for 10 min)	+++	++++	++++
50	-	-	-	Growth in presence of NaCl (gm/l):			
55	-	-	-	5	++	++	+++
Cell wall chemical structure:				20	++	++	++++
Sugar pattern	Galactose glucose mannose xylose	glucose mannose xylose	galactose glucose arabinose xylose	40	+++	+++	+
				50	++++	++++	++++
				60	++++	++++	+++
				80	++	++	+
Type of Diaminopimelic acid (DAP)	L-DAP	L-DAP	L-DAP	100	-	-	-
Inhibitory compounds (% w/v) (after 7 days)				(Antagonistic activity)Test organism			
Na.azide (0.01)	+	+	+	Gram negative bacteria *(E.coli)*	-	-	-
Na.azide (0.02)	±	±	±	Gram positive bacteria	-	-	-
Crystal violet (0.001)	+	±	+	*(Staphylococcus aureus*)			
Inhibitory compounds(% w/v) (after 14 days)							
Na.azide (0.01)	+	+	+				
Na.azide (0.02)	-	-	+				
Crystal violet (0.001)	+	±	+				

(± low growth, + light growth, ++ moderate growth, +++ good growth,++++ very good growth,- no growth).

Also results in the same table revealed that the three isolates possessed an ability to resist both of heat and salinity. Growth intensity was reduced at high concentrations of sodium chloride (NaCl). Temperature is one of the most effective factors influenced on the growth of actinomycetes. In the present study, the growth intensity was occurred at 25°C and above 45°C with maximum growth at 30°C. In the present study isolate 5C and isolate 4F have the ability to produce gelatinase, nitrate reductase, urease and aesculin but no production of both of hydrogen sulphide and cellulase.

The isolate6A has the ability for production of urease, aesculin, cellulase and hydrogen sulphide but can´t produce gelatinase, nitrate reductase. Le Roes-Hill *et al*., (2018) reported that some other *Streptomyces* species like *Streptomyces swartbergensis* could also produce cellulase, esculin hydrolysas and gelatinase, and has weakly production of nitrate reductase and H_2S and couldn't produce urease. In the present study 5C and 4F are similar to *S. thinghirensis* S10 in milk coagulation and peptonization, but couldn't produce melanin pigments neither on peptone-yeast extract-iron agar nor on tyrosine agar supporting the obtained results; *S. thinghirensis* S10 also couldn't produce melanin pigments (Loqman *et al*., 2009). 6A couldn't make neither milk coagulation nor peptonization and but produced melanin pigment in tyrosine agar, but couldn't produce melanin pigment neither on peptone yeast iron agar nor tryptone yeast iron agar (Loqman *et al*., 2009). Some other *Streptomyces* as *S. lienomycini* could produce dark brown melanin pigments (Labeda *et al*., 2012; Le Roes-Hill *et al*., 2018).

In the present study, 5C and 4F can utilize many carbon sources including L-arabinose, D-fructose, D-glucose, D-galactose, starch, sucrose, raffinose and D-xylose. It is weakly utilize maltose, meso-inositol, sodium acetate and cellulose while 6A can utilize many carbon sources such as L-arabinose, D-fructose, D-glucose, D-galactose, starch, sucrose, maltose, meso-inositol, and D-xylose. 6A weakly utilized sodium acetate, raffinose, and cellulose. Vézina *et al*., (1975) revealed that the isolated *Streptomyces* species had good growth on starch, D-glucose, D-fructose, D-mannitol and glycerol, while it showed weak growth on L-rhamnose, raffinose, D-xylose, L-arabinose,

lactose and D-maltose, but no growth on sucrose. *Streptomyces canus* also can utilize many nitrogen sources such as L-methionine, L-threonine, L-hydroxy proline, L-serine and potassium nitrate. Le Roes-Hill *et al*., (2018) reported that some other *Streptomyces* species were able to utilize different carbon sources such as xylose, , galactose, glucose, meso-inositol, arabinose, cellobiose, and mannose but showed weakly utilization of sucrose and sodium acetate with no utilization of fructose, lactose, and mannitol; and could utilize different nitrogen sources as L-threonine, L-hydroxyproline, L-methionine, L-valine, L-histidine, potassium nitrate, L-cysteine, L-arginine, L-serine, and L-phenylalanine. In the present study, *Streptomyces* strains can decompose cellulose by producing cellulase enzyme. Some strains have the ability for cellulose decomposition (Taddei *et al*., 2006).

The 5C isolate can grow in the presence of 0.0001% (w/v) crystal violet and sodium azide (0.01%, w/v) for 7 days and 14 days, but showed weak growth in the presence of sodium azide (0.02%, w/v) for 7 days and no growth in 14 days. 4F isolate could only grow in presence of sodium azide (0.01%, w/v) for 7 days and 14 days. In case of the 6A isolate can grow in the presence of 0.0001% (w/v)crystal violet and sodium azide (0.01%, w/v) for 7 days and 14 days, but showed weak growth in the presence of sodium azide (0.02%, w/v) for 7 days and in 14 days. Some *Streptomyces* strains could grow in the presence of 0.0001% (w/v) crystal violet, but not in the presence of sodium azide (0.01% & 0.02%, w/v) (Le Roes-Hill *et al*., 2018).

In the present study cell wall di-amino acid for all isolates was LL-diaminopimelic acid and its main cell wall sugars for isolate 5C were galactose, glucose, mannose and xylose, for isolate 4F, glucose, mannose and xylose, and for isolate 6A cell wall sugars were galactose, glucose, arabinose and xylose. Some other species also showed close results like *S. lienomycini* that cell wall di-amino acid was LL- di-aminopimelic acid (Labeda *et al*., 2012).

Streptomyces luteus main cell wall di-amino acid was LL-di-aminopimelic acid, and the main cell wall sugars were glucose and ribose (Luo *et al*., 2017). Aerobic actinomycetes have been assigned to four whole-cell sugar patterns (Lechevalier, 1968).Organisms belonging to pattern A have arabinose and galactose and the absence of xylose and madurose. Pattern B is characterized by the presence of madurose and absence of arabinose, galactose and xylose. Actinomycetes forming pattern C do not have any of the characteristic sugar mentioned, and those of pattern D have arabinose and xylose but lack galactose and madurose. Family Streptomycetaceae do not contain these sugars. All the identified streptomycetes in this work are no characteristic sugar (Figure 4 &5) .

All previous results revealed that these isolates can identified by using morphological, physiological and chemotaxonomy criteria as *Streptomyces canus*, *Streptomyces libani* and *Streptomyces cyaneus* as coded as 5C, 4F and 6A respectively.

Therefore *Streptomyces Streptomyces canus*, *Streptomyces libani* and *Streptomyces cyaneus* strains seem to have some major advantages for biotechnological applications. Future works will focus on production of the lignin degrading enzymes and biotransformation of lignin related.

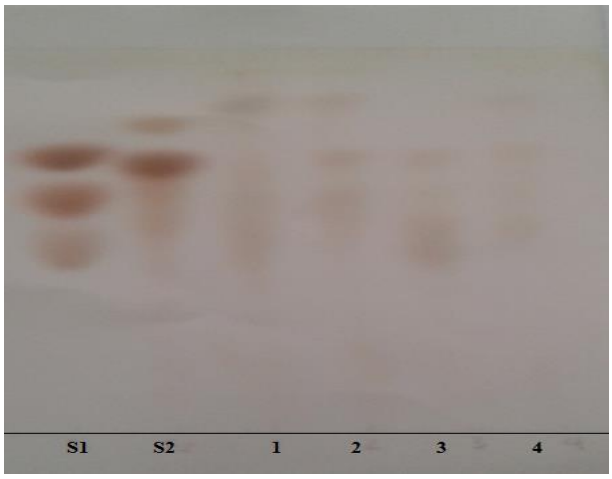

Figure 4. Thin Layer Chromatography of sugar content of some streptomycetes .carbohydrates of standard S1 and S2 are listed in the order of migration, strarting at the origin (slowest to fastestmoving components (S1) standards: glucose, mannose, ribose and rhamnose. (S2) standards: galactose, arabinose and xylose. (1 and 2) isolate 6A. (3) isolate 5C .(3) isolate 4F.

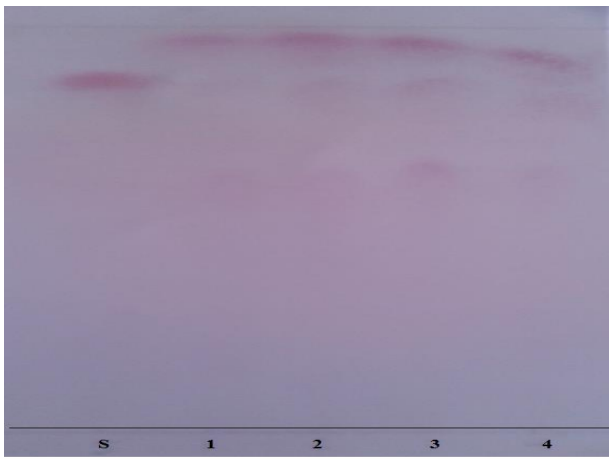

Figure 5. Thin Layer Chromatography analysis of diaminopimelic acid (DAP) of the cell hydrolysates of some streptomycetes . (S) DAP standard containing DL-DAP and LL-DAP. (1and 2) isolate 6A, (3) isolate 5C and (4) isolate 4F.

REFERENCES

Ball, A. S., Betts, W. B. and McCarthy, A. J. (1989): "Degradation of lignin-related compounds by actinomycetes," *Applied and Environmental Microbiology*, vol. 55, No. 6, pp: 1642–1644.

Bradford, M.M. (1976): A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal. Biochem. 72:248-254.

Chamberlain, K. and Crawford, D.L. (2000): Thatch biodégradation and anti-fungal activities of two lignocellulolytic *Streptomyces* strains in laboratory cultures and in golf green turf grass. Can. J. Microbiol. 46: 550-558.

Crawford, D. L., Barder, M. J., Pometto, A. L. and Crawford, R. L. (1982): Chemistry of softwood lignin degradation by *Streptomyces viridosporus*. Arch. Microbiol.131:140-145.

Crawford, D. L., Pometto, A. L. and Deobald, L. A. (1983): The pathway of lignin degradation by *Streptomyces*. Chemist. Enzymol: 78-95.

Euzeby, J. P. (2008): Genus *Streptomyces*. List of Prokaryotic names with Standing in Nomenclature. A Folder Available on the Internet. Int. J. Syst. Bacteriol., 590-592.http://www.bacterio.cict.fr/s/streptomycesa.html.

Godden, B. Ball, A. S. Helvenstein, McCarthy, P. A. J and Penninckx, M.J. (1992): "Towards elucidation of the lignin degradation pathway in actinomycetes," J. Gen. Microbiol., 138, (11): 2441 -2448.

Johnson, I.F Curl, E.A., Bond, J.H. & Fibourg, H.A. (1960): Methods for studying soil micrroflora. Burgess publishing Co., Minneapolis, USA, 15.

Kampfer, P., Kroppenstedt, R. M. and Dott, W. (1991): A numerical classification of the genera *Streptomyces* and *Streptoverticillium* using miniaturized physiological tests. J. Gen. Microbiol., 137: 1831 – 1891.

Labeda, D.P., Goodfellow, M., Brown, R., Ward, A.C., Lanoot, B., Vanncanneyt, M., Swings, J., Kim, S.B., Liu, Z., Chun, J., Tamura, T., Oguchi, A., Kikuchi, T., Kikuchi, H., Nishii, T., Tsuji, K., Yamaguchi, Y., Tase, A., Takahashi, M., Sakane, T., Suzuki, K.I. and Hatano, K. (2012): Phylogenetic study of the species within the family Streptomycetaceae. Anton. Van Leeuwen., 101(1): 73-104.

Lechevalier, M.P. (1968): identification of aerobic actinomycetes of clinical importance. *J. Laboratory and clinical Medicine*. 71: 934-944.

Le Roes-Hill, M., Prins, A. and Meyers, P.R. (2018): *Streptomyces swartbergensis* sp. nov., a novel tyrosinase and antibiotic producing actinobacterium. Antonie van Leeuwenhoek Int. J. Gen. Mol. Microbiol., 111: 589–600.

Loqman, S., Bouizgarne, B., Barka, E.A., Clement, C., von Jan, M., Sproer, C., Klenk, H.P. and Ouhdouch, Y. (2009): *Streptomyces thinghirensis* sp. nov., isolated from rhizosphere soil of Vitis vinifera. System. Evolution. Microbiol., 59: 3063-3067.

Luo, F., Zou, Y., Huang, T. and Lin, S. (2017): Draft genome sequence of Streptomyces sp. B9173, a producer of indole diketopiperazine maremycins. Gen. Ann., 5: e00447-17.

Lynd, L. R., Weimer, P. J., Van Zyl, W. H. and Pretorius,I. S. (2002): Microbial cellulose utilization: fundamentals and biotechnology. *Microbiol. Molecul. Biol. Rev.*, 66: 506–577.

Madigan, M. and Martinko, J. (2005): Brock Biology of Microorganisms. 11th Edn., prentice Hall, New Jersey, USA.

Miller, G.L. (1959): Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem., 31:426-428.

Nallamuthu, I., Devia, A. and Khanum, F. (2015): Chlorogenic acid loaded chitosan nanoparticles with sustained release property, retained antioxidantactivity and enhanced bioavailability. Asian Jour. Pharmaceut. Scien. 10(3): 203-211.

Ninawe, S., Kapoor, M. and Kuhad, R. C. (2008): "Purification and characterization of extracellular xylanase from *Streptomyces cyaneus* SN32," *Bioresource Technology*, vol. 99, no. 5, pp: 1252– 1258.

Nonomura, H. (1974): Key for classification and identification of 458 species of the streptomycetes included in ISP. Ferment. Technol., 52: 78-92.

Pandey, B., Ghimire, P. and Agrawal, V.P. (2004): International Conference on the Great Himalayas: Climate, Health, Ecology, Management and Conservation, Kathmandu, Organized by Kathmandu University and the Aquatic Ecosystem Health and *Management Society, Canada*.

Pasti, M.B., Hagen, S.R., Korus, R.A. and Crawford, D.L. (1991): The effects of various nutrients on extracellular peroxidases and acid precipitable polymeric lignin production by Streptomyces chromofuscus A2 and *S. viridosporus* T7A.Appl. Microbiol. Biotechnol., 34:661-667.

Saini, A., Aggarwal, N.K., Sharma, A. and Yadav, A. (2015): Actinomycetes: A Source of Lignocellulolytic EnzymesHind. Pub. Corporat., 279381, 15.

Schon, R. and Groth, I. (2006): Practical thin layer chromatography techniques for diaminopimelic acid and whole cell sugar analyses in the classification of environmental actinomycetes. Basic Microbiol., 46: 243–249.

Taddei, A., Jose, M., Ernesto, R. and Cristina, M. (2006): Isolation and identification of *Streptomyces* spp. from Venezuelan soils: Morphological and biochemical studies. Microbiol. Res., 161(3): 222- 231.

Vézina, C., Kudelski, A. and Sehgal, S.N. (1975): Rapamycin (AY-22,989), a new antifungal antibiotic. I. Taxonomy of the producing streptomycete and isolation of the active principle. J Antibiot (Tokyo)., 28(10):721-6.

Williams, S.T., Goodfellow, M. and Alderson, G. (1989): Genus *Streptomyces* Waksman and Henrici 1943, 339AL. In: "Bergey's Manual of Systematic Bacteriology" (1sted.), (eds., Williams, S.T., Sharpe, M.E. and Holt, J.G.), Baltimore, Lippincott Williams & Wilkins Company. 4: 2452-2492.

Wilson, D. B., (1992):"Biochemistry and genetics of actinomycete cellulases," *Critical Reviews in Biotechnology*, vol. 12, no. 1-2:45–63.

Yamac, M. and Tamer, U. (2008): Lignin Degradation and Acid Precipitable Polymeric Lignin (APPL) Accumulation by Selected *Streptomyces* Strains in Submerged and Solid State Culture Systems. J. Appl. Biol. Sci. 2: 55-61.

Zimmermann, W., (1990): Degradation of lignin by bacteria. J. Biotechnol. 13:119-130.

مسح وتعريف بعض الإستربتوميسيتات المنتجة للّجنين المتعدد والمترسب حامضيا

محمود متولي نور الدين ، محمد إسماعيل أبو دبارة و نهى السيد أبو الحمد

قسم النبات والميكروبيولوجى – كلية العلوم – جامعة دمياط – جمهورية مصرالعربية.

يهدف هذا البحث إلى عزل وتعريف بعض الأنواع من الإستربتوميسيتات المنتجة للّجنين المتعدد والمترسب حامضيا، وقد تم عزل الأكتينوميسيتات من تربة مصرية محافظة (دمياط) بواسطة بيئة أجار نترات النشا ثم تم عمل مسح لتلك العزلات بزراعتها على وسط مستخلص الخميرة والمحتوى على أملاح بكميات قليلة ومزودة بقش الأرز وقش القمح لدراسة قدرتها على إنتاج اللجنين المتعدد والمترسب حامضيا.أظهرت عملية المسح وجود ثلاث عزلات من الأكتينوميسيتات التي تنتج اللجنين المتعدد والمترسب حامضيا وتنتج أيضا انزيمى السليلوز والزيلانيز بمعدلات مختلفة. تم تعريف تلك العزلات باستخدام الخصائص المورفولوجية والفسيولوجية والتصنيف الكيميائى وأوضحت النتائج احتمالية انهم إستربتوميسيس كانيس، إستربتوميسيس ليبانى وإستربتوميسيس سيانيسز.