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STREPTOMYCES SPECIES ABLE TO UTILIZE SOME HERBICIDES AS NITROGEN AND CARBON SOURCES

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ABSTRACT

The present work was designed to isolate and identify some actinomycetes able to degrade Basta (glufosinate) and Sencor (metribuzin) herbicides, which are widely used for weed control in Egypt. Results showed that 100 isolates of actinomycetes were isolated and purified from the rhizosphere soils of 11 different crops (barley, broad bean, clover, cotton, corn, grape, cantaloupe, pepper, sesame, tomato and wheat) treated with pesticides. The tolerance of the actinomycete isolates for Basta and Sencor herbicides were determined. Results showed that, 70 out of the 100 actinomycete isolates were able to grow on the recommended dose of Sencor (0.75 g/L) but 24 out of them were showed a good growth on the ten folds of the recommended dose of Sencor (7.5 g/L). At the same time, 38 actinomycete isolates grew on the recommended dose of Basta (2 g/L) and 18 of them appeared a moderate growth on 20 g/L of Basta herbicides. In addition, the ability of the 70 isolates to utilize the Sencor as carbon and/or nitrogen source was studied. Results showed that, 9 out of 70 actinomycete isolates gave a good growth on the starch nitrate agar medium containing the Sencor as a sole nitrogen source, while no isolates were found to be able to grow on the same medium with the Sencor as a sole carbon source.In this study, 5 isolates were biologically identified and found to be strains of *Streptomyces rectiviolaceus, S.roseolus, S.albosporeus subsp abilomycaticus, S. herbaricolor and S. aureomonopodiales.*

Key words: Actinomycetes, *Streptomyces*, Pesticides, Sencor, Basta, Herbicides.

INTRODUCTION

Microorganisms degrade herbicides by a number of biochemical reactions (Kaufman,1974, Kearney and Kaufman, 1975 and Kaufman *et al*., 1976), including utilizing herbi-cides as a carbon or nitrogen sources (Shelton *et al*., 1996, Gill and Sunita Gill, 1999, Hu *et al*., 2004, Li *et al*., 2008, Monika *et al*., 2009, Sebiomo *et al*., 2011 and Ngigi *et al*., 2012).

Regarding microbial metabolism of triazines, Cook and Hütter (1981 a and b) discussed the degradation of triazine herbicides. They reported that the rapid and complete bacterial degradation of the s-triazines by two strains of *Klebsiella pneumoniae* and three *Pseudomonas* spp. isolated from sewage and soil. Vandenbergh *et al*., (1981) isolated a bacterium, *Pseudomonas cepacia* from soil of a landfill area and found that the organism could utilize 2,6-dichloro toluene as a carbon source for growth. Giardina *et al*., (1982) described the degradation of the pre- and post- emergence herbicide atrazine by a *Nocardia* sp. Grossenbacher *et al*., (1984) reported

that the bacterium *Rhodococcus corallinus* isolated from the soil utilized the deethylsimazine molecule as source of nitrogen for growth. Shelton *et al*., (1996) showed that *Streptomyces* (strain PS 1/5) metabolized atrazine (metribuzin) contaminated soil.They showed that within 28days from *Streptomyces* inoculation, ca. 78% of 20µg/g of atrazine was removed. Ma *et al.*, (2006) isolated three strains of *Actinomyces* from soil planted with Panax and which had been applied with atrazine for several years. They showed that the half life of Panax was 26.6-28.4 days, and after *Actinomyces* inoculation in the soil, the half life became 2-20 days.

Considering the herbicide glufosinate,Tachibana (1987) reported that bialaphos was a tripetide exhibiting herbicided properties, provided two alanine molecules and L-2-amino-4-hydroxymethylphosphinol butyric acid called phosphi- nothricin (PPT) or glufosinate (Basta), a phosphinic analoge of glutamate. Quinn *et al.*, (1993) reported that the glufosinate was utilized by mixed bacterial population as a sole phosphorus source. Allen *et al*., (1995) reported that the herbicide glufosinate ammonium was persistent in aerobic sandy aquifer material in laboratory batch and field in situ microcosms when added at concentrations of 50-400µg/L. The microbio- logical tests showed that glufosinate ammonium and sodium glufosinate was used as a nitrogen source in the presence of sufficient carbon. The aim of present work was to isolating and identifying some actionmycetes able to degrade Sencor and Basta herbicides and use them as sole carbon and/or nitrogen sources.

MATERIALS AND METHODS

Isolation of actinomycete isolates: Rhizosphere soil samples of different crops namely, barley, broad bean, clover, cotton, corn, grape, cantaloupe, pepper, sesame, tomato and wheat was collected from soil previously treated with pesticides and were used as sources for isolation, purification and maintenance of actionmycete isolates using starch nitrate agar medium (Waksman and Lechevalier, 1961). Isolation was carried out by plate technique. Inoculated plates were incubated at 28°C±2 for 10 days. Actinomycete colonies appearing on plates were picked up and purified by streaking technique and maintained on agar slant at 4-5°C until used, while subculturing was usually carried out every two month.

Herbicide-tolerance of actinomycetes: All purified actinomycete isolates were *in vitro* tested for their abilities to grow in the presence of Sencor and Basta herbicides in starch nitrate agar medium. For each herbicide, two concentrations were applied, *i.e.*,equivalent of the recommended dose (0.75g/L Sencor and 2 g/L Basta) and 10 folds of the recommended dose (7.5g/L Sencor and 20g/L Basta). Inoculated plates were inoculated at 28°C±2 for 15 days. The growth of actinomycete isolates with standing the toxicity of the herbicide was determined and recorded as no (-), weak (+), moderate (++) and abundant (+++) growth.

***In vitro* utilization of Sencor by actinomycete isolates as carbon and/or nitrogen source:** Isolates tolerating Sencor herbicide were tested for their abilities to utilize the herbicide as a sole source of carbon and/or nitrogen. Growth of each isolate was determined when carbon or nitrogen or both were omitted from the starch nitrate agar medium supplemented with Sencor at 0.75 g/L

(equivalent recommended dose). Two controls were used, complete medium with and without Sencor. Inoculated plates were incubated at 28°C±2 for 10 days. Then growth of the each isolate was determined as mentioned before.

Identification of selected actinomycetes isolates: Isolates which were able to degrade and/or utilize Sencor as a sole carbon and/or nitrogen source were completely identified according to the standard methods adopted by Shirling and Gottlieb (1966). The keys proposed by Küster (1972) and Pridham and Tresner (1974) were consulted. The descriptions of *Streptomyces* species of the International *Streptomyces* project (I.S.P.) introduced by Shirling and Gottlieb (1968 a, b and 1972) were also used.

RESULTS AND DISCUSSIONS

Environmental contamination by nitroaromatic compounds such as 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-tri nitro-1,3,5-s-triazine (RDX), atrazine, and/ or simazine (TRAS) generated as waste from military and agricultural activities was a serious worldwide problem (Cho *et al*., 2008).

In this study, rhizosphere soil samples of different crops grown in soils previously treated with pesticides were used as sources for isolating actinomycetes. Results showed that a number of 100 *actinomycete isolates from the rhizosphere of differe*nt crops were obtained. Of these 22 were isolated from barley, 11 (broad bean),7 (clover),4 (cotton),9 (corn),9 (grape), 4 (kantaloupe), 5 (pepper), 3 (seasmy), 9 (tomato) and 17 (wheat). These isolates were purified and tested for their tolerance to Sencor and Basta herbicides at recommended and ten folds of the recommended dose for each.

Data in Table-1 reveal that actinomycete isolates varied greatly in their tolerance to the two herbicides used and/or their concentrations applied. Sencor at recommended dose (0.75g/L) suppressed the growth of 30% of action-mycete isolates, while the remainder 70% isolates showed moderate to abundant growth. Only 38 out of these 70 isolates tolerated Basta herbicide at the recommended dose (2g/L) (Table-2**).** Generally, the growth of each isolate was more abundant in the presence of Sencor than in Basta treatment. This was more pronounced for actinomycete isolates obtained from barley rhizosphere where 20 isolates out of 22 showed abundant growth in Sencor treatment while only 12 of them showed moderate or weak growth in Basta treatment. Similarly, 6 out of 9 isolates obtained from tomato rhizosphere showed weak growth in Sencor treatment while only one of them tolerated Basta toxicity. In other mean, Basta herbicide was more toxic to actinomycete isolates than Sencor and the majority of tested isolates tolerated Sencor at recommended dose (70%), while relatively small portion of them could withstand the toxicity of Basta (38%) at its recommended dose. With increasing Sencor and Basta concentrations to ten folds of the recommended dose, the inhibitive effect was severe and more pronounced in the case of Basta herbicide. Only 24 out of 70 actinomycete isolates showed scanty growth at 7.5g/L Sencor and 18 of these 24 isolates showed weak growth at 20g/L Basta herbicide.

Actinomycete isolates from different rhizospheres varied greatly in their tolerance to tested herbicides (Table-3). Barley isolates were the most tolerant to Sencor at recommended dose (91%), followed by those of wheat (88%), others were more sensitive. In case of Basta the situation differed where isolates obtained from pepper rhizosphere showed highest tolerance

(60%) followed by those of grapes (56%), cotton and cantaloupe (50%), the remainder isolates showed lesser degrees of tolerance.

It was also noted that Basta was more inhibitive to actinomycete isolates than Sencor when both herbicides were applied at their recommended dose. This inhibitive effect was more severe as concentration of each increased to 10 folds of the recommended dose. However, the *in vitro* effect of herbicides in general and triazines in particular on actinomycetes when applied at equivalent of recommended dose in culture was varied greatly. Gusterov *et al.*, (1972) showed that Desmetryne inhibited 50% of actinomycetes in culture at concentrations of 8-100ppm, while atrazine and simazine at the same rates showed no effect on actinomycetes (Krezel and Kosinkiewicz, 1972). As far glufosinate (Basta), Quinn *et al.*, (1993) reported that only 84 out of 227 actinomycete isolates grew at 1mM, while only 38 grew at 3mM.

Table-1: Growth of actinomycete isolates obtained from different rhizospheres in the presence of different concentrations of Sencor herbicide.

	Growth in the presence of Sencor herbicide							
AI/R	RD	10RD	SI/R	RD	10RD	SI/R	RD	10RD
Barley			35	++	+	67	+	-
1	+++	+ +	36	-	-	68	-	-
2	++	-	37	+	-	69	+	-
3	+++	+++	38	+ +	+	70	-	-
4	+++	+ +	39	-	-	71	+++	+ +
5	++	-	40	+++	++	Sesame		
6	+++	+ +	Cotton			72	+++	+ +
7	++	-	41	-	-	73	-	-
8	+++	++	42	-	-	74	+	-
9	+ +	-	43	-	-	Tomato		
10	-	-	44	+++	+ +	75	+	-
11	-	-	Corn			76	+	-
12	++	-	45	-	-	77	-	-
13	+++	++	46	-	-	78	-	-
14	+++	++	47	++	+	79	+	-
15	++	-	48	-	-	80	+	-
16	++	-	49	+	-	81	+	-
17	++	-	50	+ +	+	82	+	-
18	+ +	-	51	-	-	83	-	-
19	+ +	-	52	-	-	Wheat		
20	+ +	-	53	-	-	84	+	-
21	+ +	-	Grape			85	+++	+ +
22	+ +	-	54	+++	+ +	86	+++	+ +
Broad bean			55	+ +	+	87	-	-
23	+ +	-	56	+	-	88	+	-
24	+++	++	57	-	-	89	+	-
25	+	-	58	+	-	90	+	-
26	+	-	59	++	+	91	+	-
27	+	-	60	+	-	92	+++	+ +
28	+	-	61	+	-	93	+	-
29	+	-	62	-	-	94	+	-
30	-	-	Cantaloupe			95	+++	+ +

Table-1: Continue.

31	-	-	63	++	+	96	+	-
32	-	-	64	+	-	97	-	-
33	-	-	65	-	-	98	+	-
Clover			66	-	-	99	+	-
34	-	-	Pepper			100	+	-

RD: Recommended dose (0.75 g/L). 10 RD: Ten folds of recommended dose (7.5g/L). Densities of growth: -, No growth; +, Weak growth; ++, moderate growth. +++: abundant growth. AI/R: Actinomycete isolates/ Rhizospheres.

Table-2: Growth of actinomycete isolates obtained from different rhizospheres in the presence of different concentrations of BASTA herbicide.

	Growth in the presence of BASTA herbicide							
AI/R	RD	10RD	SI/R	RD	10RD	SI/R	RD	10RD
Barley			35	+	+	67	+	-
1	++	+	36	-	-	68	-	-
2	-	-	37	-	-	69	+	-
3	++	+	38	+	+	70	-	-
4	++	+	39	-	-	71	++	+
5	-	-	40	++	+	Sesame		
6	++	+	Cotton			72	-	-
7	+	-	41	+	-	73	-	-
8	-	-	42	-	-	74	-	-
9	+	-	43	-	-	Tomato		
10	-	-	44	+	+	75	-	-
11	-	-	Corn			76	-	-
12	+	-	45	-	-	77	-	-
13	++	+	46	-	-	78	-	-
14	+	-	47	++	+	79	-	-
15	+	-	48	-	-	80	-	-
16	+	-	49	+	-	81	-	-
17	+	-	50	+	-	82	+	-
18	-	-	51	+	-	83	-	-
19	-	-	52	-	-	Wheat		
20	-	-	53	-	-	84	+	+
21	-	-	Grape			85	-	-
22	-	-	54	+	-	86	++	+
Broad bean			55	++	+	87	-	-
23	-	-	56	-	-	88	-	-
24	++	+	57	-	-	89	-	-
25	-	-	58	-	-	90	-	-
26	-	-	59	+	+	91	-	-
27	-	-	60	-	-	92	+	-
28	-	-	61	+	-	93	+	-
29	-	-	62	+	-	94	-	-
30	-	-	Cantaloupe			95	-	-
31	-	-	63	+	-	96	++	+
32	-	-	64	+	-	97	-	-
33	-	-	65	-	-	98	-	-

Table-2: Continue.

Clover			66	-	-	99	-	-
34	+	-	Pepper			100	-	-

RD: Recommended dose (2 g/L). 10 RD: Ten folds of recommended dose (20 g/L). Densities of growth: -, No growth; +, Weak growth; ++, moderate growth. +++: abundant growth. AI/R: Actinomycete isolates/ Rhizospheres.

Table -3: Actinomycete isolates tolerating different concentrations of Sencor and Basta herbicides.

Rhizosphere soil	Total No. of isolates	Total numbers of isolates tolerating herbicides			
		Sencor		Basta	
		0.75 g/L	7.5 g/L	2 g/L	20 g/L
Barley	22	91	0	14	5
Broad bean	11	64	9	9	9
Clover	7	57	43	43	43
Cotton	4	25	25	50	25
Corn	9	33	22	44	11
Grape	9	78	33	56	22
Cantaloupe	4	50	25	50	0
Pepper	5	60	20	60	20
Sesame	3	67	33	0	0
Tomato	9	67	0	11	0
Wheat	17	88	24	29	18
Total	100	70	24	38	18

In vitro* ability of actinomycete isolates to grow on Sencor herbicide as a sole carbon and/or nitrogen source:* Gill and Gill (1999) showed that atrazine was utilized as a carbon and energy source by *Pseudomonas aeruginosa* when added as a sole source to the synthetic medium. Atrazine degradation was maximum with an inoculum size of 8.0 ml (OD600=1.0) for a 48 h incubation period at pH 6.5 and 30^{o}C. In 2011, Sebiomo *et al*., determined the abilities of 12 bacterial isolates to utilize atrazine and primextra and the degradation dynamics of the two herbicides in soil. Utilization of atrazine and primextra were determined by monitoring growth rates of the bacteria, *Actinomyces* and *Streptomyces via* viable counts, optical density and pH changes.

Since Sencor was widely used for weed control in Egypt, the ability of the 70 actinomycete isolates which tolerated the toxicity of this herbicide at the recommended dose were tested for their abilities to utilize this herbicide as carbon and/or nitrogen source *in-vitro*. In fact, the 70 actinomycete isolates included all the isolates tolerating both Sencor and Basta herbicides at the two concentrations applied. In general, the growth of most tested isolates in the presence of Sencor was less than that on the complete starch nitrate medium without Sencor (Table-4). As expected no growth was attained in the medium supplemented with Sencor and deficient in either carbon and nitrogen sources or carbon source. When nitrogen was omitted from the medium, only 9 isolates (12.9%) showed less growth as compared with that observed in complete medium supplemented with Sencor. Such growth in the absence of nitrogen source in growth medium might indicate the biodegradation of Sencor and its utilization as a sole nitrogen source for

growth. In addition, these isolates failed to use the herbicide as a sole carbon source.

Shelton *et al.*, (1996) reported that some *Streptomyces* strains were capable of slowly degrading simazine or triazines using the herbicide molecule as a source of either carbon or nitrogen. Moreover, they revealed that the biodegradation of triazines occurred concurrently with growth suggesting the selective induction of certain metabolic enzymes. However, s-triazine compounds were recorded to serve as a sole nitrogen source for a variety of bacteria (Jessee *et al.*, 1983 and Grossenbachar *et al.*, 1984). Hu *et al.*, (2004) isolated a Gram positive bacterial strain BTAH1 after domestication from herbicide contaminated soil, which used atrazine as the sole carbon and nitrogen source for growth. The strain degraded 1000mg/L atrazine within 126 hours completely. Li *et al.*, (2008) isolated a bacterial strain (AD26) capable of utilizing atrazine as a sole nitrogen source for growth from an industrial wastewater sample by enrichment culture.

This study paid an attention to the important differences in the ability of sencor-hydrolyzing streptomycetes to degrade this compound in soil, and suggested that the ability to utilize sencor as a carbon source or nitrogen sources was important to establish enhanced degradation by ecologically meaningful inoculum densities. This note could be supported by the studies of Topp (2001) and Stamper *et al.*, (2002).

Table-4: Ability of actinomycete isolates to grow on Sencor herbicide as sole carbon and/or nitrogen source.

Actinomycete isolates		Complete medium*	Growth in the presence of Sencor (0.75 g/L)			
			Complete medium*	Carbon omitted	Nitrogen omitted	Nitrogen & carbon omitted
Barley	**1**	+++	+++	-	++	-
	2	+++	++	-	-	-
	3	+++	+++	-	++	-
	4	+++	+++	-	-	-
	5	+++	++	-	-	-
	6	+++	+++	-	-	-
	7	+++	++	-	-	-
	8	+++	+++	-	-	-
	9	+++	+	-	-	-
	12	+++	++	-	-	-
	13	+++	+++	-	-	-
	14	+++	+++	-	-	-
	15	+++	++	-	-	-
	16	+++	++	-	-	-
	17	+++	++	-	-	-
	18	+++	++	-	-	-
	19	+++	++	-	-	-
	20	+++	++	-	-	-
	21	+++	++	-	-	-
	22	+++	++	-	-	-
Broad bean	**23**	+++	++	-	-	-
	24	+++	+++	-	++	-
	25	+++	+	-	-	-
	26	+++	+	-	-	-
	27	+++	+	-	-	-
	28	+++	+	-	-	-

Table-4: Continue.

	29	+++	+	-	-	-
Clover	35	+++	++	-	-	-
	37	+++	+	-	-	-
	38	+++	++	-	-	-
	40	+++	+++	-	++	-
Cotton	44	+++	+++	-	-	-
Corn	47	+++	++	-	+	-
	49	+++	+	-	-	-
	50	+++	++	-	+	-
Grape	54	+++	+++	-	-	-
	55	+++	++	-	+	-
	56	+++	+	-	-	-
	58	+++	+	-	-	-
	59	+++	++	-	-	-
	60	+++	+	-	-	-
	61	+++	+	-	-	-
Cantaloupe	63	+++	++	-	-	-
	64	+++	+	-	-	-
Pepper	67	+++	+	-	-	-
	69	+++	+	-	-	-
	71	+++	+++	-	++	
Sesame	72	+++	+	-	-	-
	74	+++	+	-	-	-
Tomato	75	+++	+	-	-	-
	76	+++	+	-	-	-
	79	+++	+	-	-	-
	80	+++	+	-	-	-
	81	+++	+	-	-	-
	82	+++	+	-	-	-
Wheat	84	+++	+	-	-	-
	85	+++	+++	-	-	-
	86	+++	+++	-	++	-
	88	+++	+	-	-	-
	89	+++	+	-	-	-
	90	+++	+	-	-	-
	91	+++	+	-	-	-
	92	+++	+++	-	-	-
	93	+++	+	-	-	-
	94	+++	+	-	-	-
	95	+++	+++	-	-	-
	96	+++	+	-	-	-
	98	+++	+	-	-	-
	99	+++	+	-	-	-
	100	+++	+	-	-	-
Total	**No. 70**	**70**	**70**	**0**	**9**	**0**
	% 100	**100**	**100**	**0**	**12.9**	**0**

*** Starch nitrate agar medium -: No growth +: Weak growth. ++: Moderate growth. +++: Abundant growth.**

Identification of actinomycete isolates: It was found of importance to find out the taxonomical variations which might exist between the actinomycete isolates able to degrade and utilize the Sencor herbicide as a sole nitrogen source. Therefore, B3, BB24, Clo40, P71 and W86 isolates which degraded Sencor into 1,2,3, 4 and 5 compounds respectively were completely identified. Isolates were microscopically examined. The cultural and the morpho-logical characteristics of these isolates revealed that all of them belong to the genus *Streptomyces*, since they formed as well developed branching non-septate aerial mycelia carrying long spore chains. The non-motile spores were not borne on verticillate sporophores (Pridham and Tresner, 1974). These five streptomycetes were completely identified as mentioned in the materials and methods.

***Streptomyces* isolate B3:** It was found that the *Streptomyces* isolate B3 belonged to the violet colour series, while the vegetative mycelium was pigmented with brown or red colour. This isolate had straight or flexuous spore chain (section Rectus-flexibilis, RF) with smooth surface (Figures-1A and 2A). Melanoid pigments were produced on tyrosine agar medium (Shinobu,1958),peptone-yeast extract iron agar medium (Tresner and Danga, 1958) and tryptone-yeast extract broth medium (Pridham and Gottlieb, 1948). It gave also a good growth on Cazpek's agar medium and actively utilized D-glucose, D-xylose, L-arabinose, L-rhamnose, D-fructose, raffinose, D-mannitol, i-inositol and sucrose as carbon sources for growth.

This isolate showed antagonistic activity against the 9 test organisms. However, good growth was observed in the presence of 4 µg/ml streptomycin antibiotic in the medium. This isolate was tolerant to NaCl up to concentration of 15%. According to the key proposed by Pridham and Tresner (1974), the experimental isolate B3 appeared to be related to *Streptomyces rectiviolaceus* although there was slight difference in the melanoid pigment production. No data concerning *S. rectiviolaceus* was reported in description of the species recorded in the I.S.P. (Shirling and Gottlieb, 1968a, b and 1972) or the key proposed by Küster (1972). Therefore, isolate B3 could be considered a strain of *S. rectiviolaceus.*

***Streptomyces* isolate BB24:** Results clearly indicate that the *Streptomyces* isolate BB24 was belonged to the red colour series and the substrate mycelium produced yellow pigment on the standard media used. Aerial spore chains belonged to section RF and the spores were characterized by smooth surface without any ornamentation (Figures 1B and 2B). Melanoid pigments were not detected on the standard media used. This isolate was characterized by good growth on Cazpek's agar medium. The physiological characteristics showed that D-glucose, D-xylose, L-arabinose, L-rhamnose and D-mannitol were used as carbon sources for growth, while growth on sucrose was very slight. D-fructose, raffinose and i-inositol did not support any growth. In addition, this isolate showed antimicrobial activities against the nine test organisms used and no sensitivity to streptomycin (4µg/ml) was observed. However, it was able to grow in the presence of 10% NaCl in the medium. Comparing the cultural, morpho-logical and physiological characteristics of the *Streptomyces* spp. in Shirling and Gottlieb (1968a), Küster (1972) and Pridham and Tresner (1974) with those of *Streptomyces* isolate BB24, the isolate

was very likely to be a strain of *S. roseolus* with slight differences in the colour of substrate mycelium, in the utilization of D-fructose and D-mannitol as carbon sources for growth.

***Streptomyces* isolate Clo40:** Results of *Streptomyces* isolate Clo40 show that, this isolate had white aerial mycelium (white colour series), while the vegetative myce-lium was pigmented with yellow colour. It had straight and long spore chains (section RF) (Figure 1C) and the spores were characterized by smooth surface without any ornamentations (Figure-2C)**.** This isolate was also characterized by moderate growth on Cazpek's agar medium, actively utilized D-glucose, L-rhaminose and raffinose for growth, slightly utilized sucrose. But it failed to utilize D-xylose, L-arabinose, D-fructose, D-mannitol and i-inositol, toleranant to NaCl concentration up to 10%, not sensitive to streptomycin (4µg/ ml) and antagonized all the test organisms used. Considering the descript-tion keys proposed by Pridham and Tresner (1974)**,** the tested isolate Clo40 was closely related *to S. alboporeus* subsp. *Labilo-mycaticus*, although there were slight differences in its utilization of D-xylose and L-rhamnose for growth and in the melanoid pigment production. Therefore, the tested isolate Clo40 could be identified as a strain of *S. alboporeus* subsp *labilomycaticus*.

***Streptomyces* isolate P71:** Results showed that the isolate P71was characterized by gray aerial mycelium (gray colour series) and the reverse side of substrate mycelium was yellow on all standard media used. A spore chain was belonged to section RF (Fig.-1D**)** with smooth surface (Fig.-2D). This isolate was also found to produce melanoid, did not produce soluble pigments and had a good growth on Cazpek's medium. Concerning the utilization of carbon sources, the isolate was able to give a good growth in the presence of D-glucose, D-xylose, L-rhamnose, raffinose or sucrose as sole carbon source, while it failed to use L-arabinose, D-fructose, D-mannitol and i-inositol. The isolate also antagonized all tested organisms, was inhibited with streptomycin (4µg/ml) and grew on NaCl concentrations up to 10%. Considering the description of *Streptomyces* spp. present in Shirling and Gottlieb (1968b) and Pridham and Tresner (1974)**,** the *Streptomyces* isolate P71 was closely related to *S. herbaricolor* and could be identified as a strain of *S. herbaricolor* with slight differences in the producing melanoid pigments, in utilization of L-arabinose or D-fructose for growth as sole carbon source and in tolerance to NaCl concentrations.

***Streptomyces* isolate W86:** Results revealed that the characteristics of *Strepto-myces* isolate W86 appeared to closely resemble to *Streptomyces aureomono-podiales* based on the description keys proposed by Pridham and Tresner (1974) with some exceptions. The cultural, morphological and physiological characteristics of the tested isolate W86 clearly showed that, the color of aerial mycelium was red (red colour series) while the reverse side of substrate mycelium was yellow. Spore chains were belonged to RF section with smooth surface (Figures-1E and 2E). No soluble and melanoid pigments were produced in all standard media used. Good growth on Cazpek's agar medium was noted. Concerning the utilization of carbon compounds as sole carbon source, it was noted that this isolate actively utilized all of the carbon sources used for growth except for the L-rhamnose. It is worthy to

mention that this isolate antagonized the nine test microorganisms used and was not affected by streptomycin (4µg/ml). In addition, it was able to grow on NaCl up to 10%. However, cultural and morphological characteristics of our isolate differed for that of *S. aureomonopodiales* noted in the key of Küster (1972) in that it gave red aerial mycelium while in Kuster it gave white aerial mycelium. Such difference could be attributed to the use of four standard media while Küster (1972) used only one of them. Therefore, it could be concluded that the *Streptomyces* isolate W86 was very likely to be a strain of *S. aureomono podiales.*

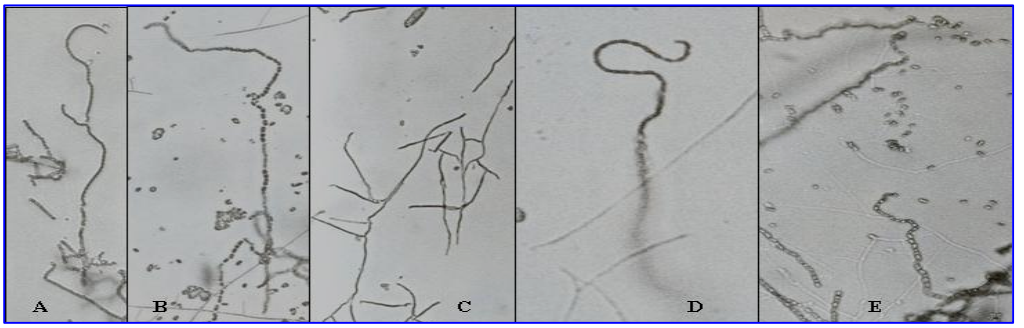

Figure-1: Microphotograph of spore chain morphology of *Streptomyces* isolates B3 (A), BB24 (B), Clo40 (C), P71 (D), W86 (E) (X-400).

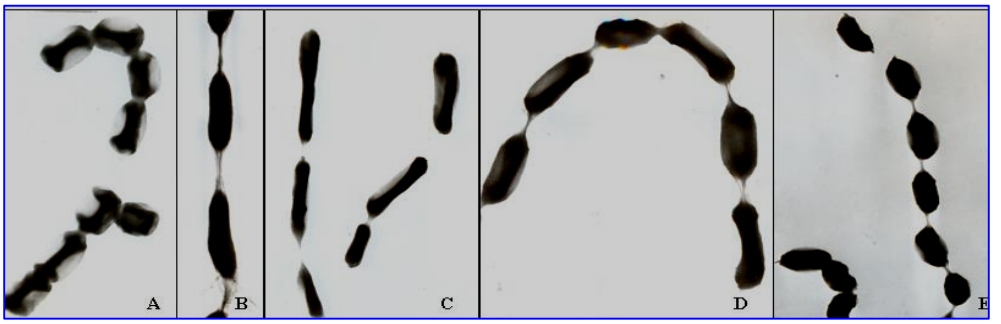

Figure-2: Electron micrograph of spore surface of *Streptomyces* isolates B3 (A), BB24 (B), Clo40 (C), P71 (D), W86 (E) B3 (X-20000).

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