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SCREENING OF ANTIMICROBIAL AND CYTOTOXIC ACTIVITIES OF SOME ACTINOMYCETES ISOLATED FROM CONSTRUCTED WETLAND SYSTEM

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ABSTRACT

A total of 174 actinomycete cultures were isolated from a constructed biological water treatment system (BIOWATSYST) established at Abu Attwa station in Ismailia city, Egypt and funded by the European Commission Grant No.IC18-CT97-0163. The isolates were identified to belong to eight genera; *Nocardia*, *Streptomyces*, *Intrasporangium*, *Micromonospora*, *Nocardioides*, *Actinomadura*, *Nocardiopsis*, *and Thermomonospora*. They were screened for their antibacterial, antifungal and cytotoxic activities against certain human and plant pathogens. Antimicrobial activities were determined by measuring bacterial and fungal growth inhibitions while cytotoxic activity was studied by using the *Artemia salina* bioassay. Thirty two percent of isolated cultures displayed antibacterial activity, 15% displayed antifungal activity and 9% displayed cytotoxic activity. Members of genus *Streptomyces* has recorded as the most frequent active isolates against tested bacteria (42%) and fungi (49%). However, the most cytotoxic activity was found with members of genus *Nocardia* (46%). Results evaluated the fact that actinomycetes isolated from such systems could be considered as promising source for antimicrobial and cytotoxic bioactive agents.

Keywords: Actinomycetes, Antibacterial, Antifungal, Cytotoxicity

INTRODUCTION

Actinomycetes have long been recognized as inhabitants of biological wastewater treatment systems **(Gerardi and Horsfall 1994).** They grow more slowly than other genera of bacteria found in the wastewater environment and are usually present in low numbers relative to other microorganisms. They possess several characteristics, which make them well suited for growth in the wastewater environment either suspended in the wastewater such as in activated ponds or fixed to the biofilms such as in constructed wetlands **(Thomsen *et al* 2002 and Nielsen *et al* 2004).** The ecological study of actinomycetes in various habitats is important for the discovery of strains that produce novel and useful bio-

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active substances **(Lee and Hwang 2002).**

They are prokaryotes with extremely various metabolic possibilities and they produce numerous substances essential for health such as antibiotics, enzymes and immunomodulators **(Iwami *et al* 1987 and Chipeva *et al* 1996)**. They are also one of the most attractive sources of other biologically active substances; such as vitamins, alkaloids, plant growth regulators and enzyme inhibitors **(Dewedar *et al* 1977; Brady & Weil 2002 and Saito *et al* 2003)**. Some strains were reported to produce metabolites of different biological activities such as cancer-fighting bioagents **(Gorman, 2003)**, antibacterial, antifungal, cytotoxic, herbicides, pesticides and insecticides **(Waugh and Long 2002)**.

Antagonistic actinomycetes can produce antibiotics or toxic metabolic byproducts that inhibit vegetative growth, spore germination, or sporulation by the pathogen; directly parasitize the pathogen; or compete with the pathogen for some limited resource it needs to cause infections. Another mechanism of biological control that does not require direct interaction of the antagonists and pathogen at the infection site is induced resistance **(Hardy and Sivasithamparam 1995)**.

Sosio *et al* (2004) stated that actinomycetes are a major source of antibiotics and have separated them into three groups, antifungal antibiotic producers, antibacterial antibiotic producers and antitumor antibiotic producers. **Moncheva *et al* (2002)** reported strains of actinomycetes which showed antagonistic activity against Gram-positive and Gram-negative bacteria. These strains possessed a broad spectrum of antibacterial activity. They were active against clinical isolates from the species *Staphylococcus aureus* and *Streptococcus pneumoniae.*

Scientists have found a large source of previously unknown strains of actinomycetes that may produce chemicals with cytotoxic properties which may promote antitumor activity. Neocarzinostatin (NCS), the first member of the enediyne family of antitumor antibiotics, was originally discovered as a macromolecular antitumor antibiotic from the culture filtrates of a *Streptomyces carzinostaticus* strain **(Ishida *et al* 1967)**.It, also, was the discovery of the Calichemicins from a *Micromonospora echinospora* strain **(Lee *et al* 1987a and Lee *et al* 1987b)** and the esperamicins from an *Actinomadura verrucosospora* strain **(Golik *et al* 1989)**.

The aim of the present study is to screen some promising actinomycetes isolated from biological wastewater treatment system for antimicrobial and cytotoxic bioactivities in relation to some ecological aspects.

MATERIAL AND METHODS

Water (influent and outlets), gravel, sand and *Phragmites australis* root samples were collected from the three treatment beds containing deep gravel, B.2 (60 cm depth), deep sand, B.4 (60 cm depth) and a mixture of gravel-sand, B.6 (20 cm sand + 60 cm gravel) as filling materials. Water samples were collected according to the method described by **American Public Health Association, APHA (1985)** while gravel, sand and root samples were collected according to the method described by **Wollum (1982)**. Actinomycetes were isolated and subcul-

tured at seasonal interval for one year on starch casein medium according to the method described by **Kuster and Williams (1964)**. Incubation was done at 28º C and 55º C for both mesophilic and thermophilic isolates respectively. The isolates were identified to the generic level according to Bergey's manual of systematic bacteriology **(Williams *el al* 1989)** and Bergey's manual of determinative bacteriology **(Holt *et al* 1994)**. Antibacterial activity of the isolates was evaluated by well-agar diffusion method according to **Case and Warner (2001)** against Gram-positive (*Bacillus subtilis* NRS-744, *Staphylococcus aureus* B-767) and Gram-negative bacteria; (*Klebsiella pneumoniae* B-3522, *Proteus vulgaris* B-123, *Escherichia coli* B-3704, *Pseudomonas aeruginosa* B-23). These isolates were kindly supplied by the Microbial Properties Research Unit, National Center for Agricultural Utilization Research, Agriculture Research Service, USA.

The antifungal activity of the isolates was proceeded by the disk-agar diffusion method **(Acar and Goldstein 1996)** using Czapek-Dox **(MacFaddin1985)** agar cultures of *Fusarium fabae*, *Alternaria alternata*, *Botrytis cinerea*, *Sclerotinia sclerotiorum* and *Rhizoctonia solani* as phytopathogenic-tested fungi and Sabouraud's Dextrose **(MacFaddin1985)** agar cultures of human pathogenic yeasts; *Candida albicans* and *C. pseudotropicalis*. These isolates were kindly supplied by plant pathology laboratory, Faculty of Science, Suez Canal University. All the assays were carried out in triplicate and the agar plates were incubated at 37ºC for 48 hours for bacterial cultures and 28 ºC for 4-7 days for yeast and fungi, respectively. The diameters of inhibition zone were measured in centimeters.

Assay for detection of cytotoxicity effect was carried out using the brine shrimp (*Artemia salina*) bioassay according to **Svoboda and Hampson (1999)**. Brine shrimp eggs obtained were hatched in sea water supplemented with 6 g/l dried yeast and oxygenated with an aquarium pump. After 48 hours incubation in a warm room (22-29ºC), viability of nauplii was tested. Culture filtrates of the isolates were applied in concentrations of 10, 30 and100 µl/ml of *Artemia* stock medium. The lethal concentration for 50% mortality after 24 hrs, LD_{50}, has been used as a criterion for cytotoxicity **(Meyer *et al* 1982; Mongelli *et al* 1996 and Doganca *et al* 1997)**. *Artemia* stock medium which containing (45-70) lived nauplii was used as control. The significance of the test lies mainly as an indicator for the possible cytotoxicity of actinomycetes or for usage as an insecticide.

RESULTS AND DISSCUSION

The survey procedure resulted in the isolation of 174 actinomycete cultures from the constructed biological wastewater treatment system. They were identified as belonging to the genera, *Nocardia* ($96.1x10^3$cfu/ml; approximately 33%), *Streptomyces* (89.2×10^3 cfu/ml; approximately 31%), *Intrasporangium* (55.4×10^3 cfu / ml; approximately 19%), *Micromonospora* (15.2×10^3 cfu/ml; approximately 5%), *Nocardioides* ($14.9x 10^3$cfu/ml; approximately 5%), *Actinomadura* (10.8×10^3 cfu/ml; approximately 4%), *Nocardiopsis* ($64.6x10^2$ cfu/ml; approximately 2%) and *Thermomonospora* (61.9 cfu / ml; approximately 0.02%), (Fig. 1). The genera, their nature, season of isolation, locality and samples in addition to antibacterial, antifungal and cytotoxic activities are given in Table (1).

Actinomycetes are undoubtedly numerous in sewage water environment and sewage water treatment systems and have important roles in their decomposition.

The growth of many actinomycetes in sewage water biological treatment system may be explained by the fact that they are able to grow on a wide range of organic compounds, including recalcitrant substrates, such as long-chain hydrocarbons, pesticides, complex aromatic compounds, and dead microbial biomass, and may actually be important in the removal of these compounds **(Dewedar *et al* 1993; Lacey 1997; Schuppler *et al* 1998; Bond *et al* 1999 and El-Shatoury *et al* 2004)**.

The screening procedure for antimicrobial activity revealed that 32% of total isolates were antibacterial active and were distinguished as, 18% showed broad spectrum activities, 9% showed activity against gram positive bacteria and 5% showed activity against gram negative bacteria (Fig.2.a). The antibacterial active members were belonging to *Streptomyces*, 42% (23 isolates), *Nocardia*, 25% (14 isolates), *Actinomadura*, 15% (8 isolates), *Micromonospora*, 7% (4 isolates), *Nocardiopsis*, 5% (3 isolates), *Nocardioides*, 4% (2 isolates) and *Thermomonospora*, 2% (one isolate), (Fig. 3.a).

Similar results were obtained by **Martin (1981) and Hosted (2001)** who stated that most of these microbial products with antimicrobial activity are produced by members of the family Streptomycetaceae, especially of the genus *Streptomyces*, and only a few by members of the families Mycobactcriaceae, Actinoplanaceae, Micromonosporaceae, Nocardiaceae and Thermoactinomycetaceae.

The screened isolates for antifungal activity showed ~15% of total isolates were active. Of that active group; 8 % were active against the human pathogens; *Candida albicans* and *Candida psuedotropicals*, 1% were active against the phytopathogenic fungi; *Fusarium*, *Alternaria*, *Botrytis*, *Sclerotina* and *Rhizoctonia* and 6% were active against both (Fig.2.b). The antifungal active members were belonging to *Streptomyces*, 49% (13 isolates), *Nocardia*, 19% (5 isolates), *Actinomadura*, 12% (3 isolates), *Nocardioides*, 12% (3 isolates) and *Intrasporangium*, 8% (2 isolates), (Fig.3.b). **El-Tarabily *et al* (1996); Waugh & Long (2002) and Podust *et al* (2004)** reported that many hundreds of compounds produced by actinomycetes show antibacterial and antifungal activities. The metabolites obtained from the actinomycetes are structurally more diverse and exhibit more interesting bioactivities compared to those of plant origin.

Results of the *Artemia salina* bioassay showed that 9% of the isolates were exhibited a cytotoxic activity (Fig.2.c). The active members were belonging to the genera, *Nocardia*, 46% (7 isolates), *Nocardiopsis*, 27% (4 isolates), *Micromonospora*, 13% (2 isolates), *Nocardioides*, 7% (one isolate) and *Intrasporangium*, 7% (one isolate), (Fig.3.c). **Gorman (2003)** recorded Salinospora strains to produce potentially therapeutic chemicals which strongly inhibited the growth of some cancer cells from human colon, lung, and breast tissues. **Zheng *et al* (2000)** isolated actinomycetes from the surface epidermis and intestines of sea plants and animals. High percent of them

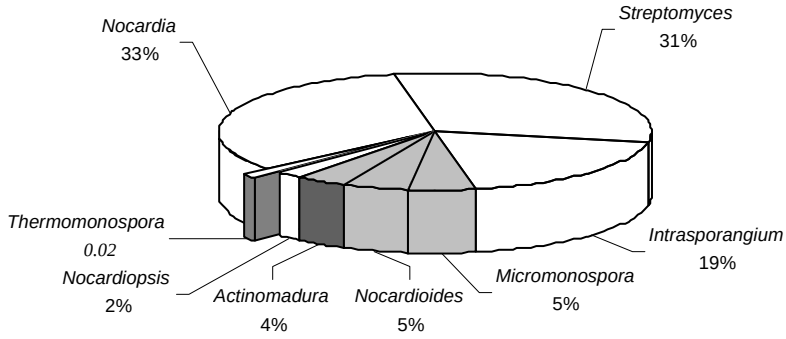

Fig. 1. Percentage of actinomycete genera (approximately) isolated form the biological wastewater treatment system.

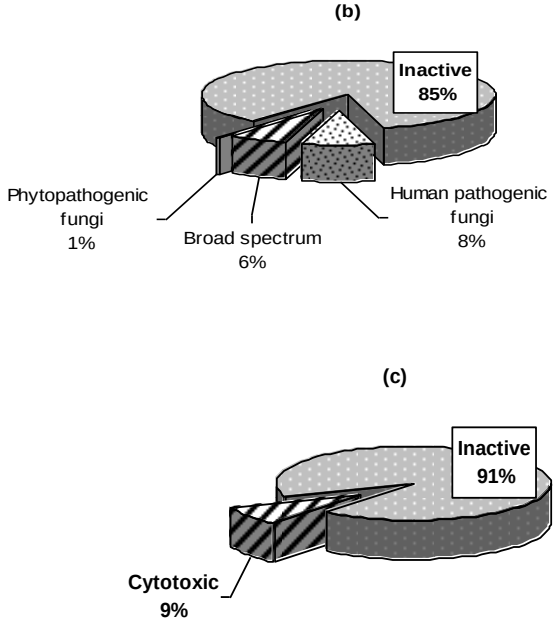

Fig. 2. Percentage of actinomycetes (%) activity; (a) antibacterial, (b) antifungal and (c) cytotoxic activities.

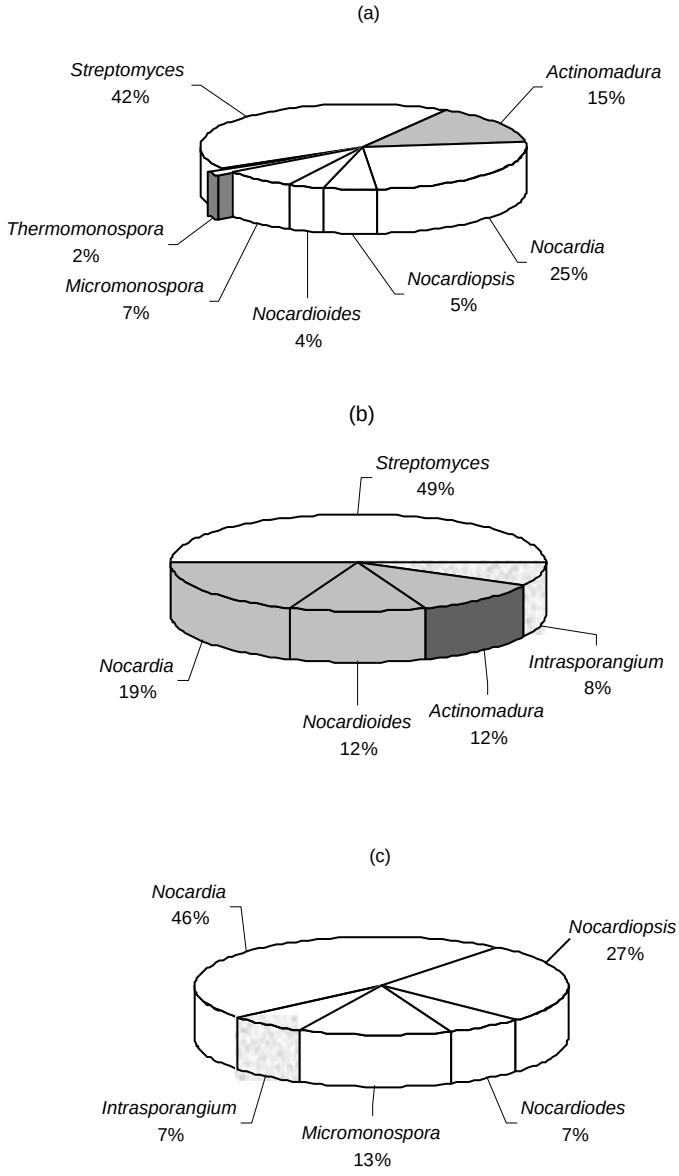

Fig. 3. Members of active actinomycetes genera (%); (a) antibacterial, (b) antifungal and (c) Cytotoxic activities

Table 1. The active actinomycete isolates, nature, season of isolation, locality, sample, antimicrobial activities (diameter of inhibition zone, cm) and cytotoxic activity (counts of living larva of *Artemia salina*)

Isolate, No.#	N.	S.	B.	Sl.	Antibacterial activity						Antifungal activity							Cytotoxic activity				
					Ba.	*St.*	*Kl.*	*Pr.*	*Ps.*	*Es.*	*C.1*	*C.2*	*Rh.*	*Bo.*	*Alt.*	*Fu.*	*Scl.*	10 µl	30 µl	100 µl	C	Toxicity
Nocardiopsis, 1	T	A	2	In	0	0	0	0	0	0	0	0	0	0	0	0	0	3	1	0		**Toxic**
Nocardia , 10	M	A	2	r	**1.9**	0	0	0	0	0	**2.3**	**1.1**	**0.8**	0	0	0	0	63	55	27		Non
Actinomadura, 18	M	A	2	R	0	**1.8**	**1.65**	0	0	0	0	**1**	0	0	0	0	0	65	52	16		Non
Thermomonospora,25	T	A	4	R	**1.5**	0	0	**1.7**	**1.5**	0	0	0	0	0	0	0	0	58	40	21		Non
Nocardiopsis, 26	T	A	4	R	0	**1.8**	0	0	0	0	0	0	0	0	0	0	0	62	40	0		Non
Intrasporangium , 34	M	A	4	R	0	0	0	0	0	0	**2.6**	0	0	0	0	0	0	60	37	4		Non
Intrasporangium , 36	M	A	4	r	0	0	0	0	0	0	**2**	0	0	0	0	0	0	55	40	0		Non
Streptomyces, 38	M	A	6	R	0	0	0	0	0	0	**2.8**	**1**	0	0	0	0	0	67	49	25	**70**	Non
Nocardia, 39	M	A	6	R	0	0	0	0	**1.6**	0	0	0	0	0	0	0	0	25	0	0		**Toxic**
Nocardia, 40	M	A	6	R	**1.5**	0	0	**1.6**	0	**1.65**	0	0	0	0	0	0	0	65	40	22		Non
Micromonospora , 41	M	A	6	R	**1.7**	0	0	0	**1.5**	0	0	0	0	0	0	0	0	68	52	12		Non
Streptomyces, 46	T	A	6	S	**1.5**	0	0	·	·	0	0	0	0	0	0	0	0	70	59	30		Non
Streptomyces, 47	T	A	6	G	**1.4**	0	0	0	**1.6**	0	0	0	**0.9**	0	0	0	0	58	43	28		Non
Streptomyces, 49	T	A	6	r	**1.5**	0	0	**1.5**	**1.4**	0	0	0	0	0	0	0	0	70	58	36		Non
Streptomyces, 52	M	W	2	G	**1.5**	**2**	0	**2**	**1.5**	0	0	0	0	0	0	0	0	40	31	5		Non
Actinomadura, 59	M	W	2	R	**1.8**	0	0	0	**1.5**	0	0	0	0	0	0	0	0	43	36	20		Non
Streptomyces, 64	T	W	2	R	**1.7**	0	0	0	**1.5**	0	0	0	0	0	0	0	0	45	36	3		Non
Streptomyces, 69	T	W	4	S	**1.5**	0	0	0	0	0	0	0	0	0	0	0	0	45	27	13		Non
Streptomyces, 79	M	W	4	R	**1.5**	0	0	**1.7**	**1.5**	0	**2.3**	**1.5**	0	0	0	0	0	40	35	0		Non
Streptomyces, 84	T	W	6	G	**1.5**	0	0	0	0	0	0	0	0	0	0	0	0	49	44	19	**45**	Non
Streptomyces, 87	T	W	6	r	**1.5**	0	0	0	0	0	0	0	0	0	0	0	0	45	34	15		Non
Nocardiodes, 88	M	W	6	G	0	0	0	0	0	0	**1.6**	0	0	0	0	0	**1.2**	35	28	0		Non
Streptomyces, 90	M	W	6	S	0	0	0	0	0	0	**2.1**	0	0	0	0	0	0	40	31	0		Non
Nocardia, 94	M	W	6	S	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0		**Toxic**
Nocardia, 96	M	W	6	R	0	**1.5**	**1.9**	**1.6**	0	**1.5**	**1.8**	0	0	0	0	0	0	37	30	0		Non
Actinomadura, 97	M	W	6	R	**1.65**	**1.5**	0	0	0	**1.6**	0	0	0	0	0	0	0	44	36	15		Non

Table 1. Cont.

Isolate, No.#	N.	S.	B.	Sl.	Antibacterial activity						Antifungal activity							Cytotoxic activity				
					Ba.	*St.*	*Kl.*	*Pr.*	*Ps.*	*Es.*	*C.1*	*C.2*	*Rh.*	*Bo.*	*Alt.*	*Fu.*	*Scl.*	10 µl	30 µl	100 µl	C	Toxicity
Nocardia, 98	M	W	6	r	0	0	0	0	**1.4**	0	0	0	0	0	0	0	0	40	35	16		Non
Nocardia, 99	M	W	6	r	0	**1.8**	0	**1.6**	**1.5**	0	0	0	0	0	0	0	0	44	27	0		Non
Nocardia, 101	M	Sp	6	O	**1.4**	**1.5**	0	**1.4**	0	**1.5**	0	0	0	0	0	0	0	37	28	0		Non
Nocardia, 102	M	Sp	2	G	0	0	0	0	0	0	**1.8**	**1.2**	**1**	0	0	0	0	48	39	8		Non
Nocardia, 103	M	Sp	2	G	**1.5**	**1.5**	**1.6**	0	**1.7**	**1.5**	0	0	0	0	0	0	0	38	30	10	**50**	Non
Streptomyces, 104	M	Sp	2	G	0	0	0	0	0	0	**2**	**1.2**	**1**	0	0	0	0	47	33	12		Non
Nocardia, 106	M	Sp	4	S	0	0	0	0	0	0	0	0	0	0	0	0	0	13	0	0	**50**	**Toxic**
Nocardiodes, 107	M	Sp	6	S	**1.3**	0	**2**	0	0	0	**2.9**	0	0	0	0	0	**1.4**	39	28	0		Non
Streptomyces, 110	M	Sp	2	R	0	0	0	0	0	**1.9**	0	0	0	0	0	0	0	40	35	0		Non
Nocardia, 111	M	Sp	2	R	**1.9**	**1.6**	0	0	**1.5**	**1.5**	0	0	0	0	0	0	0	37	29	0		Non
Nocardia, 112	M	Sp	4	R	**1.9**	0	0	0	0	0	**2.8**	0	0	0	0	0	0	45	35	5		Non
Streptomyces, 113	M	Sp	6	R	0	0	0	0	0	**1.5**	**2.1**	0	**0.9**	0	0	0	0	49	39	5		Non
Nocardia, 114	M	Sp	2	r	**1.4**	**1.5**	**1.5**	**1.4**	**1.8**	**1.5**	0	0	0	0	0	0	0	47	30	10		Non
Streptomyces, 116	M	Sp	4	r	0	**1.9**	0	0	0	0	**2**	0	**1.2**	0	0	0	0	40	27	0		Non
Nocardia, 117	M	Sp	4	r	0	0	0	0	0	0	**2.7**	**1**	0	0	0	0	0	4	1	0		**Toxic**
Nocardia, 118	M	Sp	4	r	**2.5**	0	0	0	0	0	0	0	0	0	0	0	0	45	35	5		Non
Streptomyces, 119	M	Sp	6	r	0	0	0	0	0	0	**2.5**	**1.2**	**1**	0	0	0	0	48	30	0		Non
Nocardiopsis, 120	T	Sp	2	In	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0		**Toxic**
Nocardiopsis, 121	T	Sp	2	O	0	0	0	0	0	0	0	0	0	0	0	0	0	4	1	0		**Toxic**
Streptomyces, 122	T	Sp	4	O	**1.35**	**1.9**	**2.3**	0	**1.2**	0	0	0	0	0	0	0	0	41	30	5		Non
Nocardiopsis, 124	T	Sp	6	O	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0		**Toxic**
Streptomyces, 125	T	Sp	2	G	**1.5**	0	0	0	0	0	0	0	0	0	0	0	0	50	45	20		Non
Streptomyces, 127	T	Sp	6	G	**1.8**	0	0	0	0	0	**3**	0	**1.3**	0	0	0	0	49	34	5		Non
Nocardiopsis, 128	T	Sp	6	G	**1.5**	0	0	0	0	0	0	0	0	0	0	0	0	42	36	0		Non
Actinomadura, 130	T	Sp	6	G	**1.4**	0	0	**1.6**	**1.4**	0	0	**0.9**	0	0	0	0	0	50	40	12		Non
Streptomyces, 132	T	Sp	6	S	**1.35**	**1.9**	**2.3**	0	**1.2**	0	0	0	0	0	0	0	0	40	32	0		Non
Actinomadura, 134	T	Sp	6	S	**1.6**	0	0	0	0	0	0	0	0	0	0	0	0	49	39	20		Non

Table 1. Cont.

Isolate, No.#	N.	S.	B.	Sl.	Antibacterial activity						Antifungal activity							Cytotoxic activity				
					Ba.	*St.*	*Kl.*	*Pr.*	*Ps.*	*Es.*	*C.1*	*C.2*	*Rh.*	*Bo.*	*Alt.*	*Fu.*	*Scl.*	10 µl	30 µl	100 µl	C	Toxicity
Nocardiopsis, 138	T	Sp	4	R	0	**1.7**	0	0	0	**1.6**	0	0	0	0	0	0	0	44	31	0		Non
Streptomyces, 140	T	Sp	4	R	**1.6**	0	**2**	0	0	0	0	0	0	0	0	0	0	47	43	0		Non
Streptomyces, 141	T	Sp	6	R	**1.4**	**2**	**2.3**	0	**1.2**	0	0	0	0	0	0	0	0	50	44	15		Non
Streptomyces, 145	T	Sp	2	r	**1.4**	**1.9**	**2.3**	0	**1.2**	0	**1.6**	0	0	0	0	0	0	45	30	15		Non
Actinomadura, 146	T	Sp	4	r	**1.4**	0	0	0	**1.6**	0	0	0	0	0	0	0	0	46	28	0		Non
Streptomyces, 148	T	Sp	6	r	**1.4**	0	0	0	**1.2**	0	**2.4**	0	0	0	0	0	0	50	43	17		Non
Actinomadura, 149	T	Sp	6	r	**2.5**	0	0	0	**1.4**	0	0	**1.2**	0	0	0	0	0	50	31	0		Non
Micromonospora , 153	M	Sm	4	S	0	0	0	0	0	0	0	0	0	0	0	0	0	37	23	0		**Toxic**
Micromonospora , 154	M	Sm	6	S	0	0	**2.5**	0	**1.5**	**1.6**	0	0	0	0	0	0	0	50	43	15		Non
Streptomyces, 155	M	Sm	2	R	0	0	0	0	0	0	**1.4**	0	0	0	0	0	0	40	32	6		Non
Actinomadura, 156	M	Sm	2	R	0	**1.8**	**1.7**	0	0	0	0	0	0	0	0	0	0	47	37	0		Non
Nocardia, 157	M	Sm	2	R	**1.5**	0	**1.7**	**1.4**	0	**1.5**	0	0	0	0	0	0	0	38	29	7		Non
Streptomyces, 158	M	Sm	6	R	**3**	0	0	0	0	0	0	0	0	0	0	0	0	39	30	0		Non
Nocardiodes, 159	M	Sm	6	R	0	0	**2.1**	0	0	0	**1**	0	**1.7**	0	0	0	0	40	29	0	**50**	Non
Nocardia, 161	M	Sm	6	R	0	**1.6**	**1.9**	**1.7**	**1.3**	**1.6**	0	0	0	0	0	0	0	41	32	0		Non
Intrasporangium,162	M	Sm	6	R	0	0	0	0	0	0	0	0	0	0	0	0	0	41	20	0		**Toxic**
Nocardia, 163	M	Sm	6	R	0	0	0	0	0	0	0	0	0	0	0	0	0	39	22	0		**Toxic**
Nocardiodes, 165	M	Sm	6	R	0	0	0	0	0	0	0	0	0	0	0	0	0	38	23	0		**Toxic**
Nocardia, 167	M	Sm	2	r	0	0	0	0	0	0	0	0	0	0	0	0	0	42	24	0		**Toxic**
Micromonospora , 170	M	Sm	4	r	0	0	**1.8**	0	**1.2**	**1.4**	0	0	0	0	0	0	0	34	20	0		**Toxic**
Streptomyces, 171	M	Sm	6	r	0	**1.7**	0	0	0	0	**2**	**1.5**	**1**	0	0	0	0	40	28	0		Non
Streptomyces, 172	M	Sm	6	r	0	**2.3**	0	0	0	0	0	0	0	0	0	0	0	43	32	1		Non
Nocardia, 173	M	Sm	6	r	0	0	0	0	0	0	0	0	0	0	0	0	0	37	18	0		**Toxic**
Micromonospora, 174	M	Sm	6	r	0	0	**2**	0	0	**1.5**	0	0	0	0	0	0	0	47	31	10		Non

N.: Nature **SP:** Spring **O:** outlet water ***St.:*** *Staphylococcus aureus* ***C.2*** *Candida pseudtropicals*
S.: Season **Sm:** Summer **G:** Gravel ***Kl.:*** *Klebsiella pneumonia* ***Rh.:*** *Rhizoctonia solani*
B.: Bed **Sl:** Sample **S:** Sand ***Pr.:*** *Proteus vulgaris* ***Bo.:*** *Botrytis cinerea*
L.: Locality **M:** Mesophilic **R:** Rhizosphere ***Ps.:*** *Pseudomonas aeruginosa* ***Alt.*** *Alternaria alternata*
A: Autumn **T:** Thermophilic **r:** Rhizoplane ***Es.:*** *Escherichia coli* ***Fu.:*** *Fusarium fabae*
W: Winter **In:** Inlet water ***Ba.:*** *Bacillus subtilis* ***C.1:*** *Candida albicans* ***Sc.:*** *Sclerotina sclerotiorum*
C: Control

displayed a cytotoxic activity. Among all actinomycetes isolated, the genus *Micromonospora* was found to have the highest positive rate of induction activity. **Bernan *et al* (2004)** isolated actinomycetes and found cultures produced potent antitumor activity. One of these isolates is a novel halophilic Micromonospora. **Oku *et al* (2003)** reported strains of *Streptomyces*, *Micromonospora echinospora* and *Actinomadura verrucosospora* to produce promising antitumor compounds.

In this study results indicated that, the LD_{50} was being less than 10 ul/ml for most cases and 30 ul/ml for few cases (Table 1). Similar cytotoxic activities of actinomycetes were reported by many authors. **Svoboda and Hampson (1999)** had studied the cytotixic activity of 5 volatile oils and had stated the ideal LD_{50} was being less than 40 ppm as an indicator for the possible antitumor activity of the compounds while it was being around 1ppm in case of using as an insecticide. Also, most of antibacterial, antifungal and cytotoxic activities were observed in mesophilic isolates (69.33%) and were isolated mostly during spring (42%).

The number of active isolates from different localities and samples that showing antibacterial, antifungal and/or cytotoxic activities are given in Table (2). On the basis of the total numbers of isolates (174), results cleared that 55 actinomycete cultures (32%) showed antibacterial activity, 26 cultures (15%) exhibited antifungal activity and 15 cultures (9%) reported cytotoxic activity.

Table 2. Number of isolates have antibacterial, antifungal and cytotoxic activities in the samples collected from the different localities

Locality	Sample	No. of active isolates		
		Antibacterial	Antifungal	Cytotoxicity
Gravel bed (B.2)	Inlet water	0	0	2
	Outlet water	0	0	1
	Gravel	3	2	0
	Rhizosphere	7	2	0
	Rhizoplane	3	2	1
Sand bed (B.4)	Inlet water	0	0	0
	Outlet water	1	0	0
	Sand	1	0	2
	Rhizosphere	6	3	0
	Rhizoplane	4	3	2
Gravel / Sand Bed (B.6)	Inlet water	0	0	0
	Outlet water	1	0	1
	Gravel	5	4	0
	Sand	5	2	1
	Rhizosphere	10	4	4
	Rhizoplane	9	4	1
Total Number		55	26	15

The high numbers of active isolates from the rhizosphere samples in all localities may be due to the presence of plant roots exudates such as amino acids, simple sugars, and organic acids that provide a continuous energy supply to actinomycetes living in that zone **(Zenova and Zvyagintsev 2003)**. **Hatano *et al* (1993)** stated that plants significantly affect the actinomycetes populations of constructed wetlands by conducting gases to and from the sediments through their gas exchange mechanisms. Oxygenation, however, is achieved only in area surrounding the root. These aerobic zones support some actinomycetes in such treatment system **(Wetzel, 1993)**. Results, also, indicated that some isolates were exhibited either antibacterial or antifungal activity while other isolates were exhibited both antibacterial and antifungal activities. The most interesting result is that all the cytotoxic active isolates, with one exception only (*Nocardia*, 117 and *Nocardi*a, 39), were detected to have no antimicrobial activities. The problem is more complicated and needs further studies for separation and identification of these biologically active compounds.

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قدرة بعض الاكتينوميسيتات المعزوله من نظام المعالجه البيولوجيه لمياه الصرف الصحى على انتاج مضادات حيويه و مواد سامه للخلايا [٥]

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تم فى هذه الدراسة عزل وتعريف ١٧٤ عزلة اكتينوميسيتات من مشروع المعالجه البيولوجيه لمياه الصرف الصحى بمنطقة أبوعطوة – الاسماعيليه.

اظهرت نتائج تعريف العزلات انها تنتمى الى ٨ اجناس من الاكتينوميسيتات هى: *Nocardia* (33%), *Streptomyces(31%)*, *Intrasporangium (*19%) *Micromonospora(*5%), *Nocardioides*(5%), *Actinomadura*(4%), *Nocardiopsis*(2%), *Thermomonospora (*0.02%)

تم دراسة العزلات لتحديد قدرتها على انتاج مواد ذات طبيعة سامه (مضادات البكتيريا – مضادات الفطريات – مضادات الخلايا السرطانيه) وذلك من خلال دراسة تأثيرها على نمو بعض البكتيريا والفطريات الممرضه للانسان وبعض الفطريات الممرضه للنبات باستخدام تقنية منع النمو Growth (inhibition) فى حالة المضادات الحيويه وتقنية *Artimia salina* bioassay فى حالة مضادات الخلايا السرطانيه.

أظهرت النتائج ان ٣٢ % من المعزولات سجلت نشاطا موجبا ضد البكتيريا Antibacterial و ١٥% منها سجلت نشاطا موجبا ضد الفطريات Antifungal و ٩% منها سجلت نشاطا ساما للخلايا Cytotoxic acivity كما اظهرت النتائج ان العزلات التى لها القدره على انتاج مضادات بكتيريه ينتمى

معظمها الى جنس *Streptomyces* (42%) وينتمى الى نفس الجنس ايضا العزلات التى تنتج مضادات فطريه(49%).

بينما تنتمى معظم العزلات التى لها القدره على انتاج مواد سامه للخلايا Cytoxic activity إلى جنس *Noccardia* (46%).

وبصفه عامه فانه يمكن استنتاج ان الاكتينوميسيتات المعزوله من انظمة المعالجه البيولوجيه لمياه الصرف الصحى تعتبر من المصادر الواعده لانتاج مضادات ميكروبيه ومضادات الخلايا السرطانيه.

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