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Decontamination of Pollutants in Aquatic System: 1. Biodegradation Efficiency of Isolated Bacteria Strains from Certain Contaminated Areas

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Abstract: In the present study, biodegradation of organophosphorus compounds (OP's) using various bacteria strains isolated from specified contaminated area along Marriute Lake in Alexandria was investigated. Wild bacteria were isolated from the collected water and sediment samples from different lake locations. Six strains of isolated bacteria were identified as *Escherichia coli* (*E. coli*) type using morphological and biochemical characteristics. The efficiency of the isolated strains either *E. coli* or non-*E. coli* for paraoxon biodetoxification were evaluated. The biodegradation activities of the *E. coli* type isolated from sediment samples were found to be superior to those isolated from water samples. However, non-*E. coli* type of isolated strains from water samples demonstrated an increase in the activity percentages than those of same type strains isolated from sediment samples. Generally, paraoxon biodegradation by *E. coli* isolated strains was higher than those of non-*E. coli* isolated strains.

Key words: Biodegradation, organophosphorus, bacteria strains, contaminated areas

INTRODUCTION

According to data reported by The Egyptian Central Agency for Public Mobilization and Statistics, approximately 3,330 tons of OP pesticides were used in 1994. El-Sebae *et al.*[1] reported that 13,500 metric tons of chlorpyriphos were used in Egypt between 1955-1990. As it is well documented, OP's are recognized as one of three most toxic categories used as pesticides and nerve gases[2-4]. These group of compounds acting by inhibiting acetylcholinesterase (AChE) activity, which occurs in vertebrate erythrocytes and nervous system synapses of living organisms. The inhibition action results in acetylcholine (ACh) accumulation, which interferes with muscular responses and in vital organs produce serious symptoms and eventually death[4].

Using of OP pesticides, though very important to agricultural industry success, affects the environment phases. Recently there is worldwide public concern regarding OP contamination of food products and water supplies, particularly the large quantity of pesticide wastes generated from washing of pesticide holding tanks and application machinery used by farmers[5,6]. The continuous increasing of selection pressure due to pollution factors developed in many environmental phases such as water and soil, promptly induces the formation of modified bacteria strains, specifically characterized by their capability for bioremediation such inducing chemicals[7].

Therefore, the present study aims to isolate various modified bacteria strains in specified contaminated regions, detecting their optimum growth media, as well as detected their OP's bioremediation capabilities for using such strains in decontaminating environmentally harmful OP residues.

MATERIALS AND METHODS

Chemicals

Paraoxon: (diethyl-p-nitrophenylphoshate) standard (98%) was purchased from Chem. Service, Inc, West Chester, PA, USA.

Used media: Luria-Bertani (LB) media was composed in 1 L of distilled water as: bacto-tryptone 1 g; bacto-yeast extract 5 g; sodium chloride 10 g; potassium dibasic phosphate 1 g and potassium monobasic phosphate 3 g. Nutrient broth (NB) and Soya broth (SB) were also used.

Samples collection and storage: Water samples (1 L each) and sediment samples (0.5 kg each) were collected from eight sectors that are located along Marriute Lake of

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Table 1: Physicochemical properties of water and sediment samples

			Soluble cations				Soluble anions			
Samples	pH	E.C. (dS m^{-1})	Na^+	K^+	Ca^{++}	Mg^{++}	CO_3^-	HCO_3^-	Cl^-	SO_4^-
3S (sediment)	7.7	8.5	37.4	2.4	26.0	32.2	0	14	44	40
Sx_3 (sediment)	7.5	11.5	77.8	2.6	25.0	24.6	0	6	120	4
Wx_3 (water)	7.6	2.6	18.4	0.6	2.0	7.0	0	8	20	0
Wx_2 (water)	7.6	2.8	19.2	0.4	2.6	6.8	0	6	23	0
A_3 (water)	8.0	3.5	27.0	0.6	2.2	8.2	0	4	33	1

Alexandria according to waste drainage outlets. Water and sediment samples (3 replicates for each) were collected from each specified location in screwed glass bottles for water samples and plastic bags for sediment samples. Samples were transferred after collecting immediately to the laboratory, divided to sub-samples and stored at 4°C. Physicochemical properties of water and sediment samples were analyzed (Table 1).

Isolation, identification and characterization of *E. coli* in collected samples

Isolation of *E. coli*: Loopful from each sample was streaked onto the differential media Glycerol agar. Also, isolation was conducted on commercial media such as: Maconkey agar and Brilliant green agar supplemented with 4-methyl umbellifery-β-D gluconate (M U G). Such media is hydrolyzed to give a fluorescent compound, which detected by exposure to UV light.

Identification of *E. coli*: Identification process was based on cultural and physiological characteristics.

Cultural characteristics: Cultural characteristics including shape of bacterial cells, sporulation, colony type and gram stain, were utilized for bacteria identification according to Bergey's manual of systematic bacteriology[8].

Physiological characteristics: Physiological characteristic methods were used to identify *E. coli* through different reactions according to Bergey's manual of systematic bacteriology[8]. These reactions included aerobiosis, catalase activity, Indole formation, oxidase test, H_2S production, acetyl-methyl carbinol production (VP), reactions to methyl red (MR) and fermentation of carbohydrate (lactose and glucose).

Confirmatory testes for *E. coli*: *E. coli* was determined by MUG test according to Mates and Schaffer[9]. When the substrate (MUG) cleaved by the enzyme, a fluorescent product is produced a nd detected using UV light. This additional test for β-glucuronidase activity is positive only for *E. coli*. Brilliant green bile agar supplemented with 2% (MUG) was placed in petri dish, inoculated with *E. coli* and incubated for 18-24 h, at 35°C and observe periodically for the development of fluorescence using UV light.

Capability of isolated bacteria for organophosphorus detoxification: After isolation, identification and characterization of the isolated bacteria from water and sediment samples, assessment of their growing ability in different media was carried out to explore the optimum media for growing the isolated strains. Capabilities of isolated bacteria for OP's biodegradation were assayed according to Kearney *et al.*[10] and Mulbry *et al.*[11]. One colony of each of isolated strains were grown in 5 ml of different liquid medias: NB, SB or LB media, supplemented with 5 µl of 100 µg ml^{-1} ampicilin and shaked at 37°C, 250 rpm on an orbital incubator-shaker along for 48 h. Growth was recorded by solution turbidity intensity observation. Then one ml of cell suspension was transferred to 50 ml of the same liquid media enriched by 50 µl ampicilin and repeated shaking under the aforementioned conditions. Finally, 3 ml of resulted cell suspension was transferred to 150 ml of the same media, supplemented with 150 µl ampicilin in 250 ml flask and shaken at the previous conditions. Cells were harvested by centrifugation at 4°C, 5000 rpm for 10 min, washed with 40 ml of 150 mM buffer phosphate pH 8.0 twice and re-centrifuged at the same conditions. The resulted pellets were weighted and diluted in 10 ml of buffer phosphate.

Ten µl (≈2.0 mg) of the harvested bacteria suspension were added to a serial paraoxon solutions ranged from 10 to 100 µg ml^{-1}, prepared in 50 mM buffer phosphate, pH 8.0 and incubated at 37°C for 48 h. Samples were analyzed for *p*-nitrophenol production by measuring optical densities spectrophotometrically at 410 nm as an indication for paraoxon degradation.

RESULTS AND DISCUSSION

Isolation and identification of *Escherichia coli*: Isolated bacteria from the collected water and sediments samples showed different colony morphologies dependant upon shape of bacterial cells, sporulation, colony type and gram stain. Six of these strains appeared to be similar colony morphology, which identified as *E. coli* type (Table 2)

Table 2: Particular isolated bacteria strains from contaminated resources samples

E.coli type		Non-*E.coli* type	
Sediment	Water	Sediment	Water
Sx3	Wx2	Sx1	3A
Sx2	3W	2Sd	2W
1Sd			Wx1
3S			

Table 3: The influence of different media on the growing of isolated *E. coli* strains

	Type of mediaSediment		
Strains	L.B	S.B	N.B
3S	-	++	+
1Sd	-	-	++
Sx_2	++	-	+
Sx_3	++	-	+
3W	-	-	++
Wx_2	++	-	+

Growth codes: ++ highly, + moderate, - non growth
Nutrient broth (NB), Soya broth (SB) , Luria-bertani (LB) media

according to Bergey's manual of systematic bacteriology[8].

The selected isolates were gram negative, motile show pink or red colonies, 2-3 mm in diameter on Maconkey agar and have a metallic sheen by transmi Hed to light. Colonies on glycerol agar smooth, low convex with shine surface.

The biochemical characteristics of these strains type are showed that catalase activity is positive, H_2S production is negative, Indole formation is positive, voges-proskaur test is negative, methyl red test is positive and these selected isolates produced acid and gas from glucose and lactose fermentation. MUG test is positive and a fluorescent compound detected by exposure to UV light.

Differential tests for identification must be used with the knowledge that all strains taxonomically assigned to the coliform group. The traditional tests (i.e. Indole, methyl red, voges and proskauer and citrate utilization) are useful for coliform differentiation, but do not provide complete identification. The genera *Enterobacter*, *Klebsiella*, *Citrobacter* and *Escherichia* are usually represented in the majority of isolations made from raw water. So, MUG test was carried out as an additional test, which is positive only for *E.coli* with support for inclusion in the *E. coli* as defined by Sueiro *et al.* [12], Mates and Schaffer[9].

Capabilities of isolated bacteria for organophosphorus detoxification

Detecting the optimum media for each isolated strain: The present investigation estimated the influence of different media on growing of isolated bacteria to find out the optimum media for each isolated strain. Table 3 showed differences in bacteria growth of each isolated strain in the three tested media. Thus the chosen of the optimum media for growing and assaying the detoxification capability of each isolated strain was done according this result. Moreover, the results indicated that the NB media showed to be the suitable media for growing the isolated non-*E. coli* type of isolated bacteria. While, the LB media was suitable for growing the most isolated *E. coli* type. Kaneva *et al.*[13] and Mansee *et al.*[6] who used LB media as optimal media for growing *E. coli* cells supported this result.

Paraoxon detoxification using isolated strains: The results in Fig. 1a and b showed the capabilities of wild *E. coli* isolated strains from sediment and water samples, respectively for paraoxon biodetoxification. The biodetoxification percentages of wild *E. coli* isolated strains from sediment samples were ranged between 84 and 4.5%, whereas these values were ranged between 44 and 2.2% for the isolated strains from water samples. Moreover, Sx3 and Wx2 showed the maximum activity for isolated strains from sediment and water samples, respectively while, 1Sd and 3W were the less active isolated strains for biodetoxification. The biodetoxification percentages for all strains found to be higher for the lower concentration than those of the higher one. Generally, the biodegradation activity of the *E. coli* type isolated from sediment samples were superior to those isolated from water samples.

From the previous results, *p*-nitrophenol production is an indication for paraoxon degradation by the isolated *E. coli* type. These results are comparable with the results of Gilbert *et al.*[14] who assembled a consortium comprised of two engineered microorganisms including, *E. coli* for biodegradation of the organophosphate insecticide parathion. The co-culture effectively hydrolyzed 500 μM parathion (146 mg L^{-1}) and prevented the accumulation of *p*-nitrophenol in suspended culture. Das *et al.*[15] studied the influence and persistence of phorate and carbofuran insecticides on *Escherichia* as well as other microorganisms in rice field. They found that phorate induced *Escherichia* growth. They added that both insecticides persisted in the rhizosphere soil for a short period of time and the rate of dissipation of carbofuran was higher than that of phorate in the soil depicting the half-life (T1/2) 9.1 and 10.4 days, respectively. Qiao *et al.*[16] immobilized recombinant *E. coli* and investigated its biodegradation efficiency for pesticides residues. Mulchandani *et al.*[17] reported that improved whole-cell technology for detoxifying organophosphate nerve agents was recently developed based on genetically engineered *E. coli* with organophosphorus

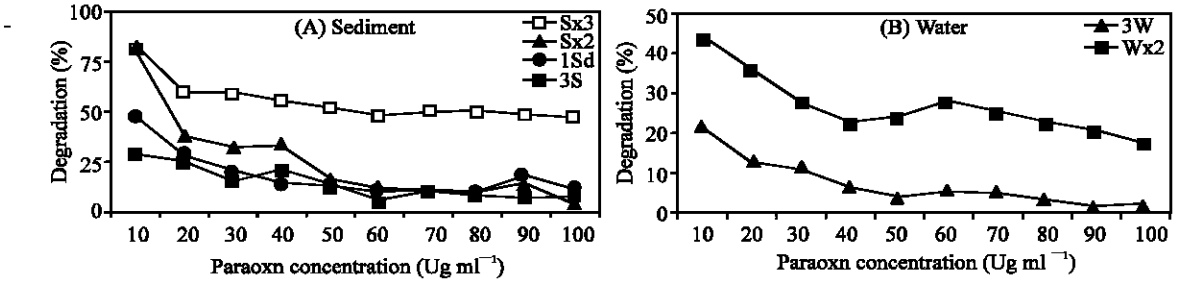

Fig. 1: Biodegradation capabilities of wild *E. coli* isolated strains from sediment and water samples

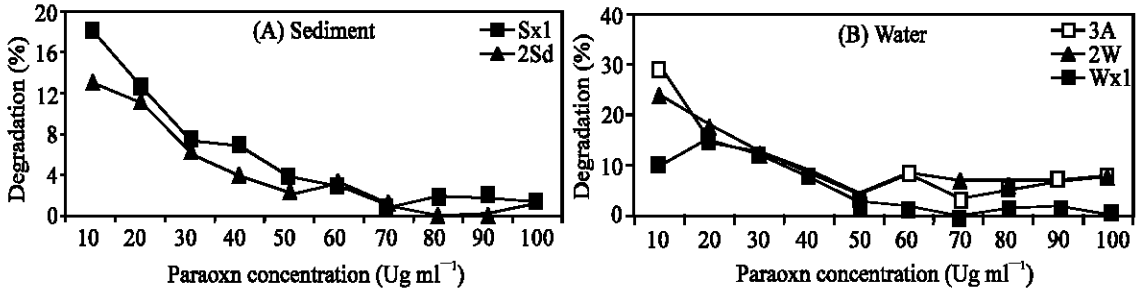

Fig. 2: Biodegradation capabilities of wild non-*E. coli* isolated strains from sediment and water samples

hydrolase anchored on the surface. Kaneva *et al.*[13] cited that there are several factors influencing parathion degradation. Since, they found that the resulting cultures grown under the optimized conditions had an eight-fold increase in parathion degradation.

The biodetoxification activity of non-*E. coli* type isolated strains from sediment and water samples was assayed and data showed an increasing in degradation powerful of these isolated strains with decreasing the concentration of paraoxon (Fig. 2a and b). Thus, the highest activity percentage was presented at 10 μg ml^{-1} of paraoxon for all tested non-*E. coli* isolated strains, which ranged from 30 to 10%. These types of isolated strains from water samples showed an increase in the activity percentages than those of same type strains isolated from sediment samples. So, the results concluded that the paraoxon biodegradation by *E. coli* isolated strains was higher than that by non-*E. coli* isolated strains.

The present investigation observed non-*E. coli* type isolated strains ability for paraoxon biodetoxification. Bhadbhade *et al.*[18] supported the present results, by using non-*E. coli* type such as *Arthrobacter atrocyaneus*, *Bacillus megaterium* and *Pseudomonas mendocina* for bioremediation of wastewater containing Monocrotophos (MCP), an organophosphorus insecticide during manufacture. They found that the bioremediation of such compound was highest at pH 8.0. They used of pure cultures for bioremediation of effluent containing MCP appears to be the first trail of such attempt. Ramanathan and Lalithakumari[7] who have isolated a soil isolate, *Pseudomonas* sp. A3, able to degrade methyl parathion (MP), malathion, monocrotophos and Diazinon. The potential of this strain to mineralize MP as a carbon and/or phosphorus source and hydrolysis MP to *p*-nitrophenol has been evaluated. Moreover, Bano and Musarrat[19] isolated and characterized phorate degrading soil bacteria of environmental and agronomic significance. The HPLC analysis of the organophosphorus pesticide, phorate in bioaugmented soil revealed its complete disappearance within 40 days. Singh *et al.*[20] examined the hydrolysis of an insecticide/nematicide, fenamiphos immobilized through sorption by cetyltrimethylammonium-exchanged montmorillonite (CTMA-clay) by a soil bacterium, *Brevibacterium* sp. They cited that the bacterium hydrolyzed, within 24 h, 82% of the fenamiphos sorbed by the CTMA-clay complex.

The present study indicated that the OP biodetoxification percentages by all isolated bacteria strains were high for the lowest concentration. Such results are in agreement with the results of El-Bestawy *et al.*[21] who isolated and identified bacteria strains form water and sediment of Mariut Lake and investigated their ability to degrade certain pesticides. They revealed the selective ability among the tested bacteria for biodegradation of different pesticides especially at the lowest concentrations.

Other studies were reported the activity of non-*E. coli* type, that isolated from soil or water, in

biodegradation of another pesticide groups. Nam *et al.*[22] discussed a novel catabolic activity of *Pseudomonas veronii* in biotransformation of pentachlorophenol. Such strain was isolated by selective enrichment of soil samples from a timber storage yard. Shah and Thakur[23] studied the enzymatic dehalogenation of pentachlorophenol by *Pseudomonas fluorescens* of the microbial community from tannery effluent. Also, El-Bestawy *et al.*[21] investigated the ability of isolated bacteria strains from water and sediment, to degrade certain organochlorine pesticides. Their biodegradation results showed superior ability of the isolated bacteria to decompose the investigated pesticides with very high efficiency reaching 100% for most of them. Sutherland *et al.*[24], isolated and characterized of a Mycobacterium strain that metabolizes the insecticide endosulfan. They isolated the endosulfan-degrading bacterium from soil inoculum after repeated culture with the insecticide as the sole source of sulfur. They concluded that this bacterium is a valuable source of enzymes for use in enzymatic bioremediation of endosulfan residues.

The present investigation concluded that the bacteria isolated from specified contaminated locations of Marriout Lake showed capability for organophosphorus pesticides detoxification. Moreover, the OP degradation percentage is depending on the type of isolated strain as well as the OP concentration.

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