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Full Length Research Paper

Behavioral response of resistant and sensitive *Pseudomonas aeruginosa* S22 isolated from Sohag Governorate, Egypt to cadmium stress

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A total of 62 *Pseudomonas* isolates were selected from a contaminated site at Sohag district and tested for their resistance to cadmium metal. Isolate no. 22 was found to be the most resistant one and has a plasmid with molecular wt 27.491 Kbp. The minimal inhibition concentrations (MIC) for the resistant (R) and adopted sensitive (S) isolates were 1.0 and 0.119 mmol L^{-1}, respectively. The growth, protein content and maximum specific growth rate (k) decreased with increasing concentrations of heavy metal. The generation time (T) increased with the addition of divalent cations (Fe^{2+}, Mg^{2+} and Ca^{2+}) plus cadmium. Under cadmium stress, three proteins of high molecular weights (208, 78 and 33.5 KD) were lost while one protein with low molecular weight (2.5 KD) was induced. LPS profile was modified under stress of $CdCl_2$. Absence of bands in region I, which represents the O-specific antigen, and region II (core-oligosaccharide linked to lipid A) were recorded at high concentrations of $CdCl_2$.

Key words: *Pseudomonas aeruginosa*, cadmium stress, heavy metal resistance.

INTRODUCTION

The release of heavy metals into our environment is still large and causes an environmental pollution problem because of their unique characteristics (Banat et al., 2005). The sources of heavy-metal pollution are mining, milling and surface finishing industries, discharging a variety of toxic metals such as Cd, Cu, Ni, Co, Zn, and Pb into the environment (Malik, 2004). Our air, food and water often contain heavy metals (Nies, 2000). Heavy metal cations play an important role in many biochemical reactions due to their ability to form complex compounds (Rossbach et al., 2000). Toxicity occurs through the displacement of essential metals from their native binding sites, ligand interactions, alterations in the conformational structure of nucleic acids and proteins or interference with oxidative phosphorylation and osmotic balance (Bruins et al., 2000; Poole and Gadd, 1989).

A microorganism may survive toxic effects of heavy metals by means of a detoxification mechanism and these are designated as resistant. However, the microorganism which has the ability to survive toxic effect of heavy metal because of intrinsic properties and/or environmental modification of toxicity are known as tolerant microorganism (Gadd, 1992). Resistance to heavy metals is observed in a wide variety of bacteria, especially in gram negative bacteria (Poole, 2002) such as *Pseudomonas* sp., *Ralstonia metallidurans*, *Enterobacter cloacae, Thiobacillus ferrooxidans,* and mucilage producing cyanobacteria (Malik, 2004; Ünaldi et al., 2003, Piotrowska-Seget et al., 2005).

Bacteria of the genus *Pseudomonas* are well-studied and are of great interest not only because of their high resistance to heavy metals and other toxic substances, but also for their simple nutritional requirements and rapid growth on standard laboratory media (Pardo et al., 2003). *P. aeruginosa* was isolated from different heavy metal-polluted environments such as sewage, irrigation and agricultural drainage canals, and soil (Shoreit and Soltan, 1992; Soltan, 2001). Therefore, it has been considered as a water quality indicator organism. In the laboratory, *P. aeruginosa* shows resistance to high concentrations of heavy metals, for example, Zn, Cu, Ni, Pb, Cd and Hg

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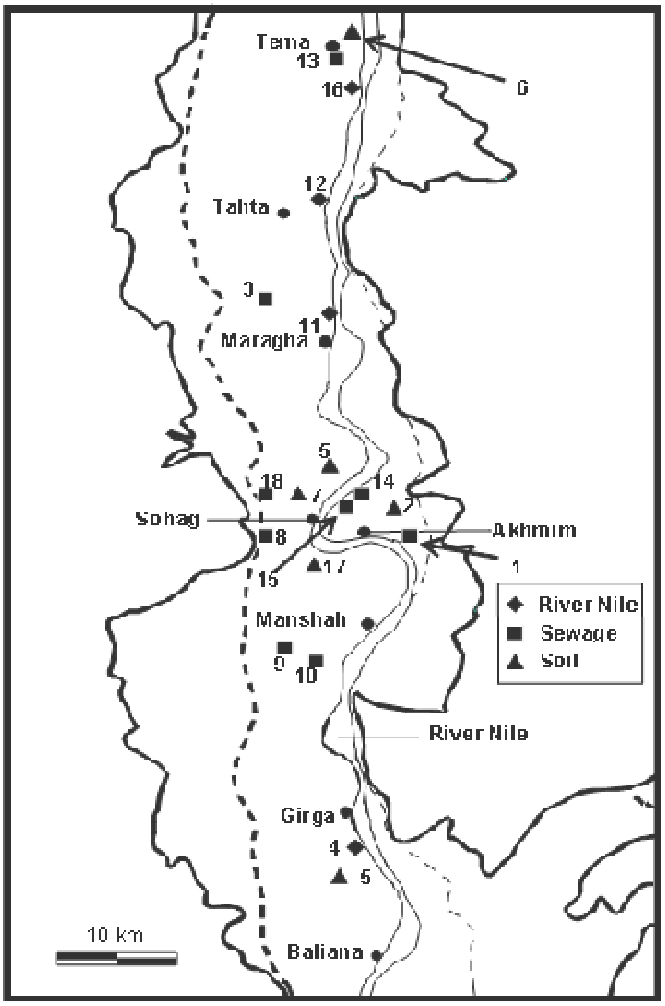

Figure 1. A map showing the different places of Sohag Governorate from which soil and water samples were collected

(Wang et al., 1997; Teitzel and Parsek 2003; Hussein et al., 2005). This demonstrates that this strain could be a good candidate for heavy metal removal from polluted sites (Filali et al., 2000).

Heavy metal accumulation is markedly affected by the presence of other ions. Cations such as magnesium and calcium can often reduce heavy metal inhibition (Issa et al., 1998). Calcium is a strong competitor for cadmium and zinc binding (Plette et al., 1996). In addition, selective permeability and ion uptake through membranes is regulated by calcium; externally added calcium acts as a membrane stabilizer and performs this function even in heavy metal-treated cells (Pessoa-de-Franca et al., 2002).

The main objective of the present study is to select a resistant and sensitive *P. aeroginosa* strain from contaminated sites at Sohag district and study their growth parameters, morphology, and the behavioral response of protein and LPS profiles to cadmium stress.

MATERIALS AND METHODS

Collection of samples

Eighteen samples (9 sewage, 5 soils and 4 River Nile) from different places at Sohag Governorate (Egypt) (Figure 1) were collected and transferred immediately to the laboratory. The samples were stored at 4 - 5˚C. Physicochemical characteristics of water and soil samples were carried out according to standard method (APHA, 1980).

Screening for heavy metal toxicity

The isolates grown on CNA medium (Havelaar et al., 1985) were tested for heavy metals toxicity by using different concentrations (100 - 2000 ppm) of $CdCl_2$.

Toxic effect of $CdCl_2$ on isolate S22

Isolate S22 was tested to $CdCl_2$ by using the disk-method. The plates containing 20 ml of tris minimal agar were inoculated with 100 µl of previously grown culture in the same medium (O.D. ≈ 0.8) by pour plate method. The discs impregnated with 40 ppm $CdCl_2$ was applied to the surface and incubated for 48 h at 37˚C.

Plasmid isolation and curing

Isolation and curing of plasmid DNA were carried out according to Ghosh et al., 2000.

Determination of minimal inhibitory concentrations (MICs)

The MICs were determined by allowing isolate 22 (R, S) to grow in tris minimal broth containing 0 - 40 ppm $CdCl_2$ for S strain and 0 - 200 ppm for R strains. It was incubated at 37°C in a rotatory shaker (150 rpm). Growth was observed after 12 h. The minimal inhibitory concentration expressed in mmol L^{-1} is the concentration at which the growth was not observed (Mergeay, 1995; Yilmaz, 2003).

Identification of isolate S22

The isolate no. 22 was identified on the basis of morphological, physiological and biochemical characteristics according to Bergey's manual 1994).

Effect of $CdCl_2$ on the growth of *P. aeruginosa* S22

P. aeruginosa S22 (R strain) was grown in Tris minimal broth containing 0, 40, 80, 120, 160 and 200 ppm of $CdCl_2$. The S-strain was grown in the same medium containing 0 and 40 ppm $CdCl_2$. All treatments were agitated at 150 rpm and incubated at 37˚C for 12 h. $CdCl_2$ was added after 3 h of incubation. Optical density at λ = 600 nm of each treatment were monitored using a Spekol 11 spectrophotometer every 3 h over 12 h of incubation (Nepple et al., 1999). Growth parameters were determined according to Mahapatra and Banerjee, 1996) after 12 h of incubation.

Effect of divalent cations on growth of *P. aeruginosa* S22 (R-strain) in the presence of $CdCl_2$

To study the effect of divalent cations on living cells of *P. aeruginosa* S22 (R-strain), it was grown in Tris minimal cations-free

Table 1. Physicochemical characteristics of soil samples collected from different sites at Sohag Governorate.

Sample No.	Physicochemical characteristics												
	Temp (°C)	pH	E.C (µhoms/cm)	OM (%)	NO_3^+ (µg/L)	PO_4^{3-} (ppm)	Cl^- (ppm)	K^+ (ppm)	Na^+ (ppm)	Pb^{2+} (ppm)	Cu^{2+} (ppm)	Cd^{2+} (ppm)	Mg^{2+} (ppm)
2	18	8.5	200	5.2	15	1.5	.06	45	285	0.5	0.09	0.1	32
7	18	8.2	220	6.8	35	1.5	0.03	35	200	0.5	0.09	0.1	35
17	20	8.5	200	8.2	41	1.65	0.028	28	187	0.4	0.08	0.02	26
5	18	7.8	170	10.6	59	1.8	0.029	20	137	0.43	0.05	0.03	28
6	18	7.7	170	14.5	82	2.1	0.025	18	126	0.2	0.04	0.05	31

WHO (2005) for cd=0.003mg/l

medium (control), or in presence of 40 ppm Cd^{2+} only or in addition to each of Fe^{2+} Mg^{2+}, or Ca^{2+}. All treatments were incubated at 37°C with shaking at 150 rpm. Heavy metals were added after 3 h of incubation. Growth curve and growth parameters were measured as stated before.

Effect of $CdCl_2$ on morphology

The Effect of $CdCl_2$ on morphology was observed by using scanning and transmission electron microscope (SEM and TEM) according to Suh et al. (1998a, b).

Effect of $CdCl_2$ on protein content

The effect of $CdCl_2$ on protein content of *P. aeruginosa* S22 (R-strain) was detected according to the method of Lowery et al (1951). Protein and lipopolysaccharides (LPS) analysis was by polyacrylamide gel electrophoresis (SDS-PAGE; Laemmli, 1970).

RESULTS AND DISCUSSION

Physicochemical characteristics of *Pseudomonas* sp.

Tables (1 and 2) show the different physicochemical characteristics of the samples. The data indicate high contamination with Cd^{2+} and Pb^{2+} (Table 2). The bacterial counts from River Nile samples ranged from 10-45 for controls and 3 - 21 cfu/g dry wt or ml/ water for $CdCl_2$ (Table 3). The highest counts were obtained from sewage samples (215 - 780 cfu/ml) followed by soil samples (60 - 155 cfu/g dry wt), whereas, the lowest counts were obtained from the River Nile samples. This was explained by high total nitrogen and biological oxygen demand values of sewage samples (116 - 190 and 263 – 340 ppm, respectively). Our data show that the higher the heavy metal content of the samples, the lower the bacterial total counts (Figure 2). This is in agreement with Kandeler et al. (2000), Kozdroj and van Elsas (2001), Suhadolc et al. (2004) and Piotrowska-Seget et al. (2005) who reported significant decreases in microbial numbers, diversity and activity due to the toxic effects of high concentrations of metals. Soltan (2001) observed that the widespread occurrence of *P. aeruginosa* reflects its role as a contaminant, as an indicator of pollution and as a member of the bacterial community in a highly polluted area.

Screening for heavy metal toxicity

Figure 2 shows that most isolates were resistant to $CdCl_2$ at low concentrations while few are resistant to higher concentrations up to 2000 ppm. These results are similar to that of Hassen et al. (1998) who reported that three strains of *P. aeruginosa* (S6, S7 and S8) isolated from natural polluted environments on nutrient agar were tolerant to $CuSO_4$, $Cr_2(SO_4)_3$ and $CoSO_4$ up to concentrations of 1.6, 1.2 and 0.8 mM, respectively. Similarly, Ünaldi et al. (2003) isolated strains of *Pseudomonas* sp. on glutamine-starch-phenol (GSP) agar which have tolerance to $CuSO_4$, $NiCl_2$, $CdCl_2$, and K_2Cr_2O up to 5250, 2594.2, 2010 and 343.44 ppm, respectively.

Among the 62 *Pseudomonas* spp., isolate no. 22 (soil sample no.7) was the most heavy metal-resistant one. It was resistant to $CdCl_2$ (1800 - 2000 ppm) and was selected for further investigations.

Isolation and curing of plasmid

A single plasmid was detected of about 27.491 kb. This suggests that the cadmium resistance of *Pseudomonas* 22 is plasmid mediated (Figure 3). This closely agrees with Malik and Jaiswal (2000). Ünaldi et al. (2003) reported that *Pseudomonas* strain WP19 which was resistant to Cu, Ni, Cd and Cr had 4 plasmids of approximately 20.8, 19.6, 8.0 and 4.7 kb. On the other hand, Malik et al. (2002) reported that the molecular size of the plasmids located in the bacterial strains isolated from agricultural and industrial soils which are re-

Table 2. Physicochemical characteristics of sewage and River Nile samples collected from different sites at Sohag Governorate.

Sample type	Sample No.	Physicochemical characteristics															
		Temp (°C)	pH	E.C (µhoms/ cm)	TSS (mg/l)	TDS (mg/l)	TN (ppm)	BOD (mg/l)	NO_3^+ (µg/L)	PO_4^{3-} (ppm)	Cl^- (ppm)	K^+ (ppm)	Na^+ (ppm)	Pb^{2+} (ppm)	Cu^{2+} (ppm)	Cd^{2+} (ppm)	Mg^{2+} (ppm)
Sewage	3	17	8.1	220	959	304	116	263	103	2.2	80	35	65	1.2	0.28	0.1	55
	15	16	7.6	200	975	300	110	260	125	2.7	56	34	35	0.8	0.28	0.09	47
	13	18	8.2	200	976	311	121	266	168	3.2	58	34	46	0.7	0.2	0.07	49
	18	23	8.5	210	984	315	115	278	275	4.1	42	20	24	0.7	0.12	0.06	32
	14	25	8.1	200	965	312	136	280	315	5.7	47	21	67	0.6	0.1	0.06	54
	8	17	8.2	180	986	410	127	300	414	5.7	51	26	54	0.6	0.1	0.05	46
	10	16	7.8	190	1050	406	150	305	495	5.8	38	15	35	0.5	0.09	0.04	68
	9	26	8.0	160	1220	414	188	311	668	6.9	65	14	45	0.5	0.07	0.03	75
	1	23	8.1	150	1300	417	190	340	705	7.5	55	14	51	0.4	0.06	0.02	58
River Nile	11	18	7.5	180	215	109	3.0	4.0	2.7	0.3	22	14	77	0.4	0.06	0.04	23
	12	19	7.6	200	221	112	5.2	4.2	4.2	0.4	23	9	59	0.3	0.05	0.02	34
	16	17	8.4	190	283	200	6.5	4.5	3.4	0.4	25	8	56	0.2	0.04	0.02	32
	4	22	8.1	160	314	215	7.4	5.5	10.8	1.3	23	1.8	45	0.2	0.01	0.014	33

Temp = Temperature; E.C = electric conductivity; TSS = total suspended salts; TDS = total dissolved salts; TN = total nitrogen; BOD = biological oxygen demand.

sistant to Hg, Cd, Cu, Cr, Pb, Zn and Ni were in the range of 7 - 70 kb. Similarly, Mahapatra et al. (2002) reported that *Acidiphilium symbioticum* KM2 is highly resistant to several metals and harbors three plasmids of 3.8, 7.1, and 56 kb in size. Moreover, Piotrowska-Seget et al (2005) found that 10 bacterial isolates out of 28 isolated from arable soil contained the plasmid of roughly 20 kb in size

Toxic effects of $CdCl_2$ on isolate S22 and its plasmid-cured strain

To confirm the previous results, the toxic effect of $CdCl_2$ (40 ppm) on the two strains of isolate 22 (plasmid-containing and plasmid-cured) was obtained. Figure 4 shows that the plasmid-cured strain formed a clear zone while the former was not affected. Thus $CdCl_2$ resistant (R) and sensitive (S) strains were obtained. Sabry et al. (1997) indicated that the resistant strains are those which were not inhibited by 1 mmol L^{-1} Cd. Mahapatra et al. (2002) reported that *Acidiphilium symbioticum* KM2 is highly resistant to several metals and harbors several plasmids. It becomes extremely sensitive to metals when it is cured of its plasmids.

Growth of *P. aeruginosa* S22 (R, S) under $CdCl_2$ stress

Figure 5 and Table 4 Show that optical density and maximum specific growth rate (k) for R, S strains decreased with increasing the concentrations of $CdCl_2$, while the generation time (T) increased. Cd^{2+} had a potent inhibitory action on growth. Mahapatra and Banerjee (1996) reported that $ZnSO_4$, $CdSO_4$, $CuSO_4$ and $NiSO_4$ decreased the growth rate (k) and increased the generation time (T) of different species of *Acidocella facilis*.

Determination of MICs of $CdCl_2$ for isolate S22 (R, S)

The obtained data revealed that the MIC for isolate no 22 R and S strains were 1.0 and 0.119 mmol L^{-1}, respectively. Wang et al. (1997) isolated a cadmium-resistant *P. aeruginosa* which tolerated cadmium up to 5 mM in artificial sea water medium. Hassen et al. (1998) and Hussein et al. 2005) isolated a Cd-resistant *P. aeruginosa* with

Table 3. Total counts of *Pseudomonas* sp. of different samples collected from different contaminated sites at Sohag district on CNA medium containing 40 ppm of $CdCl_2$.

Sample type	Sample no.	Heavy metal concentrations (cfu /g ry wt)	
		Control	$CdCl_2$ (40 ppm)
Soil	2	60	47
	7	72	54
	17	120	89
	5	145	107
	6	155	111
Sewage	3	215	110
	15	235	128
	13	260	156
	18	330	213
	14	335	224
	8	350	243
	10	355	224
	9	400	320
	1	780	687
River Nile	11	10	3
	12	20	9
	16	32	18
	4	45	21

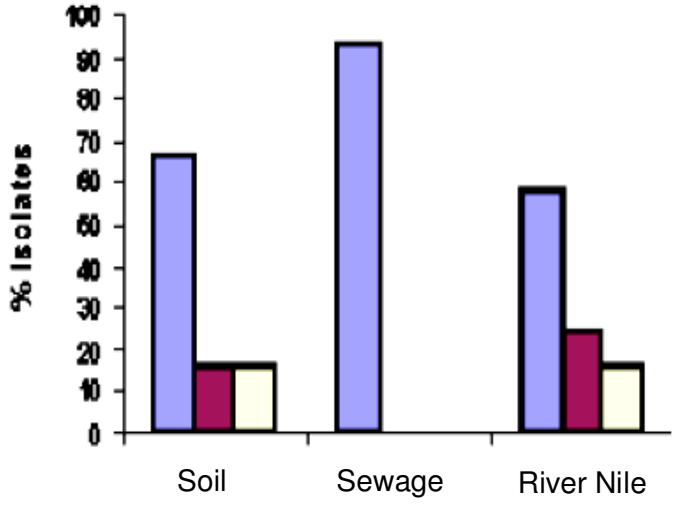

Figure 2. The percentage of the resistant *Pseudomonas* isolates to CdCl2 at low (100-700), moderate (800-1400) and high (1500-2000) concentration levels out of 18, 32 and 12 isolates isolated from soil, sewage and River Nile, respectively.

MIC of 1.5 mM in nutrient broth. On the other hand, Malik (2004) reported that *Pseudomonas* strain H1 isolated from soil was resistant to 225 ppm Cd^{2+}. Moreover, Piotrowska-Seget et al. (2005) reported that the MICs of Pb, Cd, Cu and Zn for a soil-borne *Pseudomonas gla-*

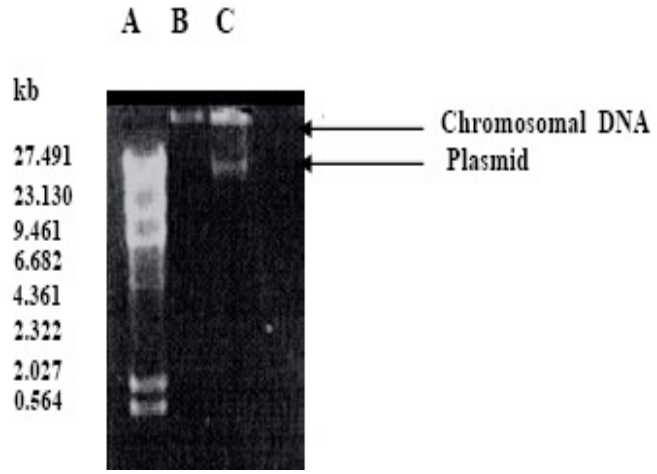

Figure 3. Lambda DNA cleaved with HindIII (A, molecular weight markers), the absence of plasmid in cured "sensitive" cells (B), and presence of plasmid in "resistant cells" (C) of *P. aeruginosa* S22 isolate.

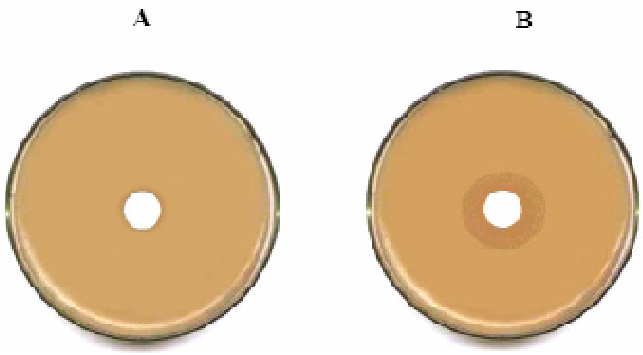

Figure 4. Toxic effect of $CdCl_2$ on isolate S22 (A) and the plasmid-cured strain (B) grown on tris minimal agar medium by using disc-method. The discs were impregnated with 40 ppm $CdCl_2$.

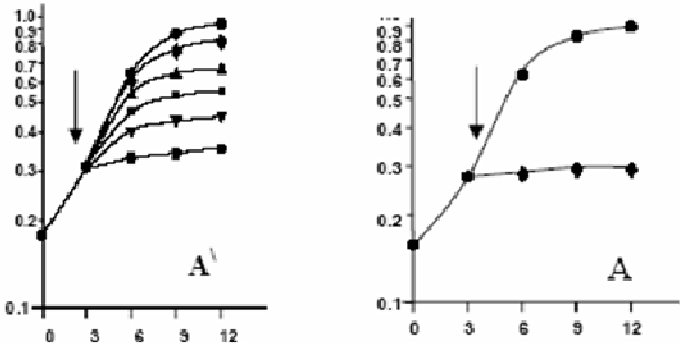

Figure 5. Growth curves of *P. aeruginosa* S22 grown in Tris minimal broth containing 0 ppm (—●—), 40 ppm $CdCl_2$(—♦—) for S-strain (A) ; 0 ppm (—●—), 40 ppm (—♦—), 80 ppm (—▲—), 120 (—■—), 160 ppm (—▼—) and 200 ppm (—●—) of $CdCl_2$ (A'), for R-strain incubated at 37 ℃ with agitation at 150 rpm for 12 h. $CdCl_2$ was added after 3 h of incubation (arrow).

Table 4. Growth parameters of *Pseudomonas aeruginosa* S22 (R, S) grown in Tris minimal broth containing 0, 40 ppm of $CdCl_2$ for S-strain and 0, 40, 80, 120, 160 and 200 ppm for R-strain of $CdCl_2$ agitated at 150 rpm and incubated at 37°C for 12 h. Heavy metals were added after 3 h of incubation.

$CdCl_2$ conc. (ppm)	T_λ (h)	T_α (h)	a R (S)	k R (S)	T (h) R (S)
Control	1.5	9.0	1.61	0.117	3.45
$CdCl_2$					
40	1.5	9.0	1.57 (0.60)	0.086 (0.01)	3.9 (>12)
80	1.5	9.0	1.52	0.085	4.35
120	1.5	9.0	1.23	0.07	5.4
160	1.5	9.0	1.16	0.04	8.75
200	1.5	9.0	1.01	0.03	12.0

T_λ (h) = Lag period; T_α(h) = the end of the exponential phase; a = asymptotic level = ln ($OD\alpha/OD_0$); k = the maximum specific growth rate = $a / T\alpha - T_0$; T = generation time; and R = resistant strain.

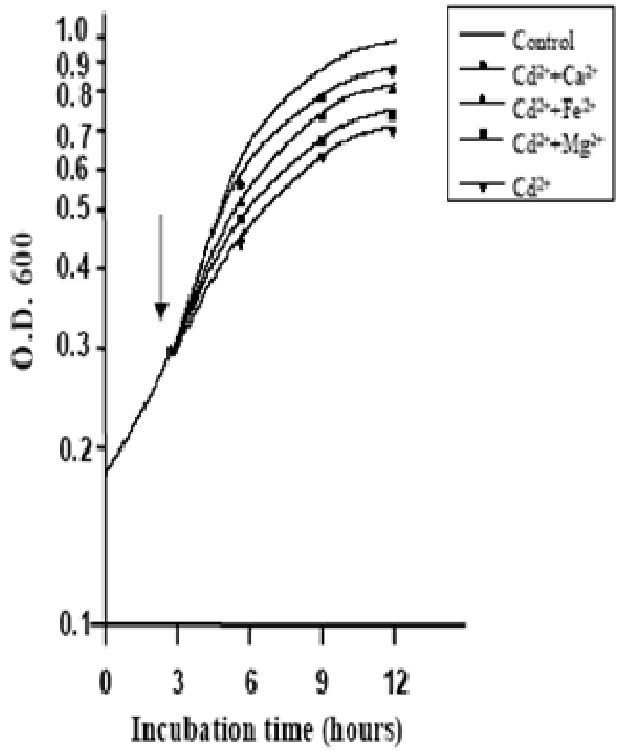

Figure 6. Effect of divalent cations (Ca^{2+}, Mg^{2+} and Fe^{2+}) on *P. aeruginosa* S22 (R-strain) grown in tris minimal medium containing 40 ppm Cd^{2+}in addition to 40 ppm of each of Mg^{2+},Fe^2 or Ca^{2+} and incubated at 37°C with agitation at 150 rpm for 12 h. Time at which $Cd^{2+}$and divalent cations were added (3 h).

diolus were 0.1, 3, 5 and 10 $mmoll^{-1}$, respectively.

Identification of isolate S22

Isolate was identified as *P. aeruginosa* S22 based on phenotypic methods. Shoreit and Soltan (1992) isolated 394 isolates on selective isolation media from different soil and water sites at Sohag district and they were identified as *Pseudomonas*; 9% of them were identified as *P. aeruginosa*. Soltan (2001) isolated 240 strains of *P. aeruginosa* from a wastewater treatment plant, agricultural drainage canals, the River Nile and irrigation canals at Sohag area 7.15, 12.9, 25.4, and 53.7% of the isolates were resistant to lead, cadmium, mercury and zinc, respectively.

Effect of divalent cations on growth of *P. aeruginosa* S22 in the presence of Cd^{2+}

Figure 6 and Table 5 depict that growth and the maximum specific growth rate (k) increased by addition Fe^{2+}, Mg^{2+} and Ca^{2+}, while the generation time (T) decreased compared to Cd^{2+} only. These results are closely related to the data obtained by Adam et al. (1997) who observed that Ca^{2+} ions increased cell multiplication of *Scenedesmus obliquus* in comparison with heavy metal treatment alone. Also, magnesium found to reduce toxicity of nickel to bacteria and yeast (Hettiarachchi et al., 2000; Sandrin and Maier, 2003).

The effect of cadmium on morphology

Scanning and transmission electron micrographs (Figure 7) of *P. aeruginosa* S22 cells grown in tris minimal broth containing 120 ppm $CdCl_2$ show malformations such as filament formation where some cells are attached together (Figure 7B) in addition to roughened cell surface (Figure 7C) in comparison with control (Figure 7A). Ultrastructural modifications such as thinner and ruptured cell walls and large intracellular vacuoles appeared within the cells (Figure 7F).

Filament formation by *P. aeruginosa* S22 under cadmium stress may be a result of cadmium interference with the division mechanisms. This agrees with Nepple et al. (1999) who suggested that heavy metals act strongly on cell division. Morita (1997) reported that cell surfaces of cultures treated with cadmium chloride tended to be rough, suggesting that the cell increased its surface to

Table 5. Effect of divalent cations (Ca^{2+}, Mg^{2+} and Fe^{2+}) on the growth parameters of *P. aeruginosa* S22 (R-strain) grown in tris minimal broth containing 40 ppm Cd^{2+}in addition to 40 ppm of each of Mg^{2+},Fe^{2} or Ca^{2+}, incubated at 37ºC with agitation at 150 rpm for 12 h.

Conc. of Cd^{2+} and divalent cations (40 ppm of each)	T_λ (h)	T_α (h)	a	K	T (h)
Cd^{2+} + Ca^{2+}	1.5	9	1.84	0.30	3.4
Cd^{2+} + Mg^{2+}	1.5	9	1.75	0.29	3.57
Cd^{2+} + Fe^{2+}	1.5	9	1.64	0.27	3.8
Cd^{2+}	1.5	9	1.61	0.26	3.87
Control	1.5	9	1.89	0.32	3.3

T_λ (h) = Lag period; T_α(h) = the end of the exponential phase; a = asymptotic level = ln ($OD\alpha/OD_0$); k = the maximum specific growth rate = a / Tα – T_0; T = generation time; and R = resistant strain.

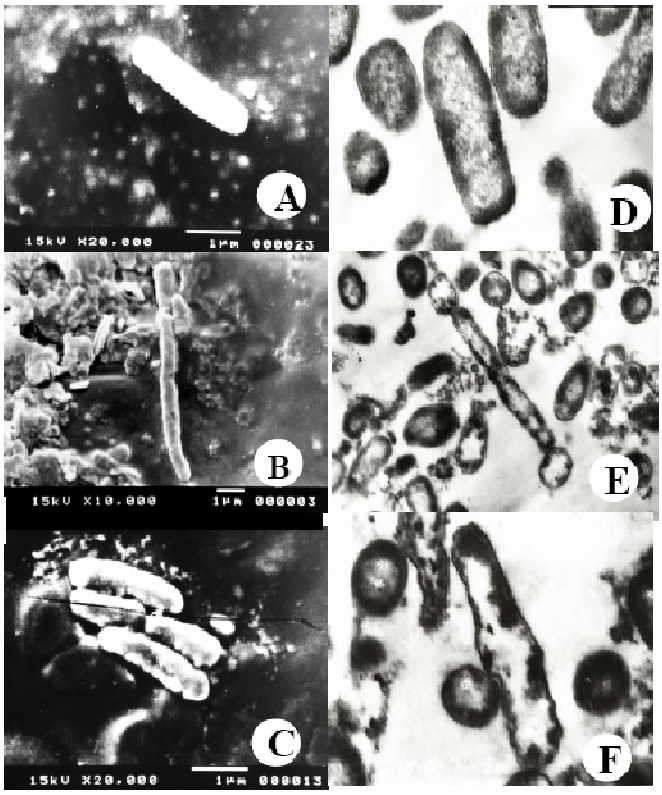

Figure 7. Scanning (A, B, C) and transmission (D, E, F) electron micrographs of *P. aeruginosa* S22 grown in Ttris minimal broth containing 0 (control) (A, D) and 120 ppm $CdCl_2$ (B, C, E and F) agitated at 150 rpm and incubated at 37°C (Bar = 1µ).

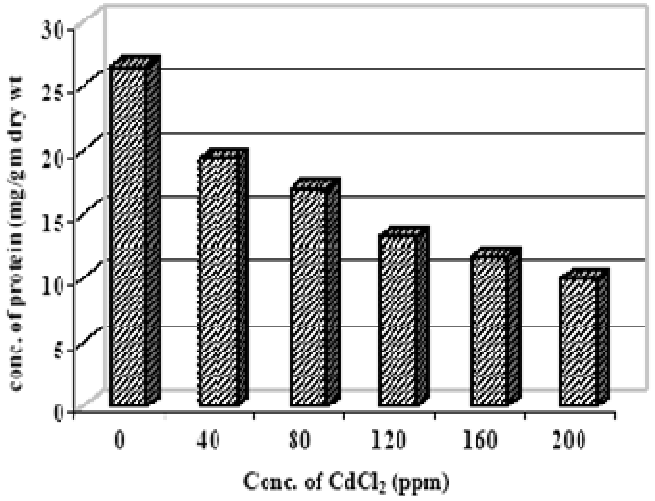

Figure 8. Toxic effect of $CdCl_2$ on the total protein content of *P. aeruginosa* S22 grown in Tris minimal broth containing 0, 40, 80, 120, 160 and 200 ppm of $CdCl_2$, agitated at 150 rpm and incubated at 37°C for 12 h.

improve the interaction of toxic substances with the cell surface.

Effect of $CdCl_2$ on protein content

Figure 8 shows that different concentrations of $CdCl_2$ inhibit protein synthesis of *P. aeruginosa* S22 compared to the respective control. The protein inhibition ratio caused by $CdCl_2$ reached about 27, 36, 48, 56 and 62% at 40, 80, 120, 160 and 200 ppm $CdCl_2$, respectively. Our obtained data indicate that Cd^{2+} decreases the total protein content of *P. aeruginosa* S22. Fahmy (2001) stated a decrease in the total protein content of two strains of photosynthetic bacteria *Rhodobacter capsulatus* B10 (capsulated strain) and 37b4 (non-capsulated strain) treated with different concentrations of Cd^{2+} and Zn^{2+}. Lima Ana et al. (2005) reported a drastic decrease in total soluble protein content of *Rhizobium leguminosarum* bv. *Viciae* traeated with high concentrations of Cd^{2+} which suggests that intracellular Cd^{2+} imposed on cellular metabolism.

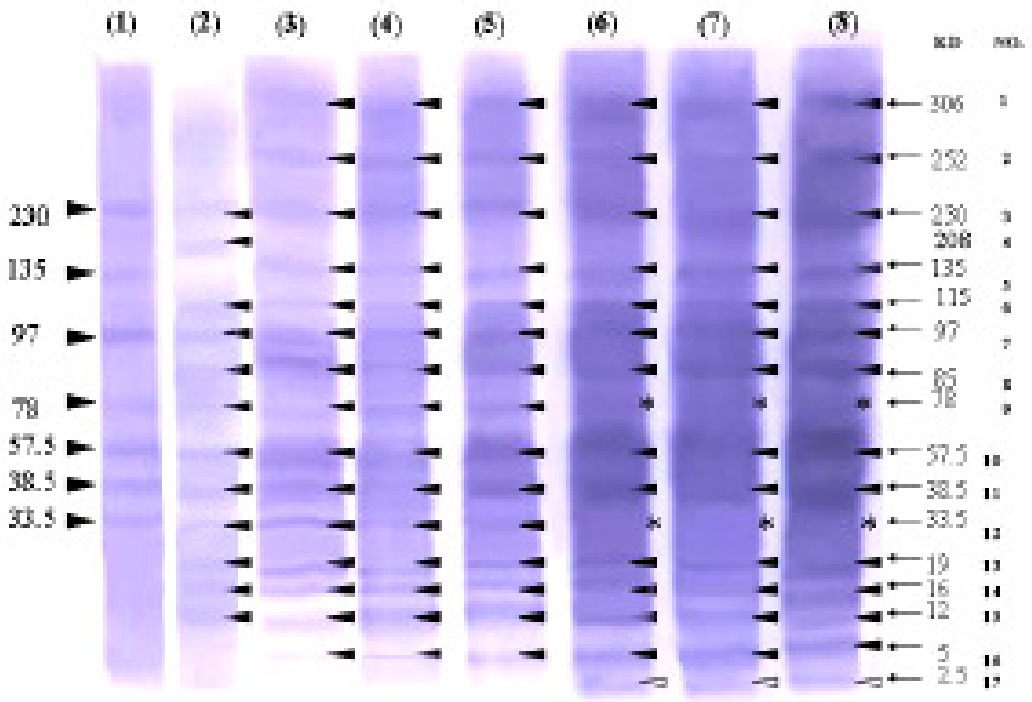

Figure 9. Soluble protein patterns (comassie blue stained 12% SDS-PAGE) of whole cell lysates extracted with lysing buffer (see materials and methods) of S-strain (lane 2) and R-strain (lanes 3 - 9) of *P. aeruginosa* S22 grown in Tris minimal broth containing 0, 40, 80, 120,160 and 200 ppm and incubated with shaking at 150 rpm at 37°C for 12 h. Lane 1: migration distance of molecular mass standards (in kilodations) are indicated in the left; lanes 2, 3 : 0 ppm $CdCl_2$ of both strains; lanes 4, 5, 6, 7 and 8 :40, 80, 120, 160 and 200 ppm $CdCl_2$, respectively, of resistant strain. Arrowheads point at some of the new bands induced during stress of $CdCl_2$. Star (*) point at some bands which disappeared at 120, 160 and 200 ppm $CdCl_2$ but appeared in control

Protein profiles of *P. aeruginosa* S22 under Cd^{2+} stress

The obtained data in Figure 9 and Table 6 reveales an alteration of protein profile when *P. aeruginosa* S22 was treated with different concentrations of $CdCl_2$. Three proteins of high molecular weights (208, 78 and 33.5 KDa) were lost and one protein with low molecular weight (2.5 KDa) was induced as a response of Cd^{2+} stress. These findings are closely related to the data obtained by Mallick and Rai (1998), who detected production of a 3.3 KD protein, rich in cadmium and –SH contents in the cyanobacterium *Anabaena doliolum* following 20 mM Cd exposure. They also observed that Cd is a strong inducer of the metal-binding proteins. On the other hand, Hamer (1986) reported that the synthesis of metal-binding proteins with low molecular weight (6 - 7 kDa) and rich with cysteine is well-known in bacteria. Fahmy (2001) also reported the induction of new sets of proteins (156, 75, 119 and 105 KDa) in *Rhodobacter capsulatus* strain B10 and 37b4, while a 67 KDa protein disappeared in both strains under cadmium stress. Similarly, Valerie and Gordon (1994) reported that the production of a copper-induced protein has been implicated in copper detoxification in a wild-type *Vibrio alginolticus*. Laplace et al. (1996) reported induction of new proteins by *Enterococcus feacalis* treated with high concentrations of Cd^{2+}. Aiu et al. (1999) found that two Cobalt-resistant strains of *Pseudomonas* [*P.* sp. BS501 and *P. putida* BS394 (pBS501)] induced protective surface proteins of the cell wall with the molecular weights of 49, 40, and 32 KDa under the stress of cobalt. Moreover, Nies (1992) observed that 116-kDa CzcA, 55-kDa CzcB and 37-kDa Czc proteins were induced by the gram-negative bacterium *Alcaligenes eutrophus* under cadmium stress.

Lipopolysaccharide (LPS) profiles of *P. aeruginosa* S22 under $CdCl_2$ stress

The data (Figure 10) revealed that the LPS profiles were modified under stress of $CdCl_2$. Absence of bands in region I which represents the O-specific antigen and region II (core-oligosaccharide linked to lipid A) occurred at high concentrations of $CdCl_2$. In this respect, Lins and Straatsma (2001) stated that lipopolysaccharides (LPS) form the major constituent of the outer membrane of Gram-negative bacteria, and are believed to play a key

Table 6. Molecular weights (Mr.wt.) and percentage amounts of protein bands of untreated S-strain of *Pseudomonas aeruginosa* S22 and R, strain grown in tris minimal broth containing 0, 40,80, 120, 160 and 200 ppm$CdCl_2$, both strains were incubated at 37°Cwith agitation at 150 rpm for 12 h.

Band no.	Marker Mr. wt. (Kda)	Mr. wt. (Kda)	Amount of protein (%)						
			S-strain	R-strain					
			Untreated (Lane 2)	Cont. (Lane 3)	40 ppm (Lane 4)	80 ppm (Lane 5)	120 ppm (Lane 6)	160 ppm (Lane 7)	200 ppm (Lane 8)
1		306	----	2.6	3.0	2.4	2.2	4.5	4.3
2		252	----	2.3	3.3	4.6	3.2	2.5	3.8
3	230	230	1.8	3.0	2.9	4.5	4.4	4.3	5.3
4		208	2.8	----	----	----	----	----	----
5	135	135	----	2.9	3.6	3.5	3.2	3.0	3.5
6		115	3.8	2.0	3.1	2.9	3.0	3.8	4.3
7	97	97	5.2	3.3	4.6	6.0	4.5	2.5	3.7
8		85	4.4	2.9	3.0	4.4	4.7	4.8	5.6
9	78	78	2.8	3.1	3.7	3.4	----	----	----
10	57.5	57.5	3.1	2.0	4.5	4.6	4.2	4.5	5.1
11	38.5	38.5	3.8	3.9	3.0	2.7	4.4	3.0	2.3
12	33.5	33.5	3.3	8.0	8.1	4.4	----	----	----
13		19	3.8	3.4	2.1	2.7	3.0	3.2	4.6
14		16	5.0	3.1	3.7	3.9	3.4	1.6	3.2
15		12	8.6	5.2	5.0	4.7	3.3	2.9	5.7
16		5	4.0	5.1	4.6	5.1	4.7	4.2	5.5
17		2.5	----	----	----	----	5.7	5.9	3.9

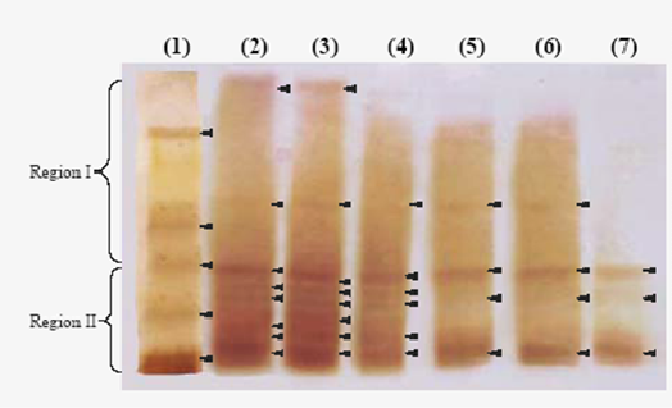

Figure 10. LPS profile in 12% SDS-PAGE of whole cell lysates extracted with lysing buffer and subjected to protinase K digestion (see materials and methods) of Pseudomonas aeruginosa S22 (untreated S-strain Lane 1) and R-strain (Lanes 2-7) $CdCl_2$. Silver strained protinase K resistant bands (controls and treated are indicated by arrows (region I and II). T he absence of arrows indicated the removal of bands under stress of $CdCl_2$.

role in processes that govern microbial metal binding, microbial adsorption to mineral surfaces, and microbe-mediated oxidation/reduction reactions at the bacterial exterior surface. Arredondo et al. (1994) concluded that raising the pH of the solution containing the cells of *Thiobacillus ferrooxidans* from 1.5 to 8.0 releases about 50% of LPS. On the other hand, Fahmy (2001) found that region I (O-specific antigen) and region II (core-oligosaccharide linked to lipid A) of *Rhodobacter capsulatus* strain B10 (capsulated strain) and strain 37b4 (non-capsulated strain) completely disappeared under cadmium stress, whereas, partial removal of region I was missing in non-capsulated strain under the same stress of heavy metal. Moreover, Poole (2002) reported that LPS mutations were associated with antibiotic treatment in *P. aeruginosa*.

Conclusion

The results obtained in this study indicate that this isolated *P. aeroginosa* S22 could be a good candidate for heavy metal removal from polluted environments.

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