Bull.Fac. Agric.,Cairo Univ.,64: 78-86(2013).

SIMULTANEOUS REMOVAL OF ZINC, COPPER AND COBALT FROM CONTAMINATED SOILS BY BIOSURFACTANT

(Received: 8. 10. 2012)

By
M.M.S. El-Shahad, W.D.Saleh and M.Z. Sedik

Microbiological Department, Faculty of Agriculture, Cairo University, Giza, Egypt

ABSTRACT

A biosurfactant-producing bacterium isolated from clay soils was investigated for its effects on the plant growth characteristics and heavy metal removal. A pot experiment was conducted for investigating the capability of the biosurfactant-producing bacterial strain *Rhodococcus* sp. to improve the plant growth and zinc, copper and cobalt uptake of tomato in soil artificially contaminated with different levels of Zn, Cu and Co (50 ppm kg^{-1} for each element).

Data revealed that bacilli gave a highly percentage of the bacterial isolates (38.7%) followed by micrococci group (34.4%). While, short rods Gram negative presented 16.1% of the total isolates. Moreover, both of the long rods Gram positive and filaments bacteria presented 5.4%, respectively.

The morphological characteristics of pure isolates indicated that Gram positive presented 84.2% of the total isolates. While, the Gram negative represented (15.8%) from the total isolates.

Thirty three isolates were tested for biosurfactant production. Only 6 isolates had potential to degrade the diesel oil after 3 to 5 days with a different degree of degradation.

The percentage of the height of emulsified layer (cm) after 3 and 5 days of incubation period of the selected six isolates showed that isolate Sm2-4 gave the highest emulsification percentage (91%) after 3 days of incubation period. While isolates Sm1-1, Sm2-3 and Sm2-8 gave the lowest emulsification % reached 71% after 3 days. Both of the two isolates number Sm2-12 and Sm2-9 recorded the second category of emulsification percentage and reached 88% after 3 days of incubation period.

Growth parameters of tomato plants grown for 60 days after treatment with heavy metals and bacterial treatments were studied. At the beginning, tomato plants of all treatments nicely grew with no nutrient deficiency symptoms.

Plants of less than 26 cm in height were detected with a plant left without treatment (control). Recommended fertilizers N, P, and K stimulated plant growth and resulted in 38.4 % increase in height of 60-day-old plants. While, plants fertilized with the recommended dose of NPK, inoculated with *Rhodococcus* sp. and treated with a mixture of Co, Cu and Zn sulphate 15 days after planting gave 57.1% increase in plant height when compared with untreated ones.

Root sizes ranged from 2.2 to 6.8 cm^3 depending on treatment and plant age-dependent.

Root and shoot biomass yields increased as a result of inoculation and / or heavy metal treatment. When *Rhodococcus* sp. and heavy metal salts (50 ppm concentration) were applied in the presence of full dose of NPK root and shoot dry weights were 84.2 and 193.3% higher than those of the control. Also, when *Rhodococcus* sp. and heavy metals were applied 15 days after planting, root and shoot dry weights were 118.4 and 222.1% higher than those of the untreated ones.

Tomato plants bore considerable number of leaves. Plenty of those (83 and 92 per plant) was produced by plants received full dose of recommended fertilizers together with incorporation into soil of the tested dose of three heavy metals.

Keywords: *biosurfactant, Rhodococcus sp., Zn, CU, Co, tomato.*

1. INTRODUCTION

Surfactants are amphiphilic compounds containing both hydrophilic and lipophilic moieties. Due to their dual nature, surfactants tend to partition into the oil-air or oil-water interface to reduce the surface and interfacial tension and stabilize newly created interfaces. Surfactants can be derived from both chemically based ("chemical surfactants" or "synthetic surfactants") and biologically based (biosurfactants) sources

(Urum and Pekdemir, 2004 and Qingyi *et al.*, 2011).

Many microorganisms have the ability to produce a wide range of biosurfactants. An initial classification of biosurfactant was made into two types; based on molecular weights, properties and cellular localizations. The low molecular weight biosurfactants *e.g.* glycolipids, lipopeptides, flavolipids, corynomycolic acids and phospholipids lower the surface and interfacial tensions at the air/water interfaces. High molecular weight ones are called bioemulsans, (such as emulsan, alasan, liposan, polysaccharides and protein complexes) and are more effective in stabilizing oil-in-water emulsions (Neu, 1996, Franzetti *et al.*, 2009 and Salihu *et al.*, 2009).These high molecular weight biosurfactants are highly efficient emulsifiers that work at low concentrations and exhibit considerable substrate specificity (Dastgheib *et al.*, 2008 and Salihu *et al.*, 2009).

Biosurfactant producing microorganisms can be used in bioremediation and oil leak clearance in soil and water environments (Head and Swannell, 1999; Mulligan, 2005; Urum *et al.*, 2006 and Ghayyomi Jazeh *et al.*, 2012).

Contamination of soil environments with heavy metals is very hazardous for human and other living organisms in the ecosystem. Due to their extremely toxic nature, the presence of even low concentrations of heavy metals in the soil has been found to have serious consequences. Nowadays, there are many techniques used to clean up soils contaminated with heavy metals. Remediation of these soils includes non biological methods such as excavation, and disposal of contaminated soil to landfill sites or biological techniques (Aşçı *et al.*, 2010 and Pacwa-Płociniczak *et al.*, 2011).

Biological methods are processes that use plants (phytoremedation) or microorganisms (bioremediation) to remove metals from the soil. The two following methods, soil washing or soil flushing, are involved in remediation of metal contaminated soil. The first technique used is *ex situ* contaminated soil is excavated, put into the glass column and washed with biosurfactant solution. In turn, soil flushing of *in situ* technologies involves use of drain pipes and trenches for introducing and collecting biosurfactant solution to and from the soil (Herman *et al.*, 1995; Singh and Cameotra, 2004 and Pacwa-Płociniczak *et al.*, 2011).

Previous work with *Rhodococcus* sp. has shown that the strain could produce biosurfactants and mobilize a mixture of zinc, copper and cobalt efficiently in the soil. However, research to determine the potential effect of *Rhodococcus* sp. on the growth of tomato in mixture of Zn, Cu and Co-amended soils has not been performed.

The major objectives of this research were to isolate and characterize the biosurfactant-producing *Rhodococcus* sp. Also, to evaluate the enhancement of plant growth improvement and Zn, Cu and Co uptake in tomato plants grown in Zn, Cu and Co-amended soil for improving the efficiency of bioremediation of Zn, Cu and Co-polluted soils.

2. MATERIALS AND METHODS

2.1. Soil samples

Ten soil samples were collected from six different sites in Giza and Ismailia Governorates. Three clay soil samples were collected at a depth of 10 cm from the Experimental Station of the Faculty of Agriculture, Cairo University and another three sandy-clay soils collected from private farms in Kerdasa Village, Giza. In addition, four sandy soil samples were collected from Ismailia Governorate.

2.2. Bacterial isolates

Serial dilution aliquots were used for the inoculation of the culture media. Enrichment and isolation of bacterial isolates were performed using nutrient agar medium. The plate cultures were incubated at 30°C for 24-48 hours (Khan and Syed, 2011). Ninety five isolates were collected from the soil samples. Streak plate procedure, complied by Grainger *et al.* (2001)was used. Selected isolates were transferred to fresh medium until identification and classification carried out. The isolates were purified by streaking plate procedure on nutrient agar medium and studied for morphological characters and biochemical tests.

2.3. Identification of bacteria

Colonies were selected from agar plates according to their morphological characteristics which include colony size, colony consistency, colony color, Gram staining and the motility tests.

2.4. Biochemical tests

The biochemical tests were applied on pure culture according to the tests recorded by Sneath (1984).

2.5. Biosurfactant activity

Thirty three isolates were tested for biosurfactant production by inoculating them in nutrient broth medium incubated at 30°C for 24 hours. After incubation period 1ml of diesel oil

was added into culture tubes and incubated at 30°C for 2-7 days.

Emulsification index (E24) was used for measuring and detecting the best isolates producing biosurfactant (Sarubbo, 2006). The E24 of six strains was measured by adding 2ml of diesel oil and 2 ml of the broth culture in a test tube, vortexes at 380 ppm for 2 min and incubated for 5 days. The percentage of emulsified layer was calculated by the formula.

E24 = Height of emulsion formed x 100 / Total height of the solution.

The E24 index is given as a percentage of the height of emulsified layer (cm).

2.6. Heavy metal resistance

The best six bacterial isolates (Sm1-1, Sm2-3, Sm2-4, Sm2-8, Sm2-9 and Sm2-12) which gave the highest emulsion layer were accomplished for resistance test in nutrient broth supplemented with three heavy metals in salt form (Cu, Zn and Co sulfate) with three concentrations for each heavy metal salt (25 ppm, 50 ppm and 100 ppm). The more resistant isolate (Sm2-4) was inoculated in broth culture medium supplemented with three heavy metals and incubated at 30°C for 24-48 hours. Another experiment was done by using nutrient agar medium supplemented with a mixture of heavy metals with the same last concentrations inoculated with the bacterial isolate (Sm 2-4) by streaking method and incubated at 30° C for 24 hours.

2.7. Application of biosurfactant in pot experiment Soil

The soil used in bioremediation of heavy metals (Zn, Cu and Co) by biosurfactant producing bacterial isolate was collected from the farm of the Faculty of Agriculture, Cairo University, Giza. Soil was characterized by: sand, 38.6 % ; silt, 19.6 %; clay, 27.0 %; WHC, 28 %; pH, 7.4; EC, 0.67 dSm^{-1}; total carbon, 0.58 % and total nitrogen, 0.05 % (Jackson, 1973). The soils were air dried and crushed to pass 2 mm sieve. Plastic pots of 30 cm diameter and 25 cm depth were filled with 8 kg of sandy loam soil: peat mixture (1:1 w/w).

Twenty one day old tomato seedlings (*Solanum lycopersicum*); kindly provided by the Vegetable Crops Department, Faculty of Agriculture, Cairo University; were planted at the rate of 3 seedlings pot^{-1}. Heavy metals (Zn, Cu and Co) were incorporated into the experimental pots in levels equivalent to 50 ppm for each metal as sulfate salts.

For bacterial inoculation, the best isolate characterized by high production of biosurfactant was separately grown in nutrient broth for one week. An aliquot of 5.0 ml of the bacterial inoculum was added over head soil adjacent to the seedling root system, this was done at planting.

A pot experiment was designed to improve tomato growth *via* biosurfactant produced by the best isolate inoculated in the soil contaminated with a mixture of heavy metals. The experimental design encompassed four treatments as follows; 1) untreated plants, 2) fully-NPK received plants, 3) fully NPK received plants supplemented with the best bacterial and mixture of Zn, Co and Cu (50 ppm) and 4) fully NPK received plants supplemented with the best isolate and a mixture of the last three heavy metals 15 days after planting. Application of the recommended doses of NPK fertilizers was done as nitrogen in the form of ammonium sulphate (20.6 % N); and PK fertilization regimes as 500 kg fed^{-1} and 400 kg fed^{-1} of calcium superphosphate and potassium sulphate, respectively. The nitrogen fertilizer was added at three equal levels after 7, 15 and 21 days of transplanting. Inoculation with the best isolate was performed by adding an aliquot of 5.0 ml of the bacterial inoculum over head soil adjacent to the seedling root system of 21-day old tomato seedlings in heavy inoculum of > 10^8 cell ml^{-1}. Plantation, incorporation into the soil of the fertilizers, inoculation with biosurfactant producing bacterial culture, irrigation and sampling procedures were applied as pot experiment.

The applications of heavy metals were applied in two sets of pot experiments. In the first set, heavy metals (Zn, Cu and Co) were introduced at planting. While in the other; the (Zn, Cu and Co) were incorporated into the soil 15 days after seedling establishment. These pot experiments were designed to clarify the effect of bioremediation of Zn, Cu and Co by biosurfactant as well as the vegetable growth in the soil inoculated with the best isolate. The adopted potting mixture facilitates an appropriate root establishment and plant standing.

Along the experimental period of 60 days, plants were watered to keep the moisture level at ca. 60% WHC. An additional inoculum was added at 15-day intervals. Four treatments were allocated for either vegetable plant in triplicates.

2.8. Statistical analysis

Data were statistically analyzed by least

significant difference (Snedecor and Cochran, 1980) as well as correlations and linear regressions among different growth variables.

3. RESULTS AND DISCUSSION

Ten soil samples were collected from two governorates for the isolation and screening of bacterial isolates producing biosurfactant. Ninety five bacterial isolates were isolated and purified.

Data obtained indicated that the majority of the isolates displayed a colony size less than 1.0 cm. Also, the consistency was ranged between aqua to rough in most bacterial isolates. Colony color, was creamy, yellow, white, brown and white brown.

The morphological characteristics of the purified isolates represented five forms of bacteria *i.e.* spore-forming, micrococci, short rods G-, long rods G+ and filamentous bacteria. Data in Table (1) and Figure (1) reveal that spore-forming bacteria represented the highest percentage of the bacterial isolates (38.7%) followed by micrococci group (34.4%). While, short rods Gram negative represented (16.1 %) of the total isolates. Long rods Gram positive and filamentous bacteria represented 5.4%, respectively.

turbidity as well as sediment in their broth culture media. Also, 20% of the total pure isolates was giving a pellicle.

The Gram staining results of pure isolates are presented in Fig. (2). The results indicated that the Gram positive presented (84.2%) of the total isolates. While the Gram negative represented (15.8%) from the total isolates.

The motility test showed that 57.9 % of the total isolates were motile but the rest were not motile (Fig. 3).

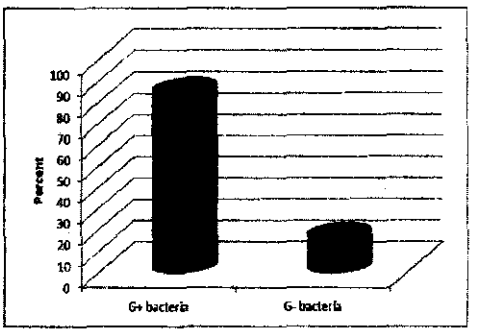


Fig. (2): Gram staining percentage of the bacterial isolates.

Table(1): Number and form of bacterial isolates of each soil sample tested

Sample No.	No of Isolate.	Isolate form				
		Spore-forming	Micrococci	Short rod G^-	Long rod G^+	Filaments bacteria
Sm1	10	5	-	3	-	2
Sm2	12	4	4	3	1	-
Sm3	11	2	6	3	-	-
Sm4	8	3	3	2	-	-
Sm5	7	2	2	1	1	1
Sm6	8	4	3	-	-	1
Sm7	12	5	4	2	1	-
Sm8	8	3	4	1	-	-
Sm9	10	5	3	-	1	1
Sm10	9	4	4	-	1	-
Total Isolates	95	37	33	15	5	5

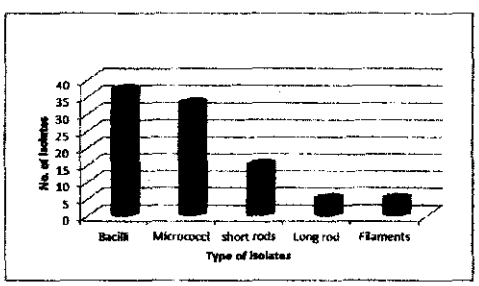


Fig. (1): Distribution of bacterial isolates isolated from ten soil samples.

The growth of pure isolates on nutrient broth showed that more than 50% of the isolates gave a

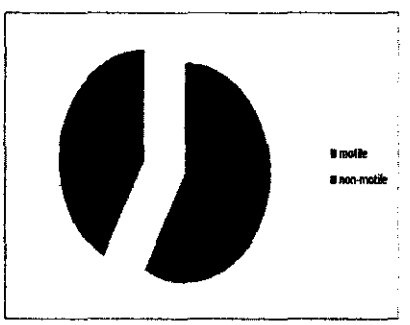


Fig. (3): Percentage distribution of motile and non-motile bacterial isolates.

3.1Biochemical tests

Data presented in Table (2) showed the results of biochemical tests for the selective and chosen isolates.

Table (2): Biochemical tests of the selective bacterial isolates.

Form	Tests and results
Spore-forming bacteria	Club shape, spore, large, strict aerobe, catalase (+), V.P. (+), cell diameter ≥ 1 µm, citrate (+), growth in 6.5% NaCl (+), starch hydrolysis (+), glucose fermentation (+) and mannitol fermentation (+).
Long rod, non- spore form	Club shape, non-spore, branching, rudimentary, catalase (-), acid fast (-), oxidase test (-) and Hugh Lefson test (+).
Short-rods form	Short rod, Gram (-), motile, non-spore, oxidase(+), indole (+), methyl red (+), catalse (+), urease (+) and glucose fermentation (-) .
Cocci form	Round and oval shape, Gram^{+}, non-motile,
Filamentous shape	Branching filaments, Gram (+), irregularly size elements, non-motile, flagellate spore, gelatin slowly liquefied, Indole (+), starch hydrolysis (+) and nitrate hydrolysis (+).

3.2.Biosurfactant activity

Thirty three isolates were tested for biosurfactant production (Sarubbo,2006). Only 6 isolates had potential to degrade the diesel oil after 3 to 5 days with a different degree of degradation (Table 3). Isolates Sm 2-4 gave the highest degradation of motor oil after 3 and 5 days of incubation period. While, two isolates only (Sm2-9 and Sm2-12) gave very good results of degradation after the same period. Moreover, the three isolates (Sm1-1, Sm2-3 and Sm2-8) gave a pass result. On the other hand, the rest of the total isolates (27 isolates) was not able to degrade the diesel oil after 3 and 5 days of incubation periods.

Table (3): Degree of degradation of the diesel oil of the thirty three isolates after 3 days.

Isolate number	Sm1- 1	Sm2-3	Sm2- 4	Sm2-8	Sm2-9	Sm2-12
Degradation diesel oil	1+	1+	5^{+}	1+	3^{+}	3^{+}

Pass (+), Very good (3+) and Excellent (5+).

The percentages of the height of emulsified layer (cm) after 3 and 5 days of incubation period of the selected six isolates are presented in Tables (4 and 5). The results showed that isolate Sm2-4 gave the highest emulsification percentage (91%) after 3 days of incubation period. While, isolates number Sm1-1, Sm2-3 and Sm2-8 gave the lowest % and reached 71% of emulsification % after 3 days. Both isolates number Sm2-9 and Sm2-12 gave the second category of emulsified percentage which reached 88 % after three days of incubation period. While, the increasing of the incubation period to 5 days led to decreasing the emulsification percentage in all isolates tested with the same percentage of decrease.

The best isolate (Sm2-4) for the production of biosurfactant was classified according to Brenner *et al.* (2005). The classification results were recorded in Table (6) and Fig.(4).

According to the morphological and biochemical tests carried out, the best isolate proved to be *Rhodococcus* sp.

3.3.Resistance to heavy metals

The best bacterial isolate (*Rhodococcus* sp.) which gave the highest emulsion layer was accomplished for resistance to heavy metal test in nutrient broth supplemented with three heavy metals in salt form (Cu, Zn and Co sulfate) with three concentrations for each heavy metal salt (25 ppm, 50 ppm and 100 ppm). The cultural medium supplemented with heavy metals was inoculated by bacterial isolate (Sm2-4) and incubated at 30°C for 24-48 hours. It was found that the Sm2-4 isolate can grow in a culture medium supplemented with 25ppm and 50 ppm of Zn and Co sulfate. While in a medium containing Cu sulfate the isolates can grow only in 25 ppm. While, in a culture medium supplemented with a concentration of 100 ppm of the three heavy metals, the bacterial isolate failed to grow (Table 7).

These results are in agreement with the data obtained by Sheng *et al.* (2008)who, reported that biosurfactant was found to exhibit different multiple heavy metals (Pb, Cd, Cu, Ni and Zn).

Table (4): Emulsification layer (%) of the six pure isolates after 3 days of incubation period.

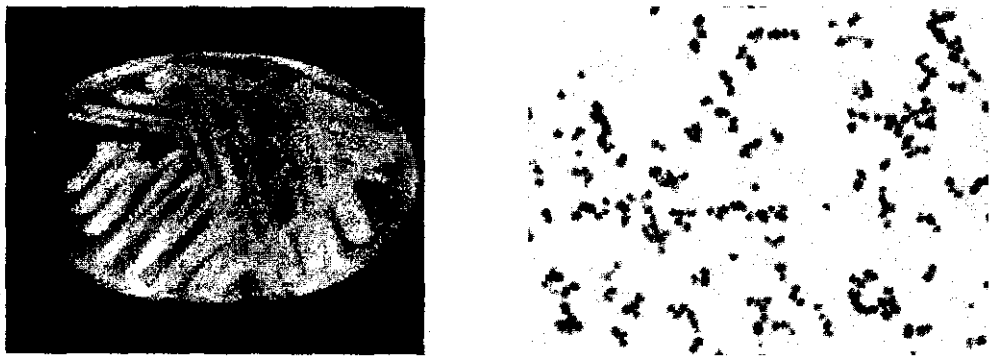
No. of isolates.	Height of emulsification layer (cm /Culture)	Height of diesel oil layer (cm.)	Total height of solution (cm)	The % of emulsification layer (E24)
Sm1-1	2	0.5	2.5	80
Sm2-3	1.5	0.6	2.1	71
Sm2-4	2.1	0.2	2.3	91
Sm2-8	2.2	0.3	2.5	88
Sm2-9	2.2	0.3	2.5	88
Sm2-12	1.5	0.4	1.9	79

Table (5): Emulsification layer (%) of the six pure isolates after 5 days of incubation period.

No. of isolates	Height of emulsification layer (cm / Culture)	Height of diesel oil layer (cm)	Total height of solution (cm)	The % of emulsification layer (E24)
Sm1-1	1.9	0.6	2.5	76
Sm2-3	1.4	0.7	2-1	67
Sm2-4	2.0	0.3	2.3	87
Sm2-8	1.9	0.4	2.3	83
Sm2-9	1.9	0.4	2.3	83
Sm2-12	1.6	0.5	2-1	76

Table (6): Morphological and biochemical results of the best isolate (Sm2-4).

Characteristics	Result
Cell shape	Rodo- cocci
Cell size	0.8-1.0 Um
Colony size	10-15 mm
Gram staining	Positive
Motility	Negative
Spore formation	Negative
Growth in O2	Aerobic
Tolerance to 6.0% NaCl	Negative
Catalase	Positive
Oxidase	Negative
Gelatin liquefaction	Positive
Nitrate reduction	Positive
Starch hydrolysis	Negative
Glucose, maltose, mannitol fermentation	Positive
Utilization of citrate	Positive



A B

Fig. (4): Colony form, (A) and microscopic examination (B) of isolate Sm2-4.

Table (7): Growth of *Rhodococcu* sp. in nutrient broth medium containing three concentrations of Zn, Co and Cu metal salt.

Heavy metal salt	Concentration (ppm)		
	25 ppm	50 ppm	100 ppm
Cu Sulfate	1^+	—	—
Zn Sulfate	2^+	2^+	—
Co Sulfate	2^+	2^+	—

No growth (-), low grow (+) and medium growth (2+).

3.4. Application of biosurfactants in pot experiment

Growth parameters of tomato plants grown for 60 days as affected by heavy metals and bacterial treatments are presented in Table (8). At the beginning, tomato plants of all treatments nicely grew with no nutrient deficiency symptoms.

Plants of less than 26 cm in height were detected with plants left without treatment (control). Recommended fertilizers N, P, and K stimulated plant growth, this resulted in 38.4 % increase in height of 60 day old plants. While, the plants fertilized with recommended dose of NPK and inoculated with *Rhodococcus* sp. as well as treated with a mixture of Co, Cu and Zn sulphate 15 days after planting gave 57.1% increase in plant height when compared with the untreated ones.

Root sizes ranged from 2.2 to 6.8 cm depending upon treatment and plant age.

Root and shoot yields significantly increased as a result of inoculation and / or heavy metals treatment. When *Rhodococcus* sp. and heavy metal salts (25 ppm concentration) were applied in the presence of full dose of agrochemical root and shoot dry weights achieved 84.2 and 193.3% higher than those of the control. Also, in the case of applying *Rhodococcus* sp. and heavy metals 15

Table (8): Tomato growth characteristics of the different treatments after 15, 30 and 60 days of planting.

Days after planting	Treatments			
	Control	Full dose of NPK	Full dose of NPK plus a mixture of Cu, Co and Zn and *Rhodococcus* sp.	Full dose of NPK plus, a mixture of Cu, Co and Zn 15 days after planting and *Rhodococcus* sp.
	Plant height (cm			
15	13.50	17.4	17.0	19.2
30	19.60	33.3	34.9	37.8
60	25.20	40.9	54.6	58.7
L.S.D. (0.05)	0.17			
	Root size (cm)			
15	2.2	2.7	2.9	3.0
30	4.3	5.4	5.8	5.9
60	5.2	6.1	6.2	6.8
L.S.D. (0.05)	2.96			
	Root dry weight (g plant^{-1})			
15	0.13	0.14	0.15	0.19
30	0.26	0.28	0.31	0.39
60	0.38	0.69	0.70	0.83
L.S.D. (0.05)	1.87			
	Shoot dry weight (g plant^{-1})			
15	0.72	0.89	0.90	0.90
30	1.34	1.65	1.77	1.85
60	1.63	4.36	4.78	5.25
L.S.D. (0.05)	3.77			
	No. leaves (per plant)			
15	20	28	29	30
30	38	56	60	68
60	43	77	83	92
L.S.D. (0.05)	48			

days after planting, root and shoot dry weights showed 118.4 and 222.1% higher than those of the untreated ones.

Tomato plants bore considerable number of leaves. Plenty of these (83 and 92 per plant) were produced by plants received the full recommended dose of fertilization rate together with the incorporation into soil of the tested dose of the three heavy metals tested.

These results are in agreement with the data reported by Sheng *et al.* (2008). They reported that a pot experiment demonstrated that the application of the biosurfactant in soil contaminated with heavy metals significantly enhanced biomass of tomato plants.

In conclusion, the application of biosurfactant and biosurfactant-producing bacteria in environmental technologies (bioremediation and phytoremediation) has been studied in some researches. Also, both organic and inorganic contaminants can be removed through different processes (physico-chemical and biological) in which biosurfactants are involved. Due to their biodegradability and low toxicity, they are very promising for the use in environmental biotechnologies. The commercial success of biosurfactants is still limited due to their high production cost. Optimized growth conditions using inexpensive renewable substrates (agro-industrial wastes) and novel, efficient methods for isolation and purification of biosurfactants could make their production more economically feasible. Another important aspect regarding biological remediation technologies is the use of biosurfactant in the process on a large scale.

Little is known about the potential of biosurfactant production by microorganisms *in situ*. Most of the described studies were done under laboratory conditions. More efforts are required to evaluate the biosurfactant production by microorganisms *in situ* and their role in biological remediation technologies. More information is required concerning the structures of biosurfactants, their interaction with soil and contaminants and scale up and cost effective biosurfactant production.

Nevertheless, careful and controlled use of these interesting surface active molecules will surely help in the enhanced cleanup of the toxic environmental pollutants and provide us with a clean environment.

4. REFERENCES

Aşçı Y., Nurbaş M. and Açıkel Y. S. (2010). Investigation of sorption/desorption equilibria of heavy metal ions on/from quartz using rhamnolipid biosurfactant. J. Environ. Manage., 91: 724–731.

Brenner D.J., Krieg N.R. and Staley J.T. (2005). Bergy's Manual of Systematic Bacteriology, 2nd Ed. Springer, New York, NY, USA. 310 pp.

Dastgheib S.M.M., Amoozegar M.A., Elahi E., Asad S. and Banat I.M. (2008). Bioemulsifier production by a halothermophilic *Bacillus* strain with potential applications in microbial enhanced oil recovery, Biotechnol. Lett., 30: 263-270.

Franzetti A., Caredda P., Ruggeri C., La Colla P., Tamburini E., Papacchini M. and Bestetti G. (2009). Potential applications of surface active compounds by *Gordonia* sp. strain BS29 in soil remediation technologies. Chemosphere. 22: 117-128.

Ghayyomi Jazeh M., Forghani F. and Deog-Hwan Oh. (2012). Biosurfactan production by *Bacillus* sp. isolated from petroleum contaminated soils of Sirri Island. Am. J. Appl. Sci., 9 (1): 1-6.

Grainger J., Janet H. and Dariel B. (2001). Basic Practical Microbiology: A Manual. The Society for General Microbiology. 201 p.

Head I.M. and Swannell R.P.J. (1999). Bioremediation of petroleum hydrocarbon contaminants in marine habitats. Curr. Opin. Biotechnol., 10: 234-239.

Herman D.C., Artiola J.F. and Miller R.M. (1995). Removal of cadmium, lead, and zinc from soil by a rhamnolipid biosurfactant. Environ. Sci. Technol., 29: 2280–2285.

Jackson M. L. (1973). Soil chemical Analysis. Contable Co., London, UK., 325 p.

Khan J. A. and Syed H.A.R. (2011). Isolation and characterization of micro-organism from oil contaminated sites. Adv. Appl. Sci. Res., (3): 455-460.

Mulligan C.N. (2005). Environmental applications for biosurfactants. Environ. Pollut.,133: 183-198.

Neu T. (1996). Significance of bacterial surface-active compounds in interaction of bacteria with interfaces. Microbiol. Rev., 60: 151-166.

Pacwa-Płociniczak M., Grażyna A.P., Piotrowska-Seget Z. and Cameotra S.S. (2011). Environmental applications of biosurfactants: Recent Advances. Int. J. Mol. Sci., 12: 633-654.

Qingyi X., Mitsutoshi N., Zengshe L. and Takeo S. (2011). Biosurfactants for preparation and application. Int. J. Molecular Sci., 1422-1430.

Salihu A.,Abdulkadir I. and Almustapha M.N. (2009). An investigation for potential development on biosurfactants. Biotechnology and Molecular Biology Reviews. 3 (5): 111-117.

Sarubbo L.A. (2006). Production and stability studies of the bioemulsifier obtained from a strain of *Candida glabrata* UCP 1002. J. Biotechnol., 9(4): 400-406.

Sheng X., He L., Wang Q., Ye H. and Jiang C. (2008).Effects of inoculation of biosurfactant-producing *Bacillus* sp. J119 on plant growth and cadmium uptake in a cadmium-amended soil. J. Hazard. Mat., 155 (1-2): 17–22.

Singh P. and Cameotra S.S. (2004). Enhancement of metal bioremediation by use of microbial surfactants. Biochem. Biophy. Res. Commun., 319, 291–297.

Sneath P. H. A. (1984). Bacterial nomenclature. In *Bergey's Manual of Systematic Bacteriology*, vol. 1, pp. 19±23. Edited by N. R. Krieg & J. G. Holt. Baltimore: Williams & Wilkins.

Snedecor G.W. and Cochran W.G. (1980). Statistical Methods. 7th Ed. Iowa State University Press. Ames, Iowa, USA, 275 p.

Urum K. and Pekdemir T. (2004). Evaluation of biosurfactants for crude oil contaminated soil washing. Chemosphere. 57:1139–1150.

Urum K., Grigson S., Pekdemir T. and McMenamy S. (2006). A comparison of the efficiency of different surfactants for removal of crude oil from contaminated soils. Chemosphere 62:1403-1410.http:// www. microeguide. comindex. asp. Micro Guide - Aseptic techniques: Plate streaking.

تحسين ازالة كل من الزنك ، النحاس ، الكوبالت من الاراضى الملوثة بالمعادن الثقيلة بواسطة مخفضات التوتر السطحى

مرام ممدوح الشاهد – وليد ضياء الدين صالح – محمد زكريا صديق

قسم الميكروبيولوجيا الزراعية – كلية الزراعة – جامعة القاهرة – الجيزة – مصر

ملخص

تم خلال هذا البحث دراسة تأثير مخفضات التوتر السطحى المنتجة من الميكروبات المعزولة من الأراضى الطينية على تحسين انتاجية نبات الطماطم وكذلك التخلص من المعادن الثقيلة. حيث تم تصميم تجربة اصص لدراسة قدرة مثل هذه الميكروبات على التخلص من كل من الزنك، النحاس، الكوبالت فى ارض زرعت بنبات الطماطم ولوثت صناعيا بمستويات مختلفة من الزنك (٥٠ جزء فى المليون/كيلوجرام تربة)، النحاس (٥٠ جزء فى المليون/ كيلو جرام تربة)، الكوبالت (٥٠ جزء فى المليون / كيلو جرام تربة).

أثبتت النتائج المتحصل عليها أن البكتريا العصوية تمثل النسب الأعلى من عدد العزلات المتحصل عليها يليها الميكروبات الكروية. أما بالنسبة للعصويات القصيرة السالبة لجرام فقد جاءت فى المرتبة الثالثة بالنسبة لعدد العزلات يليها كل من البكتريا العصوية الطويلة غير المتجرثمة والبكتريا الخيطية.

ولقد أثبتت الدراسات المورفولوجية للعزلات المتحصل عليها أن الأنواع الموجبة لجرام تمثل ٨٤% من عدد العزلات أما الأنواع السالبة لجرام فكانت تمثل ١٦% تقريبا.

ولقد تم أختيار ثلاثة وثلاثون عزلة بكتيرية من العزلات المتحصل عليها لدراسة قدرتها على انتاج مخفضات التوتر السطحى. حيث وجد أن ستة عزلات فقط تملك المقدرة على تحليل الزيت بعد ثلاثة الى خمسة ايام وبدرجات متفاوتة. ومن النتائج المتحصل عليها وجد ان السلالة (Sm2-4) أعطت أعلى نسبة تحلل وصلت الى ٩١%. أما أقل نسبة تحلل للزيت كانت للسلالة (Sm2-3).

وتم ايضا دراسة تاثير المعادن الثقيلة على نمو نباتات الطماطم المنماه فى تجربة أصص مع التلقيح ببكتريا *rhodococcus* sp. المنتجة لمخفضات التوتر السطحى بكمية كبيرة. ولقد ثبت ان التلقيح ببكتريا *rhodococcus* sp. وأضافة العناصر الثقيلة بعد خمسة عشر يوم من الزراعة مع التسميد بالجرعات الموصى بها أعطت أعلى القيم النباتية من حيث طول النبات، حجم الجذر، وزن الساق والجذر ، عدد الاوراق.

المجلة العلمية لكلية الزراعة – جامعة القاهرة – المجلد (٦٤) العدد الأول (يناير ٢٠١٣): ٧٨-٨٦.