Hazard of heavy metal resistant bacteria in polluted water and soil

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Abstract

Fifty two Chromate resistant bacterial strains were isolated from industrial sewage contaminated with Chromate, different soil sample, polluted river sediment, and many hospitals sewage. The isolates showed resistance to concentrations of Chromate, which was available as K2Cr2O7 or H2CrO3.

Forty two isolates were gram negative *bacilli* and the other gram positive *bacilli*.

In addition to resistance of Chromate as a heavy metal, they were able to resist some antibiotics. The results showed that most isolates were antibiotic multi-resistant.

Growth curve of selected isolates showed an obvious decline at high concentrations of Chromate.

Introduction :

During last few decades extensive attention has been paid to the hazard arising from contamination of the environment by heavy metals. Heavy metals are major pollutants in marine, ground, industrial and even treated water(1).

Microorganisms can play an important role in the detoxification and / or removal of heavy metals from polluted environments.

A wide variety of fungi, algae and bacteria are now under study or already in use as biosorbents for heavy metal remediation.(2) Heavy metals are such as Chromium, mercury and copper are found among hydrocarbons and increases the difficulty of biodegradation.

Lower cost and higher efficiency at low metal concentrations make biotechnological processes very attractive in comarision to physicochemical methods for heavy metals removal.

Chromium is one of the most toxic and carcinogenic heavy metals. Divalent and trivalent chromium species are the most stable and least toxic ones , while hexavalent chromate is highly toxic, readily crossing the membrane of eukaryotic and prokaryotic cells, causing oxidative cellular damage .(4)

Chromium compounds are widely used, e.g., in leather tanning, metal finishing, alloy preparation and wood preservation. Wastes are often, discharged to the environment, especially in countries which impose inadequate regulatory control; the need for effective, economic waste remediation at source is urgent.

The aim of the present work are to study some bacterial strains isolated from oil and metal polluted sites regarding the capability concentration for some heavy metals and also to identify the microbial community of resistant bacteria of metal – contaminated sites.

Chromate is soluble and thus readily spreads beyond the site of initial contaminants convert chrome to Cr(III),which is much less toxic and less soluble ;therefore, bioremediation of chromate is of considerable interest, especially given the chemical means are prohibitively expensive for large scale cleanup.(6)

Materials And Methods:

Collection of samples:

Water and soil sampling were collected from contaminated sites, industrial slug, sediment of Deala river, hospital sewage, samples from the surface and different depth of soils, were taken as sources of heavy metals resistant bacteria. These samples were stored in sterile glass bottles at 4C° for further work.

Preparation of samples:

Samples were mixed with 10 volumes of distilled water, then serially six fold diluted, and plating diluted samples on N. Agar supplemented with 0.1mM of chromate colonies were counted after 3 days incubation at 30C° . Cell growth was measured as g/L after centrifugation ,precipitating and drying. These strains were purified on N. agar without chromate.

The bacterial strains were then tested for there ability to grow on Macconkey agar, EMB, cetrimide agar with or without chromate (0.1mM,) which supplemented as K2Cr2O7 or H2Cro3 ,percentage of living bacteria measured by counting living cell in culturing media with or without chromate.

Resistant is collates were cultured on L. agar for screening of antibiotics resistance by disc method culture.

Antibiotics Test:

*Gramicidin.

*Ramfibin.

*Amoxicillin.

*Erythromycin.

*Carbencin.

*Nalexin.

*Tetracycline.

*Chloramphenicol.

Antibiotic resistance was estimated by observing the clear zone around growth of colonies after (24-48 hr) of incubation at 30C°. Selected isolates were cultured on L. agar supplemented with different concentrations of chromate to estimate bacterial growth.

Result and Discussion:

Deferent samples of contaminated locations with chromate like, sewage sludge . Industrial sewages municipal disposal were used as source of resistant bacteria to the heavy metal. The chromate sources were H2CrO3 and K2Cr2O7- .

The experiments were carried out in duplicate and each source was also assayed in duplicate .

The number of colony forming unit (CFU) in the samples was determine by making samples with 100 ml of D. water and plating on 0.1Mm Cr supplemented medium (Nutrient Agar, EM. Cetrimide, MacConkey).

Colonies were counted after 2 days incubation at 30C°. The same media without Chromate used as a control .

All samples from polluted area showed different strains at many percentage value of bacteria to the primary concentration of chromate(0.1Mn)resistant (1,2).

It can observed that the strains isolated from the metal contaminated sites were the most tolerant to Chromate. using microorganisms as biosorbents for heavy metals offers a potential alternative to existing methods for detoxication and for recovery of toxic or valuable metals from industrial discharge water.

Many bacteria including *Pseudomonas sp. Enterobacter*, showed high resistant to Chromate, especially those were originally isolated from industrial sewage polluted with Chromate (1,2) .

Pseudomonas sp., and *rhodococcus* strains presented same resistant to heavy metals used, being suitable for use in sites contaminated with high concentrations of them .(7)

Municipal sludge often contain high quantities of toxic metal and other compounds that must be removed.(8 , 9)

The sewage sample of hospitals contains bacterial strains have amulti –resistant of antibiotic in addition to its resistance to heavy metals. tab (3)

Municipal sludge contain high quantities of contaminants, such as toxic metal,

pathogenic organisms and hazardous organic compounds.(6 , 7)

Eleven bacteria strains were selected depending on its growth activity on chromate containing media tab(3).These isolated for survival with different antibiotics tab(4)using antibiotics disk method selected isolates, (MC2 , Cr15 ,SW, SW13, SW4)were tested for their ability to grow in many concentration of chromate by culturing them in specific media containing chromate concentration of (1-10)Mm.

All strains presented an efficient capability to resist Chromate at concentration (1,5) mM but they were sensitive to it at 10mM.

Bacterial strains identified as gram negative *bacillus* and one as a strain of gram postive *bacillus* .All of these strains isolated from industrial hospital sewage respectively were resistant up to (5mM) Chromate, but they were not tolerance to Chromate at 10mM conc. Many bacteria, including *Pseudomonas sp , E. coli*, and *Enterobacter* can reduce (Cr VI) to the less toxic Cr (III), which readily precipitates as Cr(OH)3.(10 , 11)

Therefore it is possible; use these tolerant microorganisms to the Chromate at heavy metal site that is being bioremediated. It can observed that the strains isolated from the metal contaminated sites were the most tolerant to Chromate.

Medium	Industrial sewage (sludge)	Soil	Hospital Sewage
Nutrient agar	85	78	65
MacC.(f)	100	100	68
MacC n.f.	100	100	87
E.M.B.	80	80	60
Cetrimide	100	100	50

Tab.(1) Percentage of chromate resistant bacteria

$K_2Cr_2O_7$ 0.1mM

Medium	Industrial Sewage (sludge)	Soil	Hospital Sewage
Nutrient agar	95	80	48
MacC.(f).	75	72	68
MacC . nf.	95	92	46
E.M.B.	70	86	56
Cetrimid	80	78	70

Tab. (2) Percentage of chromate resistant bacteria

$H_2.CrO_3$ 0.1mM

isolate	Growth g /L	
	H2CrO3	K2CrO7
Mc_2	2.4	2.5
Mc_3	1.8	0.9
Mc_8	1.3	1.0
Cr_5	1.4	1.0
Cr_7	1.4	1.8
Cr_{15}	2.9	2.5
Eb_6	1.5	1.2
Eb_{11}	1.2	0.8
Sw_6	1.2	0.8
Sw_{13}	2.5	2.6
Sw_4	2.6	2.6

Tab(3) Chromium resistant bacteria growing on chromate supplementing media with concentration of 0.1mM

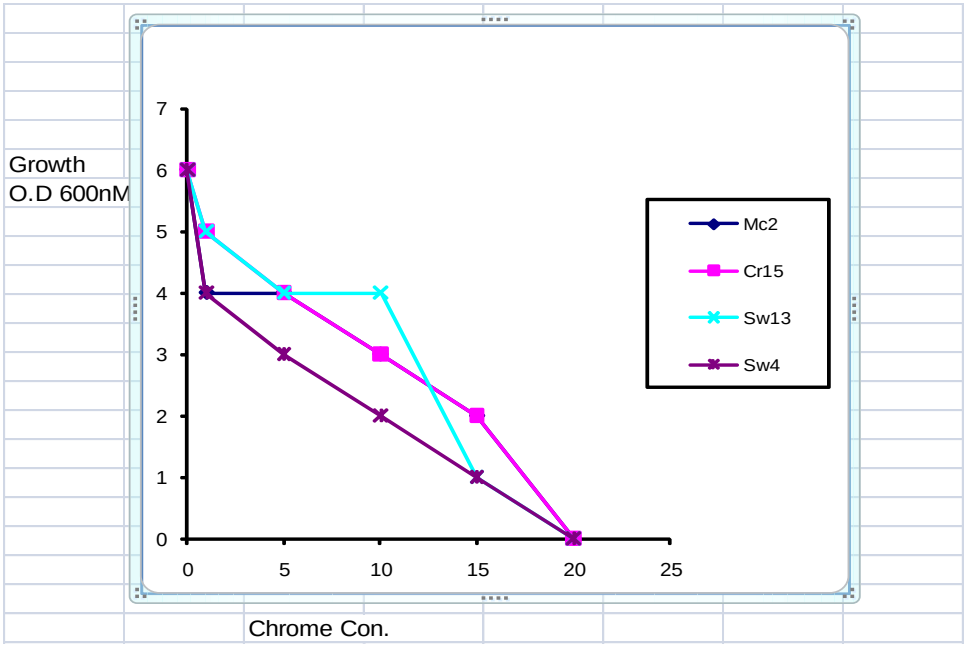

Fig.(1)Growth curve of chromate resistant isolates.

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مخاطر البكتريا المقاومة للمعادن الثقيلة في تلوث الماء والتربة

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الخلاصة

تم الحصول على 52 عزلة بكتيرية مقاومة للكروم عزلت من المخلفات الصناعية ، نماذج تربة مختلفة ، رواسب نهر ملوثة (جسر ديالى) ومياه مجاري من المستشفيات . أظهرت العزلات مقاومة للتراكيز المختلفة للكروم الذي تم تجهيزه للعزلات بشكل مادتي الدايكرومات K2Cr2O7 وحامض ألكرومك H2CrO3 .

بينت الفحوصات النظرية والمايكروسكوبية ان 42 عزلة هي عصيات سالبة لصبغة كرام والاخرى هي موجبة لصبغة كرام، العزلات المختارة أظهرت مقاومة للتراكيز الأولية للكروم من خلال منحنى النمو وانخفاض واضح في التراكيز العالية التي تصل إلى 10 mM .