Acta Microbiologica et Immunologica Hungarica, 46 (1), pp. 25–32 (1999)

CHROMIUM-RESISTANT SOIL ACTINOMYCETES: THEIR TOLERANCE TO OTHER METALS AND ANTIBIOTICS

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(Received February 16, 1998)
(Accepted October 26, 1998)

Chromium occurs widely in most soils, but generally in trace amounts. Actinomycetes, one of the important components of the microbial population in soils interact with a variety of metals including chromium. This study was aimed to evaluate the tolerance of soil actinomycetes to Cr^{6+}, other metals and antibiotics. Thirty-two actinomycete isolates were screened for their tolerance to Cr^{6+} on tryptone yeast extract agar medium supplemented with Cr^{6+} at concentrations ranging from 100 to 2000 µg ml^{-1}. Thirteen Cr-tolerant isolates were selected on the basis of their growth at the highest concentration, but their performance was not satisfactory in Cr^{6+} containing liquid salts medium. Resistance of these isolates to other metals and antibiotics was assessed using agar-cup assay and disc diffusion technique, respectively. The sequence of metal toxicity for the actinomycete isolates was in the order $Hg^{2+} > Ni^{2+} > Cu^{2+} > Co^{2+} > Cd^{2+}$, but the Cr^{6+} resistance of the isolates could not be correlated with their antibiotic-resistance profile.

Actinomycetes are widely distributed in a variety of natural and man-made environments and constitute a significant component of the microbial population in most soils. In soil they are primarily concerned with degradation of plant, animal and microbial polymers and can also respond to a range of unnatural compounds introduced to soil. They are also exposed to heavy metals in a variety of ways [13], especially when the agricultural fields are subjected to treatment with sewage sludge and industrial effluents [5, 26]. The ability of actinomycetes to survive and grow in the presence of high metal concentrations may involve adsorption to cell walls and other constituents, extracellular precipitation or complexation, internal compartmentation and transport [12]. Actinomycetes strains isolated from zinc contaminated soil showed an increased tolerance to Zn [15], but isolates from lead mine waste did not show increased lead tolerance [25]. However, it has been reported that actinomycetes are more tolerant than other bacteria to

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1217-8950/99/$ 5.00

cadmium [3]. Abbas and Edwards [1] assessed the tolerance of mesophilic and thermophilic streptomycetes to various metals and the order of toxicity was Hg > Cd > Co > Zn > Ni > Cu > Cr > Mn. *Streptomyces sp.* have been shown to remove uranium, copper and cobalt from solutions, the order of efficiency being Uo_2 > Cu > Co [19, 20]. Similarly streptomycete species that are able to detoxify Hg^{2+} to volatile Hg^{o} by means of mercuric reductase enzyme have also been isolated [23, 24].

Chromium, a transition metal occurs widely in soils. Its concentration ranges from 2–60 mg Kg^{-1} in most soils [10, 27]. Naturally occurring chromium is always present in the trivalent (Cr^{3+}) state, but almost all hexavalent chromium (Cr^{6+}) in the environment is derived from human activities. These include sewage sludge deposition and dispersal of wastes from industries that utilize chromium compounds for ferrochrome production, electroplating, pigment production and leather tanning [16].

Hexavalent chromium (Cr^{6+}) is considered much more toxic and mutagenic for most organisms than trivalent chromium (Cr^{3+}) [16]. Microbial reduction of Cr^{6+} to Cr^{3+}, therefore, could be a useful mechanism in reducing Cr^{6+} toxicity. Bacterial strains isolated from chromium-contaminated sediments and sewage sludge have been shown to tolerate high levels of Cr^{6+} [4, 14, 17, 18]. In addition to chromate reduction, Cr resistance has been correlated with the presence of plasmid DNA in several species of bacteria [6, 7]. Plasmid determined chromate resistance in *Pseudomonas fluorescence* has been reported to be due to reduced uptake of CrO_4^{2-} by the resistant cells [21].

This study was aimed to evaluate the chromium resistance potential of a range of actinomycetes strains isolated from natural soils of West Bengal during the course of screening of antifungal actinomycetes. Attempts were also made to evaluate their tolerance to other metals and antibiotics.

Materials and methods

Bacterial strain and maintenance. A collection of 32 different actinomycetes isolates were obtained from the culture collection of the Microbiology Laboratory, Department of Botany, Calcutta University, Calcutta. The actinomycete strains were isolated from natural soils of West Bengal and were maintained on glucose asparagine agar slants that contained (gL^{-1}) glucose, 10; K_2HPO_4, 0.5; asparagine, 0.5; and agar, 20 (pH 6.8).

Screening for chromate resistance. Qualitative assessment of the chromate resistance of the isolates was made following the method of Luli *et al.* [18]. The isolates were grown on tryptone yeast extract agar medium (TYE) that contained (gL^{-1}) tryptone, 5; yeast extract, 5; NaCl, 5; glucose, 1; and agar, 20 (pH 7.2) and was supplemented with Cr^{6+} at concentrations ranging from 100 to 2000 µg ml^{-1}. Homogenous spore suspensions from the sporulated cultures, prepared in 0.1% (w/v) sterile Tween 80 solution, were streaked in the form of a narrow line on Cr^{6+} incorporated plates and incubated at 30 °C for 3–7 days for visible growth.

Secondary screening. Degree of resistance of the selected isolates was also evaluated in the liquid salts medium that contained (gL^{-1}) K_2HPO_4, 6; K_2HPO_4, 2; $(NH_4)_2SO_4$, 3; $MgSO_4$. $7H_2O$, 0.02; $FeSO_4$ $7H_2O$, 0.01; glucose, 10 and was amended with 0.1% yeast extract (pH 7.4). Filter-sterilized K_2CrO_4 solution was added to the sterilized liquid salts medium to attain a Cr^{6+} concentration ranging from 250 to 2000 µg ml^{-1}. Twenty ml of medium per 100 ml flasks were inoculated with 1 ml of freshly prepared spore suspensions and incubated on a rotary shaker (120 rpm) at 30 °C for 5d. Growth of the

organisms was determined as dry weight of mycelia and the relative growth was expressed as a percentage of those obtained in untreated control cultures at the same time, which was taken as 100%.

Resistance to other metals. Resistance of Cr-tolerant isolates against a number of metals was tested by agar-cup method [22] using TYE medium. Cups (12 mm diam.) were scooped at equidistant places on TYE agar plates seeded with individual isolate and 0.1 ml of metal solutions were added to each cup, allowed to diffuse for 3–4 h at 10 °C and then finally incubated at 30 °C for 24 h. Sensitivity to different metals was scored from the diameter of inhibition zones.

Resistance to antibiotics. To determine the antibiotic sensitivity of the Cr-resistant isolates, antibiotic impregnated discs (6 mm diam., Hi-media) were placed on freshly prepared lawns of each isolate on TYE agar medium. The plates were incubated at 30 °C for 24 h. Based on inhibition zones, the organisms were categorised as resistant, intermediate and sensitive according to antibiotic disc sensitivity testing method as described in DIFCO Manual [8].

Results

Primary screening. Chromium tolerance of 32 actinomycete isolates in solid medium indicates that up to a concentration of 500 μg ml^{-1} of Cr^{6+} all the isolates showed growth almost equivalent to control (Figure 1). At the highest concentration (2000 μg ml^{-1}), 13 isolates (40% of the total number tested) were tolerant to Cr^{6+}. Higher concentrations of chromium also inhibited the sporulation of all the tolerant strains except

Fig. 1. Chromium tolerance of soil actinomycetes in solid medium

DI-06 and R.A-05; production of diffusible melanoid pigment by the strains DI-05 and DI-25, however, remained unaffected (data not shown).

Secondary screening. Thirteen isolates showing moderate to good growth at 2000 μg ml^{-1} of Cr^{6+} were secondarily screened in liquid salts medium. Only 50% of the selected isolates attained 20–65 g relative growth at 500 μg ml^{-1} of Cr^{6+} (Table I). The performance of the isolates in liquid salts medium with 1000 μg ml^{-1} of Cr^{6+} was very poor, five isolates showed only slight growth, the others were totally inhibited. None of the isolates examined showed detectable growth at 2000 μg ml^{-1}.

Table I

Chromium tolerance of some selected actinomycetes isolates

Isolate	Relative growth, % Cr^{6+} in medium μg ml^{-1}			IC_{50} values of Cr^{6+} for growth, μg/ml
	250	500	1000	
DI-01	21.00	0.00	0.00	177.8
DI-04	56.42	25.93	14.81	281.8
DI-06	61.75	16.67	0.00	295.1
DI-08	13.11	7.55	0.00	169.8
DI-22	68.00	36.96	0.00	371.5
DI-25	71.45	22.20	14.81	338.8
DI-29	78.10	64.81	0.00	588.8
KA-01	21.00	0.00	0.00	177.8
KA-02	64.00	30.56	11.11	316.2
RA-05	73.05	24.07	0.00	346.7
SA-05	49.58	19.61	13.73	251.1
SI-49	35.75	9.52	0.00	199.5
SU-05	38.00	8.33	4.16	208.9

Each value represents average of duplicates

Resistance to other metals. The selected isolates also showed varied degrees of tolerance to 5 different metals tested (Table II). Mercury was by far the most toxic metal, all the strains with the exception of one (RA-05) were totally inhibited at the lowest concentration (5 μg ml^{-1}). A number of strains for example, DI-01, DI-04 and DI-22 showed a wide range of sensitivities to Ni, Cu and Co and most of the strains tested were tolerant to Cd. Strains DI-08 and RA-05 were resistant to Cu^{2+}, Co^{2+} and Cd^{2+}. The chromium-resistant strains KA-02 and SA-05 on the other hand were sensitive to all 5 metals tested. The distribution profile of the sensitivity of all species examined against different metals revealed that the order of toxicity were $Hg^{2+} > Ni^{2+} > Cu^{2+} > Co^{2+} > Cd^{2+}$.

Resistance to antibiotics. Antibiotic sensitivity profile of the actinomycete isolates have indicated that isolate RA-05 was resistant to all the antibiotics except cycloserine (Table III). Resistance of isolate RA-05 to a wide range of antibiotics was also correlated with its multiple metal resistance character. On the other hand, isolates DI-01, DI-22 and DI-25 were highly sensitive to most of antibiotics tested. The rest of the isolates exhibited a varied degree of sensitivity and resistance to different antibiotics.

Table II

Susceptibility of Cr^{6+}-resistant actinomycetes to other metals

Isolate	Diameter of inhibition zone, mm									
	Cu^{2+}		Ni^{2+}		Co^{2+}		Cd^{2+}		Hg^{2+}	
	50	l00	50	100	50	100	50	100	5	10*
DI-01	17.5	22.0	20.0	30.5	22.0	31.0	NI	NI	25.0	28.0
DI-04	20.0	26.0	18.0	24.5	NI	20.0	NI	NI	25.0	30.5
D1-06	NI	13.5	NI	NI	NI	NI	16.0	20.0	28.0	32.0
DI-08	NI	NI	18.0	26.0	NI	NI	NI	NI	43.0	49.0
DI-22	NI	16.0	16.0	24.5	26.0	29.0	NI	NI	30.5	35.0
DI-25	NI	17.0	24.0	28.5	NI	17.5	NI	NI	25.0	30.5
DI-29	NI	25.5	22.0	24.5	NI	20.0	18.0	20.0	26.0	31.0
KA-0l	NI	18.0	20.0	24.5	NI	NI	NI	NI	28.5	32.0
KA-02	NI	22.0	22.0	27.5	22.0	29.5	18.0	20.0	26.5	31.0
RA-05	NI	NI	22.0	29.0	NI	NI	NI	NI	NI	23.5
SA-05	NI	21.0	18.0	26.0	NI	22.0	NI	20.0	34.0	40.0
SI-49	NI	16.0	NI	18.0	33.5	44.0	NI	22.0	26.0	31.0
SU-05	N1	19.0	NI	18.0	20.0	24.0	NI	NI	23.5	28.5

*Concentration of metal µg ml^{-1}

NI = No inhibition

Each value represents average of duplicates

Discussion

This study shows that actinomycetes isolated from natural soils were more tolerant to Cr^{6+} when tested in solid medium compared to that in liquid salts medium. Such a differential response may be due to the complex nature of the solid medium, the components of which could bind metal ions. The metal binding ability of the complex organic constituents of the solid medium – if any – is yet to be determined. Angle and

Chaney [2], however, concluded that the activity of free metal ions in undefined growth media is affected by processes such as binding to, or chelation and/or complex formation with organic components of the media yielding erroneously high tolerance data.

The mechanism of Cr^{6+} tolerance of the actinomycete isolates is likely to involve surface binding, intracellular transport and accumulation via a specific Cr^{6+} transport system or production of biomolecules like metallothioneins, siderophores and analogous compounds which could complex with Cr^{6+}. The reduction of chromate (CrO_4^{2-}) to Cr^{3+} and its subsequent precipitation could not be ruled out as the possible mechanism. Moreover, Cr resistance has been correlated with the presence of plasmid DNA in several isolates.

Results as shown in Table II are in good conformity with those of Babich and Stotzky [3] who found that actinomycetes as a group are more tolerant to cadmium than other bacteria. It is also interesting to note that the order of heavy metal toxicity to actinomycetes as identified in this study differs significantly from those reported by Duxbury [9] and Abbas and Edwards [1] which were ordered as Hg > Cd > Cu > Ni > Zn and Hg > Cd > Co > Zn > Ni > Cu > Cr > Mn, respectively. The major differences would appear to be the relatively low position of cadmium as a toxic metal for streptomycetes and the corresponding higher toxicity of nickel.

The antibiotic resistance may also reflect the ability of the isolates to produce the antibiotic(s) to which they are resistant. Moreover, the multiple antibiotic resistance of some of these isolates also indicates the possible acquisition of a plasmid – conferred antibiotic resistance factors by the specific isolates. The results of our study show that these resistant actinomycete isolates might be useful in the transformation of toxic Cr^{6+} to less toxic Cr^{3+} or biosorption of chromium both from natural soils and contaminated sediments. Studies on the evaluation of chromium biosorption by resistant actinomycete isolates are in progress.

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