American Journal of Biochemistry and Biotechnology, 2012, 8 (2), 111-117
ISSN: 1553-3468

doi:10.3844/ajbbsp.2012.111.117 Published Online 8 (2) 2012 (http://www.thescipub.com/ajbb.toc)

Antibiotic Resistance and Chromium Reduction Pattern Among Actinomycetes

[1]Preeti Jain, [1]Alina Amatullah, [1,2]Samiul Alam Rajib and [1]Hasan Mahmud Reza

[1]Department of Pharmacy, School of Life Sciences, North South University, Dhaka, Bangladesh
[2]Strathclyde Institute of Pharmaceutical and Biomedical Sciences, Faculty of Science, University of Strathclyde, Glasgow, United Kingdom

Received 2012-02-05; Revised 2012-05-25; Accepted 2012-06-08

ABSTRACT

Actinomycetes, one of the most important groups of microbes, exhibit many interesting activities such as degradation and transformation of organic and metal substrates together with production of antibiotics. With these bioactivities, actinomycetes would play an important role in the webs of the marine environment. The present study was designed to evaluate the antibiotic resistance pattern, antibiotic producing potential and chromium resistance as well as chromium reduction potential of a range of actinomycetes isolated from marine environments. Actinomycetes were isolated from marine sediment samples obtained from St. Martin's Island in Bangladesh. Antibiotic resistance among the selected isolates was studied against 10 different antibiotics by disc diffusion method and antibiotic producing potential was assessed by the perpendicular streak method. The isolates were screened for resistance towards heavy metal Cr(VI) on culture plates supplemented with Cr(VI) at concentrations ranging from 1-5 mM of Cr(VI). Highly resistant isolates were subjected to screening for Cr(VI) reduction activity, which was estimated using the Cr(VI) specific colorimetric reagent 1, 5-diphenylcarbazide. Out of the total 30 different selected isolates, 25 (83.33%) showed resistance against more than three antibiotics and 6 (20%) showed resistance to more than six antibiotics. Ninety three percent of the isolates showed MAR index greater than 0.2 and tolerance to Cr(VI) at 1mM of initial Cr(VI). None of the isolates displayed antimicrobial activity against the organisms tested. Among the isolates tested for chromate reduction, two were most efficient showing complete reduction of 1mM Cr(VI) within 24 h. These two isolates (SM-11, SM-20) were capable of reducing chromate even at high initial Cr(VI) concentrations. Remarkably, the isolate SM-11 was found to reduce 82.67%, 44.34% of Cr(VI) at 2.5mM, 5mM of initial Cr(VI) concentrations respectively, within 72h of incubation. The majority of the actinomycetes isolates displayed resistance to both antibiotics and heavy metal chromium which indicates the possible acquisition of resistance factors due to environmental or human activities. The study also demonstrates possible correlation between antibiotic resistance and metal tolerance. Two of the isolates which showed considerable chromium reduction activity even at high chromium concentrations, may find potential application in bioremediation approaches.

Keywords: Actinomycetes, Marine Sediments, Antibiotic Resistance, Chromium Reduction

1. INTRODUCTION

Actinomycetes constitute a significant component of the microbial population in most soils. Although distributed extensively in soil, they can also be isolated from marine sediments, marine water, marine plants and animals (You *et al*., 2005). Traditionally, actinomycetes have been a rich source of biotechnological products like antibiotics, industrial enzymes and other bioactive molecules (Lam, 2006). More than 70% of naturally occurring antibiotics have been isolated from actinomycetes. Naturally, these antibiotic producers also possess resistance for antimicrobial molecules they produce and these resistance mechanisms may be linked to antibiotic synthesis (Nodwell, 2007). Several studies have indicated a correlation between multiple antibiotic

resistance and heavy metal resistance (Bahig *et al*., 2008; Spain, 2003). Heavy metal resistance together with metabolic diversity and specific growth characteristics of actinomycetes, such as, mycelium formation and relatively rapid colonization of selective substrates indicate them as suitable agents for bioremediation (Polti *et al*., 2010).

Chromium is an essential micronutrient for living organisms. However, oxidized form of chromium [Cr(VI)] is extremely toxic and exhibits mutagenic, carcinogenic and teratogenic effects on biological systems (Poopal and Laxman, 2009). Cr(VI) is highly water soluble and more mobile, thus spreads easily beyond the site of initial contamination, while Cr(III) is relatively inert, less mobile, less bioavailable and easily adsorbed on mineral surfaces. Besides, hexavalent chromium compounds are approximately 100 fold more toxic and 1000 fold more mutagenic than trivalent chromium (Polti *et al*., 2010; 2007). Chromium containing effluents from industries like leather tanning, chrome-plating, wood preservation, dye industry are released directly or indirectly into natural water resources, mostly without proper treatment which renders chromium associated environmental pollution, a major concern. Because of the harmful effects of Cr(VI), many of which are deleterious to human health, this has been listed as a priority pollutant and classified as a class A human carcinogen by the US Environmental Protection Agency (USEPA) (Costa and Klein, 2006).

Biological reduction of Cr(VI) to and its precipitation into immobile Cr(III) by chromium resistant microbes is considered to be an effective method for detoxification of Cr(VI)-contaminated environments and have a potential use in bioremediation. Reduction of Cr(VI) has been demonstrated in various bacterial species including *Escherichia coli* (Shen and Wang, 1993), *Pseudomonas putida* (Ishibashi *et al*., 1990), *Desulfovibrio* sp. (Mabbett and Macaskie, 2001), *Bacillus* sp. (Liu *et al*., 2006), *Shewanella* sp. (Myers *et al*., 2000) and *Arthrobacter* sp. (Asatiani *et al*., 2004). However, there are only a few studies on Cr(VI) resistance and bioreduction by actinomycetes (Polti *et al*., 2010; 2007). The first report on Cr(VI) reduction by *Streptomyces* was documented by Das and Chandra (1990). Amoroso *et al*. (2001), reported on Cr(VI) bioaccumulation by *Streptomyces* strains, whereas Laxman and More (2002) determined Cr(VI) reduction by *Streptomyces griseus*. Polti *et al*. (2009), determined Cr(VI) reduction by *Streptomyces* sp. MC1 and later on characterize its chromate reductase activity (Polti *et al*., 2010).

The present study was aimed to evaluate the antibiotic resistance pattern, antibiotic producing potential and chromium tolerance as well as chromium reduction potential of a range of actinomycetes strains isolated from marine sediment samples obtained from St. Martin's Island in Bangladesh. Attempts were also taken to correlate antibiotic resistance, antibiotic production and chromium resistance.

2. MATERIALS AND METHODS

2.1. Sampling and Pretreatment

The marine sediment samples were obtained from the beaches of St. Martin's Island located in the northeast part of the Bay of Bengal, about 9 km south of the tip of the Cox's Bazar-Teknaf peninsula in Bangladesh. All the samples were collected aseptically from highly isolated areas, where there has been relatively less human intervention. From each location, five sediment samples of 5-10 g each were collected and placed in sterile falcon tubes. After collection, the samples were transported to the laboratory. Prior to the isolation of actinomycetes, the samples were air dried aseptically at room temperature for 5 days. These dried samples were then incubated at 50°C for 30 min (Takizawa *et al*., 1993; Baskaran *et al*., 2011).

2.2. Isolation and Cultivation of Actinomycetes

Pretreated samples were serially diluted 1:10 up to 10^{-5} and isolation was performed on Yeast Malt Extract Agar (YMA) medium. Briefly, an aliquot of 0.1 mL of each diluted sample was spread evenly over each separate YMA plate. For selective growth and isolation of actinomycetes, YMA medium was supplemented with 50 μg mL^{-1} of both nystatin and nalidixic acid to inhibit fungal and other bacterial contaminants respectively (Takizawa *et al*., 1993). The plates were incubated at 28-30°C for up to 14 days and observed from 7th day onwards. Morphologically different colonies that showed actinomycetes like appearance were purified by repeated streaking on YMA plates. Plates containing pure cultures were stored at 4°C until further examinations and maintained by routine subculturing in YMA agar slants and also preserved in 20% (v/v) glycerol stock solution at -80°C. Pure cultures were inoculated with 10 mL of Yeast Malt Extract Broth (YMB) and incubated at 28-30°C for 24-48 h in a rotary shaker (200 rpm) prior to screening.

2.3. Characterization of the Isolates

Several preliminary morphological and biochemical tests were carried out to characterize and differentiate actinomycetes isolates. Morphologically their growth, appearance and characteristics of colonies e.g., size, shape, color, consistency and pigment production were observed (Goodfellow *et al*., 1984). Various biochemical tests included gram-staining, growth on McConkey agar, indole production, methyl red, Voges proskauer, Triple Sugar Iron (TSI), casein hydrolysis and starch hydrolysis.

2.4. Antibiotic Susceptibility Testing

Isolates were tested for antibiotic sensitivity using the disc diffusion method (Bauer *et al*., 1966). Briefly, antibiotic impregnated discs (Himedia, India) were placed on freshly prepared lawns of each isolate onto Muller Hinton Agar plates. Antibiotic discs used in this study comprised of amoxycillin (20 µg), ampicillin (10 µg), bacitracin (10 U), chloramphenicol (30 µg), ciprofloxacin (5 µg), clindamycin (2 µg), erythromycin (15 µg), gentamycin (10 µg), streptomycin (10 µg) and tetracycline (30 µg). The plates were incubated at 28°C for 24 h. After incubation, the diameter of the zone of inhibition was measured and compared with zone diameter interpretative chart (Himedia, India) to determine the susceptibility of the isolates. Based on inhibition zone, the isolates were categorized as resistant, intermediate and sensitive to the tested antibiotics. The Multiple Antibiotic Resistance index for each isolates was also calculated (MAR index = a/b where a = number of resistant antibiotics, b = total number of antibiotics exposed) as recommended by Krumpermann (1983).

2.5. Screening for Antimicrobial Activity

The ability of isolates to produce antibiotic substances was assessed by the cross streak method on YMA medium (Takizawa *et al*., 1993; Lemos *et al*., 1985). Briefly, YMA plates were inoculated with test isolate by a single streak down the middle of the plate and incubated at 28°C for 7 days for the production of any antibiotics. Later, the plates were seeded with test organisms by a single streak at 90° angle to the actinomycetes isolates. The plates were incubated at 37°C and antimicrobial activity was evaluated by the determination of the size of the inhibition zones after 24 and 48 h. Six bacterial species, including three gram-positive (*Staphylococcus aureus, Bacillus cereus, Bacillus subtilis*) and three gram-negative (*Escherichia coli, Pseudomonas aeruginosa, Shigella boydii)* and one fugal species (*Aspergillus Niger*) were used for the evaluation of antimicrobial activity.

2.6. Screening of Isolates for Chromium (VI) Resistance and Reduction

Chromate resistance of the isolates was assessed in the following way. All the isolates were streaked onto Nutrient Agar (NA) plates supplemented with 1-5 mM of Cr(VI) and incubated at 28°C for 7 days. The source of Cr(VI) in this study was K_2CrO_4, prepared as 1 M stock solution and sterilized by filtration. Microbial growth was used as the qualitative parameter of chromium resistance. Growth of isolates on NA plates prepared without Cr(VI) served as controls. Chromium reduction ability of the isolates was determined in the following manner. The isolates were first grown at 28°C with shaking at 200 rpm in Nutrient Broth (NB) for 48h. Then a fixed amount of this vegetative inoculum (10% v/v) (Poopal and Laxman, 2009) was used to inoculate nutrient broth medium supplemented with appropriate amount (1-5 mM) of Cr(VI). The cultures were incubated at 28°C with shaking at 200 rpm. Cr(VI) reduction was assayed by withdrawing samples at regular intervals and measuring residual chromate in the supernatant after removal of cells by centrifugation at 6000 rpm for 10 min. Cr(VI) in the supernatants was determined by S-Diphenyl Carbazide (DPC) reacting (Pattanapipitpaisal *et al*., 2001) in the following way. Briefly, DPC (0.025 g) was dissolved in 9.67 mL Acetone (AR) and 330 µL of 3 M H_2SO_4 was added. The reaction was set up in a tube containing the following: 600 µL sample or standard chromate solution, 1.2mL 20mM MOPS-NaOH buffer (pH7.0), 99 µL 3M H_2SO_4, 120 µL 0.25% (w/v) DPC and 981 µL of distilled water. Spectrophotometric measurements were made immediately at 540 nm. The difference in initial and residual Cr(VI) was taken as Cr(VI) reduced and expressed as percentage of initial Cr(VI). Uninoculated controls were included for each experiment and incubated under identical conditions to determine the Cr (VI) loss of the components of the culture medium, if any.

3. RESULTS

3.1. Antibiotic Resistance Among Isolates

A total of thirty different actinomycetes isolates were selected for study based on differences in colony characteristics such as size, shape, color, consistency, texture and biochemical tests. These isolates were designated as SM-1-30. Antibiotic sensitivity profile of the selected isolates is presented in **Table 1**.

The isolates exhibited a varied degree of sensitivity and resistance to different antibiotics. Out of 30 actinomycetes isolates, none was found susceptible to all antibiotics. 83.33% isolates showed resistance against more than three antibiotics while 20% were resistant to more than six antibiotics. Only isolates SM-22, SM-28 were found to be susceptible to eight antibiotics and displayed intermediate susceptibility to two antibiotics. Antibiotic resistance pattern shown by isolates is represented in **Fig. 1**.

Values of Multiple Antibiotic Resistance (MAR) index were determined for all the isolates (**Table 1**). Furthermore, these values ranged from 0.3-0.8 except for the isolates SM-22 and SM-28.

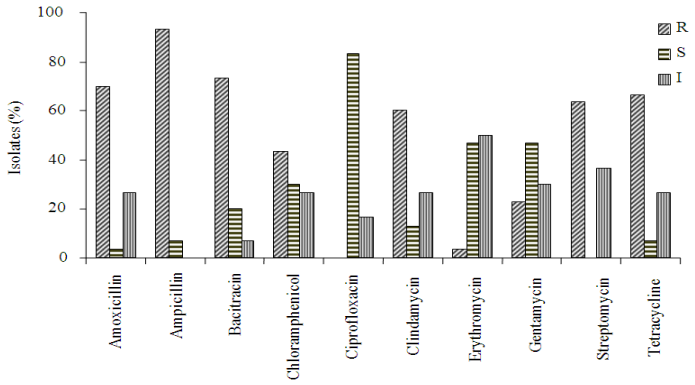

Fig. 1. Antibiotic resistance pattern as exhibited by isolates. R- Resistant; S- Sensitive, I-Intermediate

Table 1. The antibiotic sensitivity profile of 10 different isolates obtained from marine sediment samples

Isolate	Am	A	Ba	C	Cf	Cl	E	G	S	T	MAR index
SM-1	R	R	I	S	S	R	S	S	R	R	0.5
SM-2	R	R	S	S	S	S	S	S	R	I	0.3
SM-3	R	R	R	I	I	R	S	S	R	R	0.6
SM-4	R	R	R	I	S	R	S	I	R	R	0.6
SM-5	I	R	R	S	S	R	I	S	R	I	0.4
SM-6	R	R	R	R	S	R	R	S	R	R	0.8
SM-7	R	R	S	I	S	R	S	S	R	I	0.4
SM-8	R	R	R	R	I	R	S	S	I	R	0.6
SM-9	R	R	R	I	S	R	I	S	I	R	0.5
SM-10	R	R	S	I	S	R	I	S	R	I	0.4
SM-11	R	R	R	R	S	R	I	R	R	R	0.8
SM-12	I	R	R	S	S	R	I	I	I	R	0.4
SM-13	R	R	R	R	S	I	I	R	I	R	0.6
SM-14	R	R	R	R	I	R	S	R	R	R	0.8
SM-15	I	R	I	S	S	R	S	I	I	R	0.3
SM-16	R	R	R	R	S	I	I	R	R	R	0.7
SM-17	R	R	R	R	S	I	I	R	R	R	0.7
SM-18	R	R	R	R	S	I	I	I	R	R	0.6
SM-19	I	R	R	S	S	R	I	S	R	I	0.4
SM-20	R	R	R	R	I	R	I	R	R	R	0.8
SM-21	I	R	R	I	S	I	I	R	R	R	0.5
SM-22	S	S	S	S	S	S	I	S	I	S	0.0
SM-23	R	R	R	R	S	I	S	S	I	I	0.4
SM-24	I	R	R	R	S	R	S	S	R	I	0.5
SM-25	I	R	R	R	I	R	S	I	I	R	0.5
SM-26	R	R	R	R	S	I	S	I	I	I	0.4
SM-27	R	R	R	I	S	I	S	I	R	R	0.5
SM-28	I	S	S	S	S	S	S	S	I	S	0.0
SM-29	R	R	R	I	S	R	I	I	R	R	0.6
SM-30	R	R	S	S	S	S	I	I	I	R	0.3

In the above table 'S' denotes sensitive, 'R' denotes resistant and 'I' denotes intermediate response. The abbreviations for the antibiotics are as follows: Am- Amoxicillin, A- Ampicillin, Ba- Bacitracin, C-Chloramphenicol, Cf- Ciprofloxacin, Cl- Clindamycin, E-Erythromycin, G-Gentamycin, S- Streptomycin, T- Tetracycline. MAR denotes Multiple Antibiotic Resistance

3.2. Antimicrobial Activity Testing

Antibiotic production ability of all the isolates was evaluated against six bacterial species and one fungal species by a perpendicular streak method. None of the isolates displayed any antimicrobial activity against the organisms used in this study despite the fact that the actinomycetes are the best common source of antibiotics and provide approximately two-third of naturally occurring antibiotics.

3.3. Chromium Resistance and Reduction

All the actinomycetes isolates were screened on chromium (VI) supplemented nutrient agar plates. All the isolates except SM-22 and SM-28 showed visible growth similar to control at 1 mM of Cr(VI) concentration, suggesting that these isolates are resistant to Cr(VI) at 1 mM of Cr(VI) concentration. With an increase in concentration of Cr (VI), the number of resistant isolates decreased. Only eighteen isolates could

tolerate up to 2.5 mM of Cr(VI) concentration and six of them (SM-6, SM-11, SM-14, SM-16, SM-20 and SM-29) were found to grow at 5 mM of Cr(VI) concentration. One of the isolates, SM-11 showed growth even at 7.5 mM Cr(VI) concentration. Chromium reduction ability of the six isolates that tolerated up to 5 mM Cr(VI) was studied and the results obtained are presented in **Fig. 2**. Cr(VI) concentration in the culture supernatant decreased from 1 mM initial concentration to undetectable levels within 24 h by isolates SM-11 and SM-20, indicating 100% reduction (**Fig. 2**). Chromate reduction by isolates SM-11 and SM-20 was also studied at 2.5 and 5 mM of initial Cr (VI) concentrations. At 2.5 mM initial Cr(VI) concentration, the isolates SM-11 and SM-20 were found to reduce 82.67 and 70% Cr(VI) respectively while at 5 mM initial Cr(VI) concentration, only 44.34 and 31.34% of Cr(VI) was reduced after 72 h of incubation (**Fig. 3**). Concentration of Cr(VI) remained practically unchanged in uninoculated controls at all initial Cr(VI) concentrations.

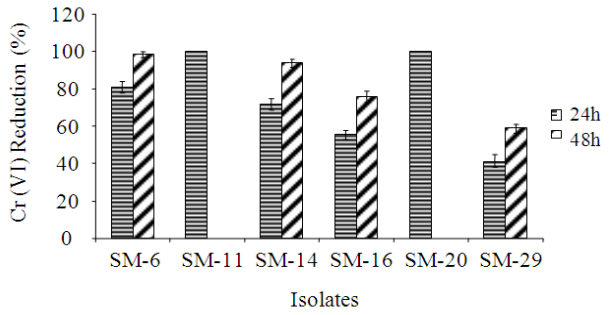

Fig. 2. Cr(VI) reduction by selected actinomycetes isolates at initial Cr(VI) concentration of 1 mM.

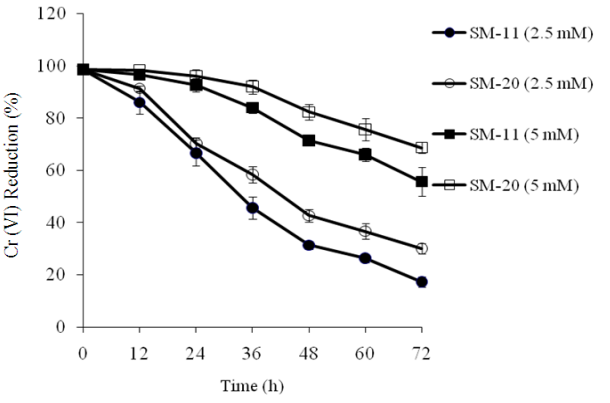

Fig. 3. Time-course of Cr (VI) reduction by isolates SM-11 and SM-20 at different Cr (VI) concentrations

4. DISCUSSION

Based on morphological characteristics and biochemical tests thirty different actinomycetes isolates (SM-1 to 30) were selected for this study. As an isolation of actinomycetes is difficult compared to bacteria and fungi (Booth, 1971), we increased the isolation number by air drying the samples followed by heat treatment for 30 min at 50°C. Isolation media were supplemented with antibiotics, cycloheximide (50 μg mL^{-1}) and nystatin (50 μg mL^{-1}) to inhibit contaminating microorganisms. The isolates obtained in this study were found to show a varied degree of sensitivity to antibiotics. None of the isolates displayed complete resistance to ciprofloxacin, although 21.5% isolates did show intermediate susceptibility to ciprofloxacin (**Table 1**). Values calculated for multiple antibiotic resistance index suggested that all the isolates exhibited multiple antibiotic resistance except SM-22 and SM-28 (**Table 1**). Multiple Antibiotic Resistance (MAR) index is a tool that reveals the spread of resistance in a given population (Krumpermann, 1983). A MAR index greater than 0.2 implies that the strains of such bacteria originate from an environment where several antibiotics are used. The MAR indices obtained in this study are a possible indication that a very large proportion of the bacterial isolates have been exposed to several antibiotics or, somehow, originated from the environment where antibiotics were irrationally used (Paul *et al*., 1997). It is interesting to note that all these isolates were obtained from highly isolated areas where human intervention is likely to be less. Therefore, we assume that the observed effects are due to "excessive" use of antibiotics in agriculture, fishing and other activities. These antibiotics probably later leach out in the soil and ultimately all reach the oceans leading to transfer of antibiotic resistance genes across species.

As, the antibiotic resistance may also reflect the ability of the isolates to produce antibiotic(s) to which they are resistant (Paul and Basu, 1999), all the isolates were tested for possible production of antimicrobial substances. Studies show that almost 70% of natural antibiotics is obtained from actinomyctes and in recent decades, several studies on the antimicrobial activity of the actinomycetes isolated from marine environment have been done (Takizawa *et al*., 1993; Zheng *et al*., 2000; Bull, 2004). Zheng *et al*. (2000), had found that 43.6% of actinomycetes isolated from the marine environment showed antimicrobial activities. Again, enhanced antibiotic resistance has been shown to be correlated with increased production of antibiotics for a number of antibiotic producing species (Khetan and Hu, 2003). Certain mutations that confer resistance to streptomycin or paromomycin can activate antibiotic

production (actinorhodin and undecylprodigiosin) in *Streptomyces coelicolor* A3(2) and *Streptomyces lividans* 66 (Okamoto-Hosoya *et al*., 2000; Shima *et al*., 1996; Okamoto-Hosoya *et al*., 2003). Novel approaches for improving antibiotic-producing strains, especially focusing on methods to induce combined drug-resistant mutations have been described by Hu and Ochi (Hu and Ochi, 2001). However, in this study we have not found any of the actinomyecetes isolate including highly antibiotic resistant ones to produce any antibiotic like substances. Several studies have demonstrated that resistance genes to both antibiotics and heavy metals may be located closely together on the same plasmid in bacteria and are thus more likely to be transferred together in the environment (Bahig *et al*., 2008; Spain, 2003). In this study, we evaluated all the isolates for their resistance towards heavy metal Cr(VI), a well known environment pollutant. Our results showed that all the antibiotic resistant strains were also resistant to chromate. The isolates with high MAR indices, exhibited higher chromium resistance and could tolerate up to 5 mM of Cr(VI). For example the isolates SM-11 and SM-20 with MAR value 0.8 (**Table 1**) could tolerate up to 7.5 mM and 5 mM of Cr(VI) concentration respectively. Six of the isolates that showed visible growth at 5 mM Cr(VI) concentration were tested for their Cr(VI) reduced ability to assess their potential in bioremediation applications. All the six isolates reduced 1 mM Cr(VI) completely within 72 h (data not shown). Since, the isolates SM-11 and SM-20, showed most efficient reduction amongst the isolates, their reduction potential was studied at higher initial Cr(VI) concentrations. Both the isolates showed an effective reduction of 2.5 mM of initial Cr(VI) concentration and the isolate SM-11 reduced 2.5 mM Cr(VI) completely within 4 days. Even at 5 mM of initial Cr(VI) concentration these isolates reduced Cr(VI) efficiently (**Fig. 3**). Thus, the observed potential of the isolates SM-11 and SM-20 to reduce Cr(VI) efficiently at high Cr(VI) concentrations could find application in bioremediation technology.

5. CONCLUSION

The results obtained revealed that the majority of the actinomycetes isolated and tested in this study displayed resistance to both antibiotics and heavy metal chromium. The multiple antibiotic resistance of the majority of the isolates indicates the possible acquisition of resistance factors. Again, our antimicrobial assay revealed that none of the isolates were active against test organisms. Therefore, it may be possible that the antibiotic resistance is not for self protection which is mostly the case with antibiotic producers or it could be that they did not produce antibiotic under the laboratory conditions tested. Among the isolates showing chromate reduction capacity, the isolates SM-11 and SM-20 were most efficient with complete conversion of 1 mM Cr(VI) within 24 h. These isolates were also capable of reducing chromate present in high concentrations and could find potential application in bioremediation. However, the use of these microbes in bioremediation should be done cautiously as this may result in transfer of metal resistance and antibiotic resistance genes in pathogenic bacteria. Hence, for use in bioremediation, chromate reduction capacity of cell free enzymes from these isolates also need to be investigated. Another point to be noted that not only the misuse of antibiotics but also the discharge of heavy metal containing wastes from industries may lead to the emergence of antibiotic resistant bacteria.

6. REFERENCES

Amoroso, M.J., G.R. Castro, A. Durán, O. Peraud and G. Oliver *et al*., 2001. Chromium accumulation by two *Streptomyces* spp. isolated from riverine sediments. J. Ind. Microbiol. Biot., 26: 210-215. DOI: 10.1038/sj.jim.7000112

Asatiani, N.V., M.K. Abuladze, T.M. Kartvelishvili, N.G. Bakradze and N.A. Sapojnikova *et al*., 2004. Effect of chromium(VI) action on *Arthrobacter oxydans*. Curr. Microbiol., 49: 321-326. DOI: 10.1007/s00284-004-4351-2

Bahig, A.E., E.A. Aly, A.A. Khaled and K.A. Amel, 2008. Isolation, characterization and application of bacterial population from agricultural soil at Sohag Province, Egypt. Malaysian J. Microbiol., 4: 42-50.

Baskaran, R., R. Vijayakumar and P.M. Mohan, 2011. Enrichment method for the isolation of bioactive actinomycetes from mangrove sediments of Andaman Islands, India. Malaysian J. Microbiol., 7: 26-32.

Bauer, A.W., W.M. Kirby, J.C. Sherris and M. Turch, 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol., 45: 493-496. PMID: 5325707

Booth, C., 1971. Methods in Microbiology. 1st Edn., Academic Press, London, ISBN-10: 0125215045 pp: 795.

Bull, A.T., 2004. Microbial Diversity and Bioprospecting. 1st Edn., ASM Press, Washington, ISBN-10: 1555812678 pp: 496.

Costa, M. and C.B. Klein, 2006. Toxicity and carcinogenicity of chromium compounds in humans. Crit. Rev. Toxicol., 36: 155-163. PMID: 16736941

Das, S. and A.L. Chandra, 1990. Chromate reduction in *Streptomyces*. Experientia., 46: 731-733. PMID: 2164957

Goodfellow, M., M. Mordarski and S.T. Williams, 1984. The Biology of the Actinomycetes. 1st Edn., Academic Press, London, ISBN-10: 012289670X pp: 544.

Hu, H. and K. Ochi, 2001. Novel approach for improving the productivity of antibiotic-producing strains by inducing combined resistant mutations. Applied Environ. Microbiol., 67: 1885-1892. DOI: 10.1128/AEM.67.4.1885-1892.2001

Ishibashi, Y., C. Cervantes and S. Silver**,** 1990. Chromium reduction in *Pseudomonas putida*. Applied Environ. Microbiol., 56: 2268-2270. PMID: 2389940

Khetan, A. and W.S. Hu, 2003. Metabolic engineering of antibiotic biosynthesis for process improvement. Cell Tissue Reactor Eng.

Krumpermann, P.H., 1983. Multiple Antibiotic Resistance indexing of *Escherichia coli* to identify high risks sources of faecal contamination of foods. Applied Environ. Microbiol., 46: 165-170.

Lam, K.S., 2006. Discovery of novel metabolites from marine actinomycetes. Curr. Opin. Microbiol., 9: 245-251. PMID: 16675289

Laxman, R.S. and S. More, 2002. Reduction of hexavalent chromium by *Streptomyces griseus.* Miner. Eng., 15: 831-837. DOI: 10.1016/S0892-6875(02)00128-0

Lemos, M.L., A.E. Toranzo and J.L. Barja, 1985. Antibiotic activity of epiphytic bacteria isolated from intertidal seaweeds. Microbial Ecol.., 11: 149-163. DOI: 10.1007/BF02010487

Liu, Y.G., W.H. Xu, G.M. Zeng, X. Li and H. Gao, 2006. Cr(VI) reduction by *Bacillus* sp. isolated from chromium landfill. Process Biochem., 41: 1981-1986. DOI: 10.1016/j.procbio.2006.04.020

Mabbett, A.N. and L.E. Macaskie, 2001. A novel isolates of *Desulfovibrio* Sp. With the enhanced ability to reduce Cr (VI). Biotechnol. Lett., 23: 683-687. DOI: 10.1023/A:1010352417399

Myers, C.R., B.P. Carstens, W.E. Antholine and J.M. Myers, 2000. Chromium(VI) reductase activity is associated with the cytoplasmic membrane of anaerobically grown *Shewanella putrefaciens* MR-1. J. Appied Microbiol., 88: 98-106. PMID: 10735248

Nodwell, J.R., 2007. Novel links between antibiotic resistance and antibiotic production. J. Bacteriol., 189: 3683-3685. DOI: 10.1128/JB.00356-07

Okamoto-Hosoya, O., T.A. Sato and K. Ochi, 2000. Resistance to paromomycin is conferred by rpsL mutations, accompanied by an enhanced antibiotic production in Streptomyces coelicolor A3(2). J. Antibiot., 53: 1424-1427. PMID: 11217811

Okamoto-Hosoya, Y., S. Okamoto and K. Ochi, 2003. Development of antibiotic-overproducing strains by site-directed mutagenesis of the *rpsL* gene in *Streptomyces lividans*. Applied Environ. Microbiol., 69: 4256-4259. DOI: 10.1128/AEM.69.7.4256-4259.2003

Pattanapipitpaisal, P., N. Brown and L. Macaskie, 2001. Chromate reduction and 16S rRNA identification of bacteria isolated from a Cr(VI)-contaminated site. Applied Microbiol. Biotechnol., 57: 257-261. DOI: 10.1007/s002530100758

Paul, A.K. and M. Basu, 1999. Chromium-resistant soil actinomycetes: Their resistance to other metals and antibiotics. Acta. Microbiol. Immunol. Hung., 46: 25-32. DOI: 10.1556/AMicr.46.1999.1.3

Paul, S., R. Bezbaruah, M.K. Roy and A.C Ghosh, 1997. Multiple Antibiotic Resistance (MAR) index and its reversion in *Pseudomonas aeruginosa*. Lett. Applied Microb., 24: 169-171. DOI: 10.1046/j.1472-765X.1997.00364.x

Polti, M.A., M.J. Amoroso and C.M. Abate, 2007. Chromium (VI) resistance and removal by actinomycete strains isolated from sediments. Chemosphere, 67: 660-667. DOI: 10.1016/j.chemosphere.2006.11.008

Polti, M.A., M.J. Amoroso and C.M. Abate, 2010. Chromate reductase activity in *Streptomyces* sp. MC1. J. Gen. Applied Microbiol., 56: 11-18.

Polti, M.A., R.O. García, M.J. Amoroso and C.M. Abate, 2009. Bioremediation of chromium(VI) contaminated soil by *Streptomyces* sp. MC1. J. Basic Microb., 49: 285-292. DOI: 10.1002/jobm.200800239

Poopal, A.C. and R.S. Laxman, 2009. Studies on biological reduction of chromate by *Streptomyces griseus*. J. Hazard. Mater., 169: 539-545. DOI: 10.1016/j.jhazmat.2009.03.126

Shen, H. and Y.T. Wang, 1993. Characterization of enzymatic reduction of hexavalent chromium by *Escherichia coli* ATCC 33456. Applied Environ. Microbiol., 59: 3771-3777. PMID: 8285683

Shima, J., A. Hesketh, S. Okamoto, S. Kawamoto and K. Ochi, 1996. Induction of actinorhodin production of *rpsL* (encoding ribosomal protein S12) mutations that confer streptomycin resistance in *Streptomyces lividans* and *Streptomyces coelicolor* A3 (2). J. Bacteriol., 178: 7276-7284.

Spain, A., 2003. Implications of microbial heavy metal tolerance in the environment. Rev. Undergrad. Res., 2: 1-6.

Takizawa, M., R.R. Colwell and R.T. Hill, 1993. Isolation and diversity of actinomycetes in the Chesapeake Bay. Applied Environ. Microbiol., 59: 997-1002. PMID: 16348922

You, J.L., L.X. Cao, G.F. Liu, S.N. Zhou and H.M. Tan *et al*., 2005. Isolation and characterization of actinomycetes antagonistic to pathogenic *Vibrio* spp. from nearshore marine sediments. World J. Microbiol. Biotechnol., 21: 679-682. DOI: 10.1007/s11274-004-3851-3

Zheng, Z., W. Zeng, Y. Huang, Z. Yang and J. Li, 2000. Detection of antitumor and antimicrobial activities in marine organism associated actinomycetes isolated from the Taiwan Strait, China. FEMS Microbiol. Lett., 188: 87-91. PMID: 10867239