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Heavy metal adsorption of *Streptomyces chromofuscus* $_{K101}$

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PEER REVIEW

Peer reviewer

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Comments

This is an interesting research work in which authors have demonstrated the metal resistance and adsorption of some heavy metal ions (Pb^{2+}, Zn^{2+}, Cu^{2+}, Cd^{2+} and Fe^{2+}) by actinomycetes isolated from polluted areas at River Nile, Egypt. In this research many methods were used like qualitative evaluation of heavy metals, MIC of heavy metal determination, metal binding assay, heavy metal assessment.

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ABSTRACT

Objective: To find the best actinomycete that has potential application value in the heavy metal remediation due to its special morphological and physiological metabolism.

Methods: In some areas of River Nile, Egypt, a total of 67 actinomycete isolates (17 isolates from surface water and 50 from sediment) were identified. In addition, the studied area was characterized by a large amount of submerged macrophyte species *Ceratophyllum demersum*, one free floating species *Eichhornia crassipes* and two emergent species *Polygonum tomentosum* and *Saccharum spontaneum* with the highest biomass production values. Many methods are used in this research like qualitative evaluation of heavy metals, minimum inhibitory concentration of heavy metal determination, metal binding assay, heavy metal assessment, *etc*.

Results: Many actinomycetes isolates were isolated from River Nile, Egypt, the absorbent efficiency of one isolate *Streptomyces chromofuscus* $_{K101}$ showed the most efficient metal binding activity. The adsorption process of Zn^{2+}, Pb^{2+} and Fe^{2+} single or mixture metal ions was investigated, where the order of adsorption potential (Zn^{2+}>Pb^{2+}>Fe^{2+}) was observed in single metal reaction. The adsorption in mixed metal reactions was the same order as in single-metal ion with a significant decrease in Fe^{2+} and Pb^{2+} adsorption.

Conclusions: In conclusion the metal adsorption reactions were very fast, pH dependent and culture age-independent, suggestive of a physicochemical reaction between cell wall components and heavy metal ions. The absorbent removal efficiency was determined as a function of ion concentration, pH and temperature.

KEYWORDS
Adsorption, Metals, Biomass, Efficiency

1. Introduction

Actinomycetes have long and branching filaments that resemble the hyphae of fungi. They are Gram positive and constitute a significant component of the microbial population in most soils. Although distributed extensively in soil, they can also be isolated from sediments, water, and aquatic plants[1].

Several features regarding to heavy metal resistance correlated with some metabolic changes and specific growth characteristics of actinomycetes, such as, mycelium formation and relatively rapid colonization of selective substrates indicate them as suitable agents for bioremediation[2]. Heavy metals are introduced to the water bodies as by-products of some industrial or mining activities. These heavy metal by-products affect the aquatic biodiversity thereby disturbing the environmental equilibrium of large areas all over the world[3].

Environment contamination by toxic heavy metals is causing a serious problem worldwide due to their incremented accumulation in food chain and continued persistence ecosystem[4]. The most common heavy metal

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contaminants (*e.g.* lead, cadmium, copper, zinc, and iron) at all concentration are difficult to remove from aqueous solutions. The large scale production of a variety of chemical compounds has caused a global deterioration of environmental quality[5].

Chemical precipitation, reverse osmosis and other methods became inefficient when contaminants are present in trace concentration[6]. Adsorption, precipitation and uptake by purified biopolymers derived from microbial cells provide alternative mechanisms[7]. Intact microbial cells live or dead and their products can be highly efficient bioaccumulators of both soluble and particulate forms of metals[8]. A variety of mechanisms are used for the removal of heavy metals from aqueous solution by different microorganisms such as bacteria[9], algae[10], mixture of dried microalgal/bacterial biomass[11], mold[12], yeast[13,14] and actinomycetes[15,16]. The heavy metal adsorption by streptomycetes, has been presumed to possess a large heavy metal binding capacity and is considered as an alternative method to recover metals from waste liquid[17], while *Streptomyces fradiae* dead biomass was found to adsorb different heavy metal ions, *viz.* Cu^{2+}, Zn^{2+}, Ni^{2+} and Pb^{2+}[17]. The present study aimed to evaluate the metal resistance and adsorption of some heavy metal ions by actinomycetes isolated from polluted areas at River Nile, Egypt.

2. Material and methods

The macrophyte vegetation reflects the trophic characteristics of the water body. So, the macrophytic flora was studied at the area of samples collected. The standing crop of macrophytes was obtained by using metal square quadrate method and the collected macrophytes species were identified[18].

2.1. Sample collection for microorganisms' detection

Water samples were collected manually using 250 mL sterilized plastic bottles; meanwhile stainless steel Ekman dredge sediment sampler was used to collect sediment samples from River Nile at an area which was contaminated with industrial wastes. Samples were stored at 4 °C and analyzed within six hours[19].

2.2. Microorganisms, culture media and characterization

Ten grams of each sediment sample was taken and suspended in 90 mL of 0.1% sodium pyrophosphate solution. The sediment suspension was agitated for six hours at (28±2) °C using rotary shaker at 150 r/min[20]. Starch casein agar[21], and yeast malt extract agar plates were inoculated with 10 μL of serially diluted water and sediment suspension[22]. All plates were incubated at (28±2) °C for two to three weeks. The culture characteristics were studied in accordance with the guidelines established by the International *Streptomyces* Project and identification was based on some criteria[23,24].

2.3. Qualitative evaluation of heavy metals utilized by Streptomyces

Stock solution of Pb^{2+}, Zn^{2+}, Cu^{2+}, Cd^{2+} and Fe^{2+} were used as $PbCl_2$, $ZnSO_4$, $CuSO_4 \cdot 5\ H_2O$, $CdCl_2$, and Fe_2Cl_3, respectively. Solutions were prepared in sterile water and sterilized by filtration, then added to molten cooled starch casein medium immediately before pouring the plates to obtain final concentration of 50 mg/L. Microbial growth was used as the qualitative parameter of mean resistance after seven days of incubation at (28±2) °C.

2.4. Determination of minimum inhibitory concentration (MIC) of heavy metals

Lowest concentration of heavy metal that cause growth inhibition was determined[25]. The metals in varying concentration ranging from 25.0 μg/mL to 100.0 μg/mL was added to starch casein agar media previously inoculated with tested strains spores (10^6 spore/mL). MIC of the tested heavy metal was determined using classical diffusion agar method. 100 μL of each metal concentration is filled into agar well.

2.5. Metal-binding assay

The binding of heavy metals was measured by biomass, mycelium and spores. One liter Erlenmeyer flask containing 200 mL of yeast malt extract broth was inoculated with six millimeter disc of three days old culture of *Streptomyces* and incubated for seven days at (28±2) °C at 150 r/min. Biomass was collected via centrifugation at 10 000 r/min for 10 min and twice washed with phosphate buffer solution (pH 7.0). Two grams of freshly harvested cells were added into 100 mL conical flask containing 20 mL of each metal salt at 100 mg/L, biomass metal mixture was shaked (150 r/min) for two hours at room temperature (28±2) °C. To investigate the pH effect, the initial concentration was adjusted to 100 μg/mL for each metal to avoid possible perception, and pH values of metal solution were adjusted to cover the pH range between five and seven using 0.1 mol/L HCl or 0.1 mol/L NaOH, before the addition of the biomass. The reaction mixture was filtered to remove mycelium using Whatman filter, (pore size 0.42 μm)[26].

2.6. Heavy metal assessment

The residual metal of the filtrate concentration was measured by atomic absorption spectrometry using Perkin Elmer model 3030 B. The metal uptake per unit mass (mg metal ions/g cells) was obtained using the following expression $Q=(C_0-C)V/M$, where Q is the amount of metal ions absorbed to the unit of the absorbent (mg/g), C_0 and

C are the concentration of the metal ions before and after adsorption (mg/L), *V* is the volume of the aqueous phase (L) and *M* is the amount of the absorbent (g)[27]. The metal binding test was also carried out for the mixed metal solution in which the five metal salts listed above were dissolved together to give the final concentration of 25.0 μg/mL for each metal ion. Biomass was mixed with metal solution and incubated for two hours at room temperature (28±2) °C at 150 r/min.

Metal binding assay was carried out in the presence of different culture age (three, five and seven days) biomass. As mentioned above biomass was removed by filtration and the metal concentration for each metal was measured. Results were expressed as mean of experiments carried out in triplicate and data were compared with those for loss of heavy metals from uninoculated control.

2.7. Statistical analysis

The data recorded in triplicate for the parameters were subjected to ANOVA using SPSS-statistical program at 5% significance level.

3. Results

3.1. Macrophytic vegetation

The study area is characterized by a large bed of submerged macrophyte species *Ceratophyllum demersum* with a biomass production value of (202.66±2.51) gross weight/m^2. In addition, three other macrophyte species were recorded, one free floating species *Eichhornia crassipes* and another two emergent species with the highest biomass production values *Polygonum tomentosum* and *Saccharum spontaneum* (304.00±4.00) and (402.00±2.64) gross weight/m^2, respectively (Table 1).

Table 1

Recorded macrophytes at the study site and their biomass production (gross weight/m^2, mean ±SD).

Macrophyte species	Family name	Biomass
Ceratophyllum demersum L.	Ceratophyllaceae	202.66±2.51
Polygonum tomentosum L.	Polygonaceae	304.00±4.00
Saccharum spontaneum L.	Poaceae	402.00±2.64
Eichhornia crassipes (Mart) Solms	Pontederiaceae	190.66±3.06

3.2. Heavy metal resistant isolates

A total of 67 actinomycete isolates (17 isolates from surface water and 50 from sediment) were isolated and tested for heavy metal resistance, only five isolates were resistant to all tested five heavy metals at concentration of 50 μg/mL (Table 2). Growth measures of 14_S, 15_S, 16_S, 17_S and 17_W (S means sediment, *i.e.* these isolates were isolated from the sediment samples, W means water samples) showed that, very low incidence (0.0%) is susceptible to copper and cadmium, where growth ranged from 0.0%-25.0%. All the isolates showed intermediate resistance to Fe^{2+}, Zn^{2+} and Pb^{2+}, the incidence of 14_S strain resistance to all five tested heavy metals was high, ranging from 25%-100%.

Table 2

Heavy metal tolerance screening in single metal suspension (50 μg/mL).

Metal ions	14_S	15_S	16_S	17_S	17_W
Lead	100	45	35	50.0	45.0
Zinc	100	50	50	75.0	48.0
Copper	25	<10	<10	0.0	0.0
Cadmium	25	<10	<10	0.0	0.0
Iron	75	37	25	25.0	25.0

3.3. Semi-quantitative analysis of inhibition of heavy metals

Increasing the metal concentration (100 μg/mL) in plate diffusion experiments resulted in marked inhibition of microbial growth. The order of metal resistance by isolate, 14_S was $Pb^{2+} \geqslant Zn^{2+} > Fe^{2+} > Cu^{2+} > Cd^{2+}$, of these zinc and lead did not inhibited the growth. As a result, all the tested isolates except 14_S turned out to be sensitive to 100 μg/mL of Zn^{2+} and Pb^{2+}, interestingly 14_S isolate used as metal uptake. The growth inhibition profile at 100 μg/mL revealed four strains, strain 17_S was the most sensitive to Cd^{2+} (37±0.58) mm and Cu^{2+} (26±1.0) mm. The growth of 14_S was not inhibited at 25.0 mg/L of all tested heavy metal ions except Cd^{2+} that inhibited the isolate growth (Figure 1).

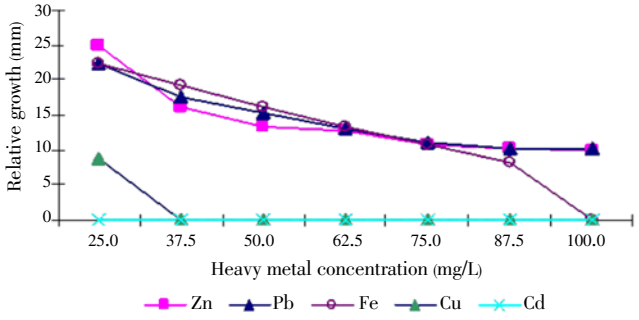

Figure 1. Effect of heavy metal concentration on the growth of *S. chromofuscusit* $_{K101}$.

3.4. Characterization and identification of the most resistance isolate

Isolate code No: 14_S produced well developed branched and non fragmented vegetative hyphae, with smooth surface spores (Figure 2A). Culture characteristics of these isolates 14_S on various agar media were summarized in Table 3. The vegetative mycelia were white to olive and no distinctive pigments were produced. The aerial mycelia were white to yellowish white and became gray on sucrose-nitrate agar. The hydrolyzed cell wall of the strain contained LL-diaminopimelic acid without characteristic sugar pattern.

Table 3

Culture characteristics of isolate 14_S on various growth media.

Growth media	Aerial mycelium	Vegetative mycelium reverse color	Soluble pigment
Yeast extract-malt extract agar (ISP-2)	Good, white	Good, yellowish-white	None
Oat-meal agar (ISP-3)	Good, white	Good, white	None
Glycerol-asparagine agar (ISP-5)	Good, yellowish-white	Good, white	None
Glucose-asparagine agar	Good, yellowish-white	None	None
Sucrose-nitrate agar	Good, grey	Good, olive	None

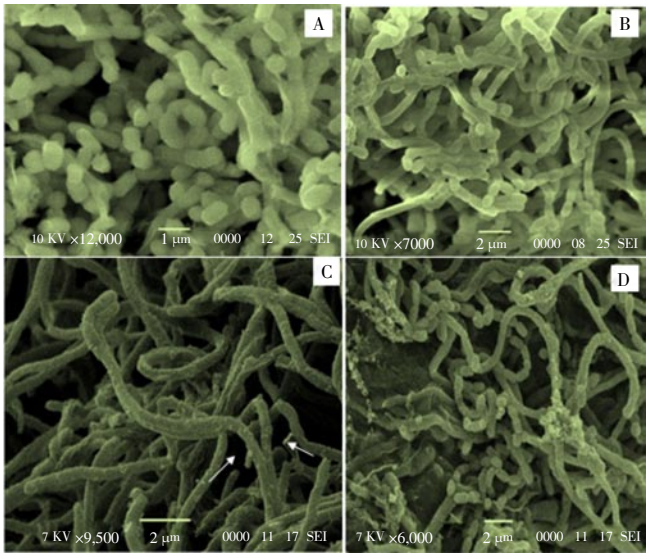

Figure 2. Electron micrograph of *S. chromofuscusit* $_{K101}$ grown in heavy metal solutions.

A: Control (without heavy metal); B,C: Growth under Zn^{2+}, Pb^{2+} (100 mg/L) stress; D: Mixture of tested heavy metal ions with 50 mg/L.

These taxonomic observations indicated that isolate 14_S belong to the genus *Streptomyces*. Melanin-like pigments or other significant diffusible pigments were not detected in any tested agar medium; the physiological characteristics of 14_S are shown in Table 4. On the basis of the previously collected data and in view of the comparative study of the recorded properties of *Streptomyces* in relation to the most close reference strain, *viz. Streptomyces chromofuscus* (*S. chromofuscus*), it could be stated that isolate 14_S is suggestive of being likely belonging to *S. chromofuscusit*, that has the name *S. chromofuscusit* $_{K101}$.

Electron micrographs of metal-treated cells showed that, neither Zn^{2+} nor the mixture of the metal solution could impair the cell morphology (Figure 2B and 2D), where no distinctive changes occurred compared to the control (Figure 2A). However *S. chromofuscusit* $_{K101}$ grown in 100 mg/L Pb^{2+} could not tolerate the metal effect, it failed to form spores (the arrow, Figure 2C).

3.5. Heavy metals uptake by S. chromofuscusit $_{K101}$

The metal adsorbing activities of the *S. chromofuscusit* $_{K101}$ were determined for lead, zinc and iron ions. Table 5 shows that the order of metal absorption was zinc>lead>iron, while iron in single metal was moderately adsorptive (0.5645 µg/mg) where Zn^{2+} and Pb^{2+} uptake were much greater than that observed for the iron, it were 0.7286 and 0.8000 µg/mg dry weigh biomass, respectively. The order of metal uptake in mixture ions was the same as in single metal ion compared with the single metal reaction (Table 5). Significantly decrease in all metal adsorption was notable, and except with zinc where no significant adsorption in single and mixed ion was detected. No reproducible changes according to cell age of mycelium were observed for metal adsorption. Thus, Zn^{2+} were the most adsorptive by the mycelium of three, five and seven days (0.78, 0.75 and 0.7980 µg/mg dry weight biomass, respectively). The mycelium from five days culture presented lower adsorption level than that from three and seven days without significant differences (Figure 3). Effect of pH on the adsorption of heavy metals after two hours incubation in each metal suspension (100 µg/mL) by *S. chromofuscusit* $_{K101}$ is showed in Figure 4, the metal adsorption reaction was pH dependent. This increase was most evident in zinc adsorption (0.83 µg/mL).

Table 5

Heavy metal uptake efficiency after two hours incubation.

Metal solutions	Heavy metal uptake (µg/mg)		
	Iron	Lead	Zinc
Single metal ion	0.5645^{aA}±0.0000	0.7286^{bB}±0.0520	0.8000^{bB}±0.0100
Mixed metal ions	0.341^{B}±0.020	0.612^{C}±0.000	0.791^{B}±0.020

Different superscripts differ significantly (P<0.05).

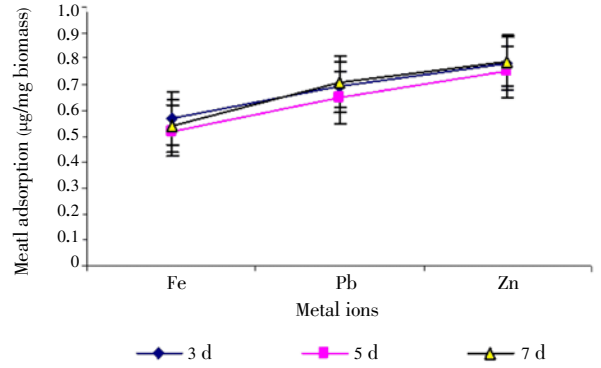

Figure 3. Effect of cell age of *S. chromofuscusit* $_{K101}$ on heavy-metal adsorption.

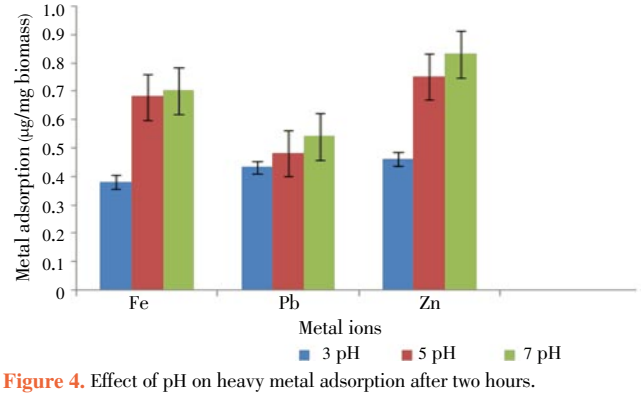

Figure 4. Effect of pH on heavy metal adsorption after two hours.

Table 4

Physiological properties of isolate 14_S.

Characteristics	Growth of isolate 14_S
Temperature range for growth	10-45 °C, optimum range at 35-40 °C
pH range of growth	5-8, with optimum at 7.5
Growth at 2%-7% NaCl; 0.01%-0.02% Na-azide and 0.1% phenol	Positive
Hydrolysis of starch, liquefaction of gelatin and nitrate reduction	Positive
Utilization of (carbon); D-glucose, D-xylose, D-fructose, L-rhamnose, sucrose, raffinose and L-arabinose	Positive
Utilization of (nitrogen); DL-valine, phenylanaline, L-serine, aspragine and arginine	Positive

4. Discussion

Macrophytes physically structure aquatic ecosystems, provide habitat for epiphytes and herbivore organisms and contribute to sedimentation of particulate organic matter. In addition, these plants serve as a substrate for microbial activity[28]. During growth and decay macrophytes release dissolved organic matter, a by-product of photosynthesis[29]. These dissolved organic substances constitute a potential source of carbon for water column metabolism and provide a high-quality substrate for bacterial growth[30]. An unknown fraction of plant derived dissolved organic matter consists of biochemically active allelochemicals. Allelochemicals are chemically diverse secondary plant metabolites and exert multifunctional properties of allelopathic interaction when released into the water[31].

During our investigation, only four macrophyte species and a total of 67 actinomycetes isolates were recorded in the studied area. Although the physicochemical analysis of water samples reflect a suitable conditions for the growth of actinomycetes [represented by a relatively high water temperature (30.23±0.21) ℃ , a slightly alkaline pH value 7.74 ±0.64 suitable for the dominance of non toxic NH_4[32] and there is no ecological stress on aquatic organisms inhabiting this area, relatively high value (5.46±0.08) mg/L of dissolved oxygen and a suitable amount of dissolved nutrients] only 17 species were isolated from water, while majority of them were isolated from sediment samples.

These findings come in agreement with the previously recorded results[33], they recorded that, majority of actinomycetes were isolated from sediment samples and very few of them were recovered from macrophyte roots, while it was not detectable in water samples. The authors reflect this variation to the effect of environmental conditions as well as to the allelopathic effect of macrophytes at the area of sample collection. As previously recorded, the macrophyte species of the area under investigation were characterized by the presence of some biologically active substances, like alkaloids, flavonoids, terpenes, glycosides and tannins[34], which were recorded to have an antimicrobial activity[35]. In addition, some compounds of these groups are known to be either bacteriostatic or bactericidal materials, based on their concentration[36]. Many researchers reported that flavonoids and phenolic derivatives have antimicrobial activities[37]. These compounds are believed to function by affecting the bacterial cell membrane integrity, resulting in inhibiting the bacterial growth[38].

This study indicated the metal resistance and metal adsorption activities of five actinomycetes tested for some heavy metals. The resistance of the tested actinomycete isolates was in the following order Zn≥Pb>Fe>Cu≥Cd for all isolates. Among the test isolates, *S. chromofuscusit* $_{K101}$ is the most effective organism for all heavy metals at all concentrations (25-100 μg/mL medium). All the tested isolates could not grow at the medium containing cadmium (Cd^{2+}) at concentration of 25 μg/mL, which was due to the toxic effect of Cd^{2+}. Kazuya M *et al.*[39] reported that the growth of *Streptomyces coelicolor* was inhibited by 50% after 16 h of culturing in the presence of 0.14 mmol/L Cd^{2+}. The growth of all tested isolates was reduced as copper concentrations increased in the medium, where no growth was observed at all tested concentrations, but *S. chromofuscusit* $_{K101}$ showed growth at 25 μg/mL. *Streptomyces coelicolor* could tolerate Cu^{2+} concentrations no higher than 0.047 mmol/L[40]. It is well known that medium composition may influence metal sensitivity[41]. Significantly, zinc adsorption was much higher than lead and iron, 0.8000, 0.7286 and 0.5645 μg/mL respectively. Generally, heavy metal binding was found to be a result of physicochemical reaction and metals were rapidly absorbed by surface structures of the cell. The surface envelopes of bacterial cells can adsorb various heavy metals by virtue of ionic bonds to their intrinsic chemical groups. The sites for metal binding are different according to bacterial species and metals. Heavy metal adsorption appears to be stronger in Gram-positive cell walls, thus due to anionic groups such as carboxylate and phosphate groups of peptidoglycan and teichoic acid that are considered the major metal binding sites[42].

The heavy metal adsorption by streptomycetes, also belong to Gram-positive bacteria, has been presumed to contribute a large heavy metal binding capacity. Adsorption activities of *S. chromofuscusit* $_{K101}$ in mixed metal compared with the single metal reaction revealed decrease in both iron and lead and no significant change for zinc, which could be attributed to zinc occupied its binding sites where adsorption of other metals relatively poorly took place[43], also this differential metal binding of cell walls might be due to the difference in binding strength or to binding selectivity of cell wall components[44], also complex ion and ion exchange with cell wall compounds could lead to removing toxic metals via adsorption to cell wall surface[45]. It means that inter particle mass transfer resistance is rate limiting. Therefore, in the presence of a mixture of the metal ions the metal ions jostle for the adsorption sites on the absorbent. This competition affects the diffusion properties of the metal ions; hence decreases the adsorption capacity of the metal ions. Thus, the metal ion that successfully reaches the adsorption site faster depends on the ionic radii of the metal ions. Generally competition among the metal ions for adsorption sites obviously affected the adsorption capacity[46]. The reason for less evident pH dependent increases in adsorption of other heavy metals is not clear, but it might correlated to that different binding sites with different ionizing patterns contribute to metal adsorption for each heavy metal[47].

Our results revealed that the strongest heavy metal absorbent was the most resistant strain that was able to survive at high heavy metal concentration. This is in contrast to the results obtained previously[48], which showed that the strongest heavy metal absorbent was the most sensitive strain. The reduction in Zn, Fe and Pb ions uptake by free nonliving cell at low pH 3.0 may be due to the alteration in the chemical and physical behavior of cells binding sites[49]. Such effect may be due to the electrostatic interaction between the cells[50].

It can be concluded that non-living biomass of *S. chromofuscusit* $_{K101}$ was found to have Fe^{2+}, Pb^{2+} and Zn^{2+} uptake properties. Therefore, the possibility of recycle and reuse the waste byproducts abundantly produced in fermentation industry can render disposal of waste to environment. Furthermore, the reusable absorbent can be used to treat the waste before being discharge properly into the environment.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Actinomycetes have long and branching filaments that resemble the hyphae of fungi. They are Gram positive and constitute a significant component of the microbial population in most soils and they can also be isolated from sediments, water, and aquatic plants. Actinomycetes are microorganisms with ability to remove heavy metals from aqueous solution.

Research frontiers

The present study aimed to evaluate the metal resistance and adsorption of some heavy metal ions (Pb^{2+}, Zn^{2+}, Cu^{2+}, Cd^{2+} and Fe^{2+}) by actinomycetes isolated from polluted areas at River Nile, Egypt. A total of 67 actinomycete isolates (17 isolates from surface water and 50 from sediment) were isolated and tested for heavy metal resistance, only five isolates were resistant to all tested five heavy metals.

Related reports

On the basis of the previously collected data and in view of the comparative study of the recorded properties of *Streptomyces* in relation to the most close reference strain, *viz. S. chromofuscus*, it could be stated that isolate 14_S is suggestive of being likely belonging to *Streptomyces chromofuscusit*, that has the name *S. chromofuscusit* $_{K101}$.

Innovations and breakthroughs

Many methods are used in this research like qualitative evaluation of heavy metals, MIC of heavy metal determination, metal binding assay and heavy metal assessment.

Applications

The possibility of recycle and reuse the waste byproducts produced abundantly in fermentation industry can render disposal of waste to environment. Furthermore, the income generated from the reusable absorbent can be used to treat the waste before being discharge properly into the environment.

Peer review

This is an interesting research work in which authors have demonstrated the metal resistance and adsorption of some heavy metal ions (Pb^{2+}, Zn^{2+}, Cu^{2+}, Cd^{2+} and Fe^{2+}) by actinomycetes isolated from polluted areas at River Nile, Egypt. In this research many methods were used like qualitative evaluation of heavy metals, MIC of heavy metal determination, metal binding assay and heavy metal assessment.

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