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Isolation and characterization of metal-resistant bacterial strain from wastewater and evaluation of its capacity in metal-ions removal using living and dry bacterial cells

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Abstract A novel strain of the genus *Micrococcus* isolated from wastewater was studied for resistance to seven heavy metals and forty antibiotics. Its capacity to accumulate metal ions was also realized at different pH. The strain exhibited high minimal inhibitory concentration values for metal ions tested and resist to 15 antibiotics. The living cells of the bacterial strain show a largest uptake capacity at pH 6–8.5 for copper, nickel, and zinc with values ranging from 51.45 to 83.90 %, 52.59 to 78.81 %, and 59.55 to 78.90 %, respectively. It was also able to absorbed 59.81–80.08 % of chromium and 58.09–79.41 % of cobalt at pH 7.3–8.5. The maximum lead uptake was obtained at pH 5.5–8.5 with an amount of 55.28–91.06 %. The significant absorption of cadmium was shown at pH 6.5 with 38 %. In 25 µg mL-1 zinc, chromium, and nickel solutions, dead cells of the isolate were able to biosorbed 20.46, 22.5, and 23.98 µg mL^{-1}, respectively, after 30 min of contact. In other solutions with higher concentrations 50 and 100 µg mL^{-1}, the amount of each metal immobilized was, respectively, as follows: 38.02 and 90.21 µg mL^{-1} for zinc, 39.78 and 89.23 µg mL^{-1} for chromium, and 47.19 and 86.83 µg mL^{-1} for nickel. Due to its high-metal accumulation capacity in aerobic conditions, these Gram-positive bacteria may be potentially applicable in situ bioremediation of heavy metals contaminating aqueous systems.

Keywords Gram-positive bacteria · Antibiotics · Heavy metals · Resistance · Accumulation

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Introduction

Heavy-metal pollution of the environment has dramatically increased in recent years due to various human activities, such as agriculture, mining, and various other industrial processes (Rajaganapathy et al. 2011). The presence of significant concentrations of these toxic elements in environment ecosystems leads to both contamination of soil and water and deleterious impact on environment life (Rajbanshi 2008). Heavy metals are recognised to be powerful inhibitors of biodegradation activities (Deeb and Altalhi 2009). These chemical elements can not be degraded and are ultimately indestructible. The toxic effects of metal ions result mainly from their interaction with proteins and inhibition of metabolic processes (Tamil Selvi et al. 2012). Though some heavy metals, such as copper, nickel, chromium, and zinc are using as micronutrients for development and growth of organisms, however, others, such as cadmium, mercury, and lead, have no physiological activity for the cell. Moreover, the higher concentrations of these toxic elements above threshold levels have a very detrimental effect on the microbial communities and their vital activities (Ahemad and Malik 2012). Thus, microbial populations exposed to heavy metals present in the environment contain bacteria which have acquired a variety of mechanism for adaptation and resistance to these toxic elements, and, among them, bioaccumulation which involves complexation of the metal ions inside and outside

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the cell with biosorption (Johncy et al. 2010), mineralisation and precipitation, enzymatic oxidation or reduction of the toxic metals, and the efflux systems of metal ions outside the cell (Nies 2003). Wastewaters are usually hazardous to human populations and the various environmental ecosystems and must be treated prior to disposal into rivers, seas, and land surfaces (Bashan and Bashan 2010). Although, the removal of these toxic compounds from them is a real challenge for waste treatment engineers and scientists (Wang et al. 2010). The traditional biological treatment processes can eliminate a large fraction of biodegradable organics compound existed in wastewater. Furthermore, the biological treatment cost is much lower than that of physical and chemical methods (Kumar et al. 2011). Thus, the heavy-metal removal ability of metal-resistant bacteria has been studied extensively and proposed to be efficient and economical alternative in uptake of heavy metal ions from wastewater (Vidali 2001).

This study deals with the characterization of the bacterial strain isolated from wastewater and testing its resistance to different heavy metal concentrations and antibiotics sensitivity. The ability of this bacterium to accumulate cadmium, lead, zinc, chromium, cobalt, copper, and nickel ions at different pH using living bacterial cells was evaluated and compared. Eventually, the biosorption of zinc, chromium and nickel was studied.

Materials and methods

The bacterium used in this study was the strain 2YB-25OH isolated from a sample of wastewater collected from Oued El Harrach in west of Algiers, Algeria, in 2010. It was identified on the basis of cells morphology, Gram-coloring, and study of some physiological and biochemical characteristics, such as respiratory type, catalase, oxydase, and nitrate reductase tests. The molecular identification of the isolate by the sequencingof 16S rRNA was done in the laboratories IRD [France] and GATC [Germany].

Bacterial heavy-metal resistance test and MIC determination

The new strain was tested for its resistance to seven heavy metals by agar dilution method Malik and Ahemad (2006); Kinare and Shingadia 2014). However, stock solutions of 10^4 μg mL^{-1} were prepared by dissolving the exact quantities of the following metal salts: CdCl$_2$ (BIOCHEM), ZnCl$_2$ (ANALAR), Pb(NO$_3$)$_2$ (BIOCHEM), K$_2$CrO$_4$ (MERCK), Ni(NO$_3$)$_2$·6H$_2$O (BIOCHEM), CuCl$_2$ (PANREAC), and

CoCl$_2$ (FLUKA) in distilled water and sterilized at 120 °C for 20 min. 20 mL of nutrient agar was poured into Petri plates and the volume of metal stock solutions was calculated by the formula: $C_1 \times V_1 = C_2 \times V_2$, where C_1 is the metal concentration in stock solution, V_1 is the volume of stock solution used, C_2 is the concentration of metal in agar, and V_2 is the volume of agar. Thereafter, the bacterial strain was streaked onto the medium-containing increasing concentrations of metal salts using sterile loops. Then, plates were sealed and incubated at 30 °C for 5 days. Plate-containing only nutrient agar was also inoculated and incubated to act as control. The lowest concentration of each metal at which no growth occurred when compared to the control plates was considered as the MIC.

Heavy-metal accumulation and removal capacity of living bacterial cells

Foremost, the evolution of the growth of the strain 2YB-25OH was monitored in the presence of metal ions at different pHs. Thereafter, the removal capacity of living bacterial cells was evaluated. In this context, the bacterial strain was grown in nutrient broth containing 100 μg mL^{-1} of each metal at different pHs (5, 5.5, 6, 6.5, 7.3, 8, and 8.5). The flasks were incubated in shaking conditions (130 rpm) at 30 °C for 48 h (Krishna et al. 2012; Sujitha and Jayanthi 2014; Abbas et al. 2014a, b). Two sets of controls crops were prepared simultaneously with the experiment flasks. The first one was prepared at different pHs and without heavy metals to measure bacterial growth and the second without bacterial biomass to evaluate the residual concentration of each metal. The growth was determined by measuring the optical density at 600 nm with uninoculated broth as control. However, the evaluation of the residual concentration of each metal was carried out after centrifugation at 6000 rpm for 10 min 10 mL of each bacterial culture and analysis of the supernatant from each sample using Atomic Absorption Spectrometry as recommended by the American Public Health Association (1998).

Study of metal-ions removal capacity of dry bacterial cells (Biosorption)

To evaluate the capacity of zinc, chromium, and nickel adsorption by the isolate, 0.2 g of cell dry weight were added to 100 mL heavy-metal solutions of 25, 50, and 100 μg mL^{-1} in 250 mL flasks. The pH of the solutions was adjusted to 6.5 and incubation was performed under

stirring (130 rpm) at 30 °C. 10 mL were removed from each flask after 30, 60, 90, and 120 min of contact and centrifuged at 6000 rpm for 10 min. The supernatants were analyzed to determine metal ions concentrations using Atomic Absorption Spectrometry (AAS).

Determination of antibiotic resistance

The strain 2YB-25OH was tested for its sensitivity to 40 antibiotics using disk diffusion method (Bauer et al. 1966). A single colony was picked and inoculated in nutrient broth media. The culture was incubated at 30 °C for 24 h. The turbidity of media was compared to the 0.5 McFarland standard, then spread on Muller-Hinton agar using sterile swabs. Thereafter, antibiotic-impregnated discs were located on the surface of the plates. After 48 h of incubation at 30 °C, the diameter of the inhibition zones was measured and the strain was classified as resistant (R), intermediate (I), and susceptible (S) following the standard antibiotic disk chart. The following antibiotic discs were tested: ampicillin (AM) 10 µg, oxacillin (OX) 5 µg, penicillin (P) 10 IU, amoxicillin (AMX) 25 µg, cefalexin (CN) 30 µg, cefotaxim (CTX) 30 µg, cefoxitin (FOX) 30ug, cefalotin (CF) 30 µg, cefuroxim (CXM) 30 µg, piperacilline (PIP) 100 µg, carbenicillin (CB) 100 µg, fosfomycin (FOS) 50 µg, streptomycin (S) 10 µg, gentamycin (GM) 10 µg, kanamycin (K) 30 µg, amikacin (AN) 30ug, tobramycin (NN) 10 µg, neomycin (N) 30UI, netilmicin (Net) 30 µg, dibekacin (DKB) 10 µg, erythromycin (E) 15 µg, spiramycin (SP) 100 µg, nalidixic-acid (NA) 30 µg, pipemidique acid (Pi) 20 µg, ofloxacin (OFX) 5 µg, tetracyclin (TE) 30 µg, doxycyclin (DOX) 30ug, chloramphenicol (C) 30 µg, bacitracin (B) 10 IU, colistin (Cl) 30 µg, lincomycine (L) 15 µg, furanes (FT) 300 µg, flumequin (UB) 30 µg, fusidic acid (FA) 10 µg, nitroxolin (Ni) 20 µg, nitofurantoin (F/M) 300 µg, vancomycin (VA) 30 µg, pristinamycin (PR) 15 µg, sulfamides (G) 200 µg, and rifampicine (RA) 30 µg.

Results and discussion

The strain 2YB-25OH was yellow pigmented with cells spherical-shaped and grouped into tetrad. It's Gram-positive, strictly aerobic, catalase and oxydase-positive, hydrolyze arginine, reduce nitrate to nitrite, non-motile, and do not form spores. It grew over a wide range of pH (3–10), temperature (20°–40 °C), and NaCl concentration (0.0–10 %); the strain was halotolerant, as it grew in the presence of 10 % NaCl, but did not require salt for its physiological activities. On account of morphological and biochemical characteristics, it was identified as *Micrococcus sp*. Analysis of 16S rDNA sequence revealed that the strain 2YB-25OH is a novel strain of the genus
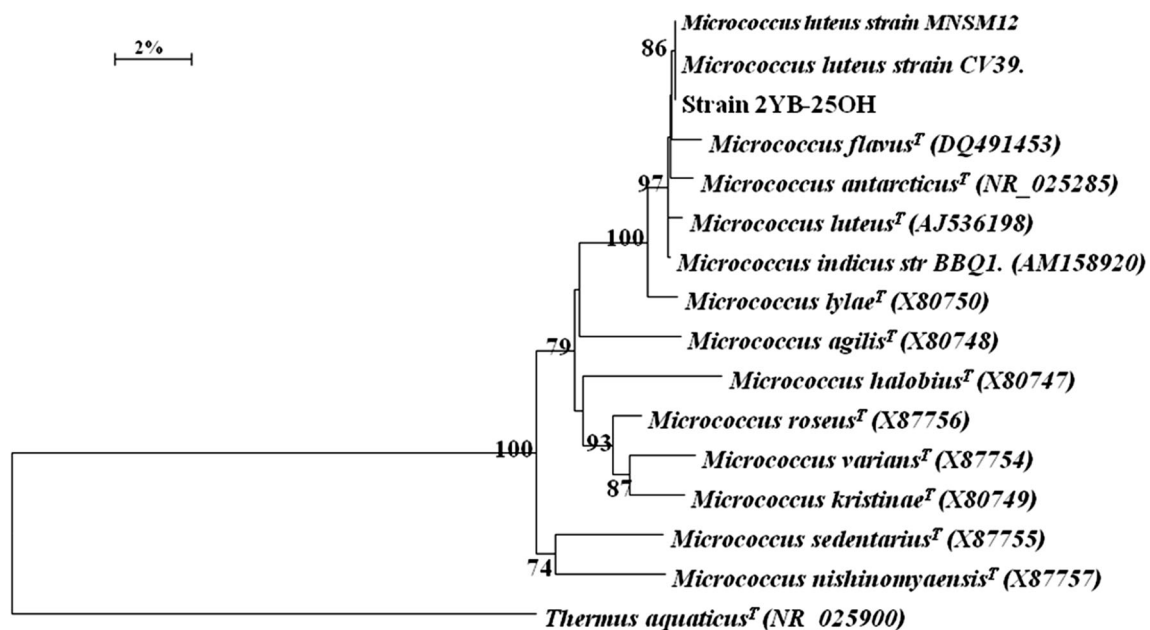

Fig. 1 Phylogenetic tree showing relation between Strain 2YB-25OH and other *Micrococcus* strains

Micrococcus with 99 % homology with *Micrococcus lu-teus* strains and was designated as *Micrococcus luteus* strain 2YB-25OH. The phylogenetic position of the species is shown in Fig. 1.

Determination of the MIC of heavy metals and antibiotic resistance

The novel strain of *Micrococcus luteus* characterized and showed a varied level of resistance to heavy metals tested. Results obtained revealed a great deal of variation in resistance pattern of the promising isolate to each metal ion studied. However, the MIC levels against cadmium, nickel, copper, and zinc up to 300, 350, 400, and 500 μg mL^{-1}, respectively. It exhibited MIC towards chromium and cobalt up to 600 and 650 μg mL^{-1}, respectively. The bacterium showed the maximum resistance against lead with MIC value 1400 μg mL^{-1}. The resistance to metal ions may be attributed to some detoxifying mechanisms developed by this strain to rap out their toxicity, such as accumulation and complexation of the metal ions inside the cell, the efflux of metal ions outside the cell, adsorption by metal ions exchange on the cell surface, and reduction of some heavy metal to a less toxic state (Issazadeh et al. 2013; Lucious et al. 2013). Thus, cadmium resistance mechanism in Gram-positive bacteria is linked to cadmium efflux systems. However, the zinc resistance is achieved

through the two general efflux mechanisms mediated by a P-type ATPase efflux system and an RND-driven trans-porters system (Spain and Alm 2003). Furthermore, resistance against copper is most often dependent upon efflux mechanism. Mancini et al. (2015) reported that P-type ATPases detoxify copper via efflux mechanism in some species. However, it has been reported that copper meta-bolism occurs naturally in all living organisms, and is generally chromosomally encoded (Ahemad 2012). In contrast, mechanisms that specify resistance to copper in bacteria is often plasmid encoded (Abicht et al. 2013). The plasmid and chromosomal systems may interact with each other to maintain copper homeostasis in bacteria (Lu et al. 1999). Moreover, the copper resistance systems of the type, usually encode four proteins which bind copper in the periplasm or close to the outer membrane (Hans et al. 2016). Mikolay and Nies (2009) reported that nickel and cobalt resistance is linked to RND-driven outer membrane efflux system and cytoplasmic membrane efflux systems.

The behavior of the strain against antibiotics studied varies according to antibiotic families. Among 40 antibi-otics tested, only 15 were active on the strain 2YB-25OH with a rate of 37.5 %. Total resistance (R_T) was observed against twelve antibiotics: cefalexin, cefuroxim, kanamy-cin, tetracyclin, piperacilline, tobramycin, furanes, flume-quin, pipemidique acid, nalidixic-acid, nitrofurantoin, and fosfomycin with an intermediate resistance (R_I) to

Table 1 Antibiotic sensitivity and resistance of the strain 2YB-25OH

| Families and number of antibiotics tested	Resistant to	Sensitive to
β lactamins: (11)	Cefalexin, Cefuroxim, Piperacilline	Penicillin, Ampicillin, Amoxicillin, Oxacillin, Cefalotin, Cefotaxim, Cefoxitin, Carbenicillin
Aminosids: (08)	Kanamycin, Tobramycin Dibekacin (R_I)	Gentamycin, Netilmicin, Neomycin Streptomycin, Amikacin
Tetracyclins: (2)	Tetracyclin	Doxyciclin
Quinolons:03	Pipemidique acid, Ofloxacin (R_I) Nalidixic-acid	
Furans +Nitrofurans: 1 + 1	Furanes, Nitrofurantoin	
Fosfomycin: 01	Fosfomycin	
Oxyquinoleines: 01	Nitroxolin (R_I)	
Fluoroquinolones: 01	Flumequin	
Glycopeptides: 01		Vancomycine
Fusidamins: 01		Fusidic acid
Sulfamides et associés: 01		Sulfamides
Macrolides: 02		Erythromycin, Spiramycin
Lincosamides: 01		Lincomycine
Phénicoles: 01		Chloramphenicol
Rifamycines: 01		Rifampicine
Polypeptides: 02		Bacitracin, Colistin
Streptogramines: 01		Pristinamycine

dibekacin, ofloxacin, and nitroxolin (Table 1). Thus, the strain was found to be sensitive to a large number of antibiotics in particular the *β* lactams. Sulaimon et al. (2015) reported that a strain of *Micrococcus luteus* isolated from Awotan Dumpsite Leachate in Nigeria had lowest resistance ability to drugs. It was sensitive to gentamycin, streptomycin, amoxicillin, rifampicine, erythromycin, and ciprofloxacin. These results were partially in agreement with our own. Based on the results of other researchers, the high sensitivity of *M. luteus* to *β*-lactam antibiotics may be due to the presence of some reduced set, such as a penicillin-binding proteins and the absence of a *wblC* gene, which plays an important role in the antibiotic resistance in other actinobacteria (Morris et al. 2005).

Study of the effect of heavy metals on the bacterial growth

As shown in Fig. 2, the strain 2YB-25OH exhibited different growth patterns in the presence of 100 µg mL^{-1} of different heavy metals. The shape of the growth curves of the Gram-positive bacteria was relatively close to the control growth curve. However, a decrease in growth (measured in terms of optical density) was observed compared to the control without metal amendment. The lower optical density values obtained with cadmium and cobalt revealed that the bacterial growth was extremely affected by these metal ions in the growth medium. Ranquet et al. (2007) reported that iron–sulfur proteins were greatly affected in cobalt-treated bacteria cells and have demonstrated experimentally that elevated intracellular concentration of cobalt leads to the inactivation of three selected iron-sulfur enzymes. However, cobalt toxicity was attributed to cobalt–thiol group interaction of enzymes. Eventually, some authors have reported that living cells to prevent damage caused by metallic toxics seem to reside in the expression of proteins that export or chelate metals, such as the membrane-bound polypeptide specific for nickel and cobalt resistance induced under cobalt stress and allows cobalt efflux (Rodrigue et al.

2005). The effect of cadmium on the growth of the strain 2YB-25OH may be related to its deleterious effects on cell division, protein synthesis, and pronounced morphological aberrations (Slaveykova et al. 2009). Moreover, the growth was not affected much or very little affected in the presence of zinc, nickel, lead, copper, and chromium. This could be related to the role and degree of toxicity of each metal and the ability to precipitate at different pH (Ahemad 2012). In general, metals to act either as a micronutrient or as a toxicant, they have to be available for uptake by the bacterial cells. The metal species determines the solubility, bioavailability, and membrane transport, besides influencing the phenomenon of adsorption, oxidation/reduction, and exposure times (Worms et al. 2006). Usually, metal speciation is governed by pH of the medium (alkalinity or acidity), presence of Ca/Mg ions, presence of organic matter, redox potential, and salinity (Markich et al. 2001). It is known that metals play a vital role in the metabolic processes of bacteria. Thus, some of them are essential and are required by the microorganisms as micronutrients and are known as trace elements (Rathnayake et al. 2009). They are involved in redox processes to stabilize molecules through electrostatic interactions, as catalysts in enzymatic reactions and regulating the osmotic balance. Thereby, zinc is structurally a vital constituent of several cellular enzymes and resistance to toxic levels of this metal can be due to extracellular accumulation, sequestration by metallothioneins, intracellular physical sequestration, or efflux mechanisms, such as P-type ATPase efflux system and RND-driven transporters system (Choudhury and Srivastava 2001). Though, some other heavy metals have no biological role and are detrimental to the organisms even at very low concentration. Their toxicity occurs through the displacement of essential metals from their native-binding sites or through ligand interactions. Eventually, toxicity can occur as a result of alterations in enzymes specificity and in the conformational structure of the nucleic acids and proteins and interference with oxidative phosphorylation and osmotic balance (Pereira et al. 2012).

Fig. 2 Evolution of the growth of the strain 2YB-25-OH in the presence of metal ions at different pH
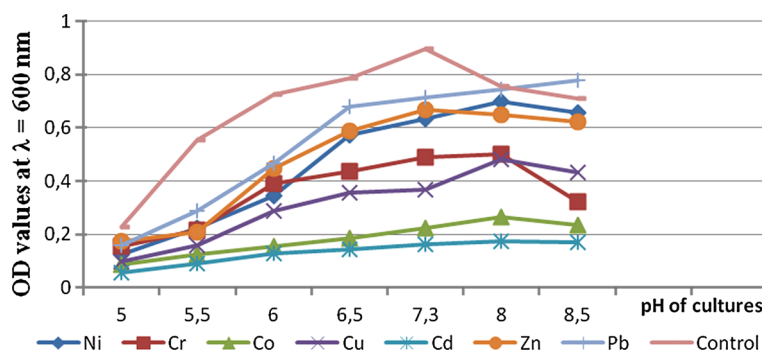
Accumulation of metal ions by living bacterial cells

Bioaccumulation is an active mode of metal uptake by living cells and depends on the metabolic activity of the cells referred to its intrinsic biochemical and structural properties, physiological and/or genetic adaptation, metal specification, availability, and toxicity (Krishna et al. 2012). The capacity of living cells to remove metal ions from culture medium is also significantly influenced by growth conditions, such as temperature, pH, medium composition, and biomass concentrations (Chen and Ting 1995). Though, the results obtained from metals absorption study using living bacterial culture of the strain YB-25OH revealed higher amount of metal ions accumulation at higher pH and the values vary depending on the pH of medium (Table 2).The largest uptake capacity was obtained at pH 6–8.5 for copper, nickel, and zinc with values ranging from 51.45 to 83.90 %, 52.59 to 78.81 %, and 59.55 to 78.90 %, respectively. The maximum rate of metal ions accumulated by the isolate was obtained at pH 7.3. The living cells of the bacterium were able also to uptake 59.81–80.08 % of chromium and 58.09–79.41 % of cobalt at pH between 7.3 and 8.5. However, it has been reported that optimal pH for bioaccumulation of chromium, copper, zinc, and nickel by *Micrococcus* sp. was located at pH 7–7.2 (Congeevaram et al. 2007). Ademola et al. (2013) reported that *Micrococcus luteus* was not only resistant against copper, but also had the extensive capability of accumulating this heavy metal ion. Eventually, it has been reported that *Micrococcus luteus* highly accumulated nickel (Sandrin and Maier 2003). Sujitha and Jayanthi (2014) reported that living cells of *Micrococcus* sp. *were able to accumulate* 38.6 mg L^{-1} of chromium, 38.4 mg L^{-1} of zinc, and 36.3 mg L^{-1} of nickel. These results show that the accumulation amount of metal ions by the *Micrococcus* strain is relatively stable for the three heavy metals and are relatively far from ours. Beside that, some studies reported that bacteria are able to accumulate

and compartmentalize copper in the cell's periplasm and the outer membrane and concluded the protective mechanism against copper due to productions of four proteins to overcome copper stress (Cooksey 1994; Mancini et al. 2015). Adebisi Musbaudeen et al. (2014) reported that *Micrococcus luteus* is not only resistant against copper and nickel, but also had the extensive capability of accumulating these heavy metals. Moreover, bacteria accumulate zinc by a fast but unspecific uptake mechanism and generally coupled with magnesium. Nevertheless, a significant absorption of lead was observed at pH 5.5–8.5 with an amount of 55.28–91.06 %. Furthermore, the bacterial strain showed the lowest activity of cadmium uptake. This indicates that the accumulation of cadmium from nutrient broth media was also influenced by pH values. The maximum cadmium uptake capacity was shown at pH 6.5 (38 %), while pH 8.5 was suppressive. Thereby, it is important to mention that the pH of the broth media plays a major role in the extent of metal binding to microorganisms and greatly affects the degree of potential ligand that contribute to metal binding. Based on the results cited above, the isolate shows a high potential to accumulate heavy metals in particular lead and copper. It was also demonstrated that the species *Micrococcus luteus* has a greater ability to absorb lead Puyen et al. (2012). Furthermore, many researchers have reported on efficiency and mechanisms of bacteria to remove different metal ions, some of their statements could be comparable to this study. Richard et al. (2002) reported that copper and lead seemed to bind to materials on their cells surface. It has also been experimentally demonstrated that lead produced no toxic effect on *Micrococcus luteus* growth, and it was found in the cells wall and membrane. This indicates that lead was precipitated in an insoluble form that is localized to the cells membrane or cells surface (Levinson et al. 1996; Roane and Pepper 1999). This investigation may partly explain our results. Noghabi et al. (2007) reported that Gram-negative bacteria showed higher bioaccumulation capacity

pH	Cadmium	Chromium	Cobalt	Copper	Lead	Nickel	Zinc
5	15.97	20.14	12.78	28.05	42.17	30.94	26.08
5.5	18.10	31.02	18.22	44.07	55.28	33.12	46.03
6	23.01	37.59	31.92	51.45	60.08	52.59	59.55
6.5	38.00	43.01	44.50	54.22	73.06	63.01	66.47
7.3	20.04	59.81	58.09	83.90	78.45	78.81	78.90
8	10.17	66.57	79.41	78.13	89.20	72.57	72.70
8.5	06.05	80.08	62.01	70.52	91.06	67.08	51.03

Table 2 Heavy metals accumulated rates (%) by living bacterial cells at different pHs

to heavy metals than the Gram-positive counter parts due to their higher level of intrinsic metal resistance. This difference was based essentially on the chemical composition of their cell wall (Ahmad et al. 2005).

Study of metal-ions removal capacity of dry bacterial cells (Biosorption)

In general, biosorption is defined as an attribute of the inactive or dead microbial biomass to bind and concentrate metal ions even from highly dilute solutions (Vasudevan et al. 2001).The interactions between heavy metals and functional groups on the cell wall surface of bacterial biomass were confirmed by the Fourier transform infrared spectroscopy analysis, which indicate the possible removal of metal ions from the solution (Uniyal et al. 2013). This mechanism is based on non-enzymatic processes and characterized by the non-specific binding of metal ions to extracellular/cell surface-associated polysaccharides and proteins (Issazadeh et al. 2013). Though, the passive uptake is a rapid and reversible sequestering process of chemical compounds (Shim et al. 2015). Thus, the figures below show that the concentration of metal ions adsorbed by the strain in various metal concentrations decreases with the extension of the incubation time. According to the results obtained, the total bacterial adsorption in 25 $\mu g \ mL^{-1}$ of zinc, chromium, and nickel solutions was very important immediately after inoculation. After 30 min of incubation, the amount of metal ions immobilized and captured by dead cells of the bacterium was also considerable and range from 20.46, 22.5, and 23.98 $\mu g \ mL^{-1}$, respectively. A very slight decrease in the amount adsorbed after 60 min was noted. Beyond the mentioned removal times, zinc, chromium, and nickel concentrations sorbed decrease rapidly. Heavy-metal removal by the isolate in other solutions with higher concentrations 50 and 100 $\mu g \ mL^{-1}$ started just after inoculation and the amount of metal ions adsorbed was relatively larger. The adsorption reached its maximum at 30 min and continued until 60 min of incubation but with a very discreet decrease. Finally, the amount of each metal immobilized in each solution was, respectively, as follows: 38.02 and 90.21 $\mu g \ mL^{-1}$ for zinc, 39.78 and 89.23 $\mu g \ mL^{-1}$ for chromium, and 47.19 and 86.83 $\mu g \ mL^{-1}$ for nickel. These results appear to be linked directly to the pH of the solution, the time of contact, the heavy-metal concentrations, and the biomass. Leung et al. (2000) reported that *Micrococcus luteus* was able to adsorb heavy metals and indicated that the metal biosorption increased with increasing pH from 2 to 6. Likewise, some studies reported that metal biosorption is
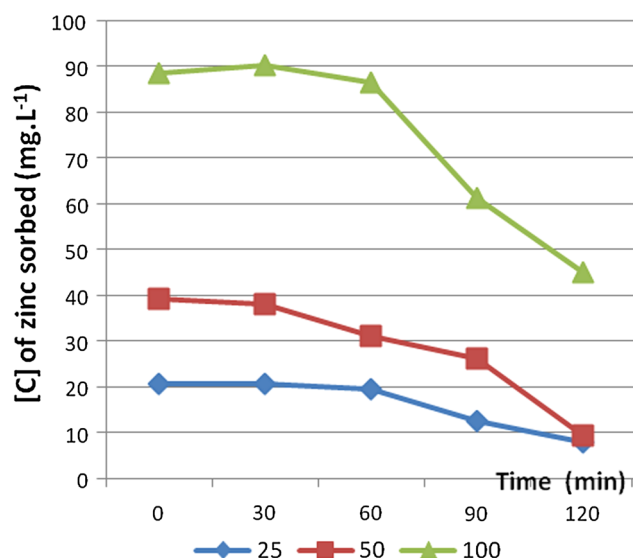

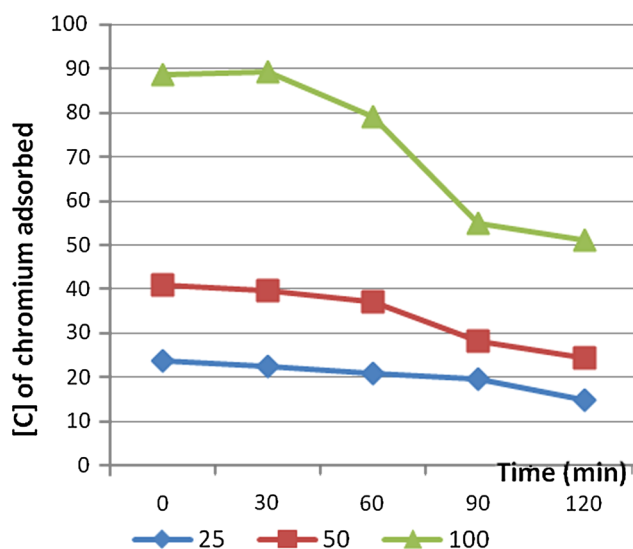**Fig. 3** Biosorption of zinc by dry cells of *Micrococcus luteus*

Fig. 4 Biosorption of chromium by dry cells of *Micrococcus luteus*

strongly pH dependent and demonstrate its effect on the nature of biomass binding sites, the activity of the functional groups in the biomass, the metal solubility, and the competition of metallic ions (Olaniran et al. 2013).Thereby, it is very important to clarify that heavy-metal uptake for most of the biomass types, including bacteria, decline significantly when pH of the metal solutions is decreased from 6.0 to 2.5, but increases when pH increases from 3.0 to 5.0 (Abbas et al. 2014a, b). However,
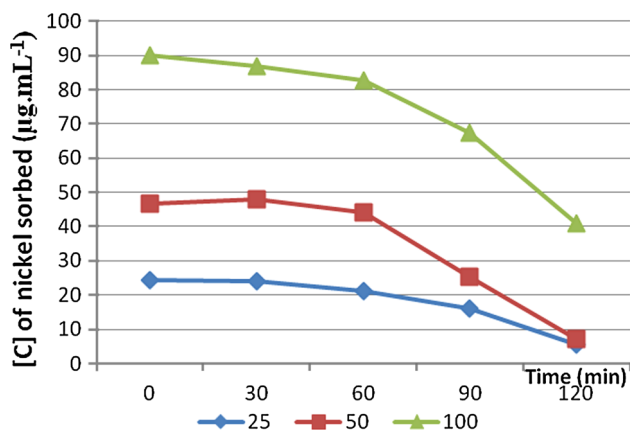
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Fig. 5 Biosorption of nickel by dry cells of *Micrococcus luteus*

the biosorption of nickel, zinc, and chromium is often reduced at low pH values (Deng and Wang, 2012; Singh and Chopra 2014). Moreover, the results obtained between 90 and 120 min of incubation showed a significant amount of metal ions in the medium which could be explained by a reversible reaction, hence desorption of metal ions. Abyar et al. (2012) reported that glycoprotein materials in the cell wall of dead bacteria might be degraded with the extension of the incubation time which leads to the release of the adsorbed metals into the aqueous solution. Furthermore, it should be mentioned that the cell walls of the Gram-positive bacteria attach higher concentrations of metals than that of the Gram-negative bacteria, because of their thickness and anionic character which is mainly due to peptidoglycan, theichoic acid, and theichuronic acids (Vasudevan et al. 2001) (Figs. 3, 4, 5).

Conclusion

The bacterial strain 2YB-25OH isolated from wastewater of Oued EL HARRACH is multi-heavy-metal ions and antibiotics resistant. Its capacity to accumulate copper, nickel, zinc, chromium, cobalt, cadmium, and lead at different pH using living cells is considerable. Furthermore, the biosorption of zinc, chromium, and nickel by dry bacterial cells is also important. In all the cases, non-living cells showed higher activity to remove metal ions than living cells. However, further studies are needed; these results are very promising as a starting point for a potential application of this new strain of *Micrococcus luteus* in bioremediation of industrial effluent, sewage sludge, and industrial wastes.

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