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ISOLATION AND IDENTIFICATION OF CADMIUM -RESISTANT BACTERIA FROM CEMENT PLANT SOIL IN ALGERIA

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ABSTRACT

The bioremediation of metallic pollutants using heavy metal-resistant bacteria is a very important aspect of environmental biotechnology. This biological process leads to the removal of heavy metals from contaminated areas. The first step in bioremediation is the screening of metal-resistant bacteria. In this study, we assessed the soil contamination level of the cement plant by carrying out measurements of certain heavy metals. A total of twelve cadmium-resistant strains were selected and studied among twenty three isolated from the soil of cement plant. The strains showed great resistance to cadmium, minimal inhibitory concentrations (MIC) oscillate between 400 and 1800 $\mu g.mL^{-1}$. The most resistant strain, designated YL-QS1, was selected and the sequencing of the 16S rRNA gene and the phylogenetic analysis revealed that the isolate is identified as *Raoultella ornithinolytica*, with a MIC of 1800 $\mu g.mL^{-1}$The objective of this study was to isolate and identify cadmium-resistant bacteria from contaminated soil, for use in bioremediation.

KEYWORDS: Pollution; Soil; Bacteria; Bioremediation.

INTRODUCTION

Heavy metals contamination due to natural and anthropogenic sources is a global environmental concern. In Algeria, the problem of population growth, urbanization and industrialization has always been ahead of measures to control pollution and environmental degradation, despite the efforts made. The effluents discharged from industries, health establishments, research centers and the exploitation of mineral deposits are most often responsible for the emergence of pollution of the environment by toxic mineral elements such as heavy metals. Cadmium is the most toxic and dangerous metal ion for human health due to its hazardous characteristics and non biodegradability.

Release of heavy metals without prior treatment poses a significant threat to public health. Several chemical technologies are used to remove metal ions such as cadmium, lead, mercury, zinc, copper and chromium. Most of them are ineffective or excessively expensive when the metal concentrations are less than $100mg.L^{-1}$ (Ahluwalia and Goyal, 2007**)**. Due to these constraints, alternative treating techniques such as those using microbial biomass are gaining more serious considerations (Nanda et al., 2011). Although, heavy metal-Resistant bacteria have been demonstrated to exhibit high metal ions accumulation capacity and some of them have been successfully applied in remediating contaminated sites elsewhere in the developed world (Owolabi and Hekeu, 2015).

Hence, the aim of this study was isolation and identification of cadmium-resistant bacteria in cement plant soil and determination of minimum inhibitory concentration (MIC).

MATERIALS AND METHODS

Substrates and Chemicals

Unless specified otherwise, all substrates, chemicals, and reagents were of the analytical grade or highest available purity, and were purchased from Sigma Chemical Co. (St. Louis, MO, USA)

Study Areas and Collection of Soil Samples

Soil samples were taken from a cement plant soil in Bologhine. It was located in an urban area in the north of Algiers and functional since 1914. Soil samples were taken 4-5 cm from the surface in sterile glass flasks for microbiological analysis and polyethylene bottles for measuring heavy metal concentrations. The first samples were transported in a cooler at 4 °C, while the others at ambient temperature.

Physicochemical Properties of Soil Samples

The concentrations of metal-ions in the soil (Cd^{2+}, Pb^{2+}, Zn^{2+}, Cu^{2+}, Ni^{2+}, Cr^{2+}) were measured after acid digestion of 1g of the soil dry weight (DW) in 10 mL of HCl and Supra Pure-concentrated HNO_3 (3:1, v/v; Sigma-Aldrich), using atomic absorption spectrometry (AAS) with a flame or graphite furnace nebulizer (Perkin-Elmer). The accuracy of the mineralization process was assessed using blank samples and samples of standard certified metals. The electrical conductance was determined by conductivity meter (Roane and Kellogg, 1995).

Isolation and Classical Taxonomy Identification of Cadmium-Resistant Bacteria

Five grams of soil were suspended in 45mL of nutrient broth then flasks were incubated at 30 °C for 48 h, with shaking at 100 rpm.

Thereafter, Ten-fold serial dilutions were prepared and an aliquot (1mL) was spread in mass of sterile nutrient agar plates amended With 25µg.mL^{-1} of cadmium. After the incubation period(48 h at 30°C), the distinct colonies obtained were sub-cultured on the same Media for purification. Cadmium resistant Bacteria were identified by conventional biochemical methods according to standard microbiological techniques.

Determination of Minimum Inhibitory Concentration (MIC)

MIC of the cadmium-resistant isolates was determined by gradually increasing the concentration of cadmium 100µg. mL^{-1} each time on nutrient-agar plate until the strains failed to grow on plates even after ten days at 30°C.

RESULTS

Physico-Chemical Properties of Soil Sample

The results obtained from the measurement of metal-ions in the soil showed significant concentrations. The value of cadmium determined was important 3.355mg.Kg^{-1}, exceeding the standards designed by AFNOR. However, lead, nickel, chromium, and copper were present with normal grades (Table 1). The electrical conductivity of this soil was 0.575 ds/m with a pH of 6.828.

Table 1: Concentrations of Heavy Metals Determined in the Soil Collected from the Cement Plant Floor Soil

Metal-Ions Measured	Values Obtained ($Mg.Kg^{-1}$)	AFNOR NF U44-041 ($Mg.Kg^{-1}$)
Zinc	87.50	150 - 300
Chromium	77.90	150
Lead	51.46	100
Nickel	44.68	50
Copper	29.70	100
Cadmium	03.36	02

Isolation, Identification and MIC Determination of Soil Cadmium-Resistant Bacteria

Given that conventional microbiological methods can detect only a small proportion of the microorganisms present, less than 1% of the bacteria can be cultivated (Amann et al. 1995).

A total of twelve cadmium-resistant strains were selected and studied among twenty three isolated from the soil of cement plant. According to the results obtained and summarized in Table 2, Gram-negative bacteria were predominant with only one Gram-positive. The isolates characterized and identified by api 20E, api 20NE and api 50H showed wide varieties of species. They belonged to eight genera, represented by *Aeromonas* (33%), *Pseudomonas* (17%), *Burkholderia* (8.3%), *Pasteurella* (8.3%), *Shigella* (8.3%), *Salmonella* (8.3%), *Raoultella* (8.3%) and *Bacillus* (8.3%). However, these results should be taken with caution because the cultivation methods impose constraints allowing only limited access to biodiversity compared to molecular methods.

The soil isolates showed a very high level of resistance to cadmium with MIC oscillating between 400 and 1800µg.mL^{-1}(Table 2). The species *Pasteurella spp* (YLSS1) and *Pseudomonas fluorescens* (YLTS1) were highly resistant to cadmium with MIC values of the order of 1400 and 1500 µg.mL^{-1} respectively. The isolate YL-QS1 was the most resistant to cadmium with a MIC of 1800µg.mL^{-1}. On the basis of this strong resistance, this bacterium was identified by the 16S rDNA sequencing. Using representative sequences from databases revealed that the isolate YL-QS1 had maximum sequence similarity (98%) with the species *Raoultella ornitinolytica*. In a nutshell, all the results obtained strongly suggested that this isolate should be assigned as *Raoultella ornitinolytica* strain YL-QS1. The morphological characterization on nutrient agar showed that this strain is in the form of medium colonies, white, mucoid, circular with a regular contour. It was distinguished mainly by its capacity to grow at 10°C, thus characterizing the group Klebsiella ornitinolytica (Jordan, 2015).

Table 2: Cadmium-Resistant Bacteria Isolated and Identified with MIC Values

Soil isolates	Similarity rate	MIC (µg.mL^{-1})
Burkholderia cepacia (YL-CS1)	99%	800
Aeromonas hydrophyla /caviae (YL-DS1)	100%	700
Aeromonas hydrophyla /caviae (YL-ES1)	99%	400
Aeromonas hydrophyla /caviae (YL-OS1)	98%	800
Aeromonas salmonicida (YL-US1)	90%	700
Pseudomonas luteola (YL-KS1)	99%	700
Pseudomonas fluorescens (YL-TS1)	98%	1500
Pasteurella spp (YL-SS1)	98%	1400
Shigella spp (YL-XS1)	90%	400
Salmonella spp (YL-WS1)	90%	1100
Bacillus cereus (YL-VS1)	90%	1100
Raoultella spp (YL-QS1)	99%	1800

DISCUSSIONS

The metallic pollution of the soil taken from the Bologhine cement plant could be directly linked to the release of dusts loaded with mineral pollutants including cadmium. These pollutants are most often dispersed in the atmosphere and also fall on the soil and other ecosystems, leading to a general pollution of the environment

As the cement plant has been operational since 1914, the accumulation of pollutants in the soil could be considered as important in particular for cadmium. The impact of heavy metals on the diversity of the bacterial community in the soil and its activities is related to their solubility and bioavailability which depends on a large number of soil parameters (Sharma and Raju, 2013).Thereby, the presence of heavy metals in the soil leads to the disappearance of the most sensitive populations and subsequently to the adaptation of the most resistant populations. Thus, the equilibrium can tilt and the dominances reverse (Turpeinen et al., 2004).

However, the considerable level of resistance to cadmium observed in bacteria studied could be attributed to the important concentration of this metal in this soil (Aboushanab et al., 2007). Resistance to cadmium has been reported for both Gram-positive and Gram-negative bacteria, which is in agreement with our results (Mahler *et al*., 1986). According to Nies, (1999), resistance to this toxic metal in bacteria is based on cadmium efflux. Though, in Gram-negative bacteria, cadmium seems to be detoxified by RND-driven systems, but in Gram-positive bacteria by the P-type ATPases "CadA and CadA-like proteins" (Nies 1995 ; Nikaido, 1996).

The bacterial isolates characterized in this study were mostly Gram-negative, the group that has been often found in metal polluted soils. These results could be linked to the presence of cell wall which confers resistance to a large number of toxic compounds such as heavy metals (khafilzadeh, 2013). Beside that, some studies reported that species of the genera *Pseudomonas, Bacillus, Burkholderia and Aeromonas were* frequently isolated from polluted sites by metal ions due to their high resistance to metallic stress (Joonu et al., 2012; Marzan, 2016).

The genus *Aeromonas* represents the highest proportion of cadmium-resistant bacteria in the soil of cement plant. This preponderance could be linked to the capabilities of its species to develop mechanisms to adapt to stress, and therefore to resist and survive in soil contaminated with heavy metals (Miranda & Castillo, 1998). Some authors reported that members of this genus tolerate up a significant concentrations of heavy metals (Matyar et al., 2009).

Furthermore, the genus *Pseudomonas, Pasteurella, Salmonella, Bacillus and Burkholderia* showed a great potentiality in resistance to cadmium (Singh *et al.*, 2011). It has been reported that the greater ability of these genera to grow in soil which contains numerous heavy metals, may be related to the fact that they contains different resistance functions such as intra and extra-cellular sequestration and efflux systems (Gupta, 2012 ; Bushra, 2016), or many predicted open reading frames involved in stress responses (Pal et al., 2014 ; Gómez-Ramirez et al., 2015).

It has been demonstrated that the species *Burkholderia dabaoshanensis* isolated from the Dabaoshan mining area in Guangdong Province in China tolerate up 2467µg.mL^{-1}of cadmiumand (Zhu et al., 2012).

The species *Raoultella ornithinolytica* isolated from cement plant soil showed the highest level of resistance to cadmium with a MIC value of 1800µg.mL^{-1}.

The reservoir of the genus *Raoultella* is the gastrointestinal tract and upper respiratory, but can inhabit natural environments such as water, soil and plants (Henriques et al, 2006). It is distinguished from other members of the family of *Enterobacteriaceae* by its ability to grow at low temperature and do not produce gas from lactose at 44.5 °C, and differs from the genus *Klebsiella*, by its capacity to use histamine as the only source of carbon in the medium (Sękowska, 2017). *Raoultella ornithinolytica* and *Raoultella planticola* are the most frequently encountered human pathogens among the genus *Raoultella.*Some studies reported that the species *Raoultella planticola* isolated from surface water was both multi-drug and multi-metal resistant. This bacterium displayed resistance to eleven heavy metals (Serkan et al., 2013).

CONCLUSIONS

The resistance of bacteria isolated from the cement plant floor, which is translated by the strong CMI recorded, represents an ecological advantage for bacteria, especially in sites contaminated by heavy metals. This capacity to accumulate cadmium is interesting to exploit in bioremediation. The contaminated soil of the Bologhine cement plant is an ideal ecological niche for resistant cadmium bacteria and a source of bacteria with high bioremediation potential.

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Issue 5: 827-836

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