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Responses of Bacteria to Heavy-Metal-Stress and Its Influence on The Antioxidant Enzymes Activity

Yousra Benghait[1], Mohamed Blaghen[1, 2]

[1]Laboratory of Microbiology, Pharmacology, Biotechnology, and Environment, University Hassan II, Faculty of Sciences Aïn Chock, Km 8 route d'El Jadida, B.P. 5366 Mâarif, Casablanca, Morocco.

[2]Laboratory of Plant Biotechnology, Ecology and Ecosystem Valorization, Faculty of Sciences, University Chouaïb Doukkali, 24000 El Jadida, Morocco.

*Corresponding Author
E-mail: benghaity@gmail.com

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Abstract

The aims of this study, *Bacillus subtilis, Aeromonas salmonicida, Exiguobacterium aurantiacum* and *Burkholderia cepacia* were isolated from Marchica lagoon in Morocco, and studied their resistance toward heavy metals (Cd^{2+}, Cr^{6+}, Ag^{+}, Cu^{2+} and Hg^{2+}) and antibiotics. Effects of the metals on growth kinetics of strains and the antioxidant enzymes activities produced by some strains were evaluated. The minimal inhibitory concentrations (MIC) of the metals at variant concentrations (10-38 mM) were determined in liquid and solid medium. Thus, the obtained results in solid medium were higher than those in liquid medium. Strains were showed an extreme resistance against metals with the MIC values of Cu (9 mM), Cd (6.25 mM) and Cr (2.5 mM). Meanwhile, different concentrations of Cd had stimulated and increased the activity of catalase and superoxide dismutase in *Bacillus subtilis*, whereas the activity of these enzymes decreased when used 2mM of Cd in *Burkholderia cepacia.*

Keywords: Marchica lagoon; Heavy metal resistance; minimal inhibitory concentrations; antibiotics; antioxidant activity.

INTRODUCTION

Environmental pollution is the presence of a pollutant in the environment, which may be mortal or poisonous and will cause harmful to living organisms [1]the biotoxic effects of many of them in human biochemistry are of great concern. Hence, there is the need for proper understanding of the conditions, such as the concentrations and oxidation states, which make them harmful, and how biotoxicity occurs. It is also important to know their sources, leaching processes, chemical conversions and their modes of deposition to pollute the environment, which essentially supports lives. Literaturesources point to the fact that these metals are released into the environment by both natural and nanthropogenic sources, especially mining and industrial activities, and automobile exhausts (for lead. Chemical pollution caused by heavy metals as inorganic contaminants in marine and terrestrial environment is nowadays considered as a major problem. Some of metals, trace elements or essentials such as Cobalt (Co), Copper (Cu), Iron (Fe), Magnesium (Mg), Nickel (Ni) and Zinc (Zn), have useful biological functions and therefore indispensable in weak concentrations [2]. We report the concentrations of 28 PAHs, 15 oxygenated PAHs (OPAHs. The importance and availability of these metals are essential and associated with necessary proteins to their growth and cellular metabolism. Once their concentration threshold is over passed, most of them have nefarious effects on organism. Human activities such as urban and industrial discharges, as well as natural processes are the source of the large quantity of heavy metals found in the environment. These activities are the main factors of environmental contamination by heavy metals potentially toxic such as Arsenic (As), Lead (Pb), Cadmium (Cd), chrome (Cr) and mercury (Hg) [3].

Many conventional methods have been developed to remove toxic metals from the aqueous stream such us precipitation, evaporation, electrochemical treatment, ion exchange, membrane processes, coagulation [4,5]evaporation, electroplating, ion exchange, membrane processes, etc. However, these conventional technologies are providing expensive due to non- regenerable materials used, high cost and generation of toxic sludge. Biosorption is a process which represents a biotechnological innovation as well as a cost effective excellent tool for removing heavy metals from aqueous solutions. It represents a typical technique for using economical alternate biological materials for the purpose. Today, biosorption is one of the main components of environmental and bioresource technology. Application of microorganisms (specifically bacteria, algae, yeasts and fungi. However, these methods have several limitations including unpredictable metal ions removal, high cost, and generation of toxic sludge [6]. These last were conducted to search for alternative methods, including bioremediation by using biological resources such as bacteria, which are highly adapted to nature and their cellular mechanism can tolerate and absorb different types of heavy metals [7–9].

In the excessive presence of heavy metals, bacteria generate physiological stress such as reactive oxygen species (ROS), including hydrogen peroxide (H_2O_2), superoxide radical (O_2^-) and hydroxyl radicals ($HO^{\cdot}$) [10,11]. In order to reduce the oxidative damage, bacteria produce several antioxidant enzymes of which Superoxide dismutase (SOD), Catalase (CAT), peroxidase (POD) and ascorbate peroxidase (APX).

In this study, we report the capacity of strains to respond to heavy-metal-stress and antibiotics. The isolated strains heavy-metals-resistant, could be useful in the field of treatment of water contaminated by heavy metals.

MATERIAL AND METHODS

Microorganism strain

The strains *B. subtilis, A. salmonicida, E. aurantiacum and B. cepacia* were isolated from water and soil from the lagoon of Marchica located in the north-west Mediterranean coast of Morocco. Bacterial identification was performed by biochemical analysis using API 20E and 20NE (Biomeriaux) and the genomic DNA was extracted using *GenElute™ Bacterial Genomic DNA Kit and* ***ABI 3130xl*** *Genetic Analyzer* and the (16S rDNA) was amplified by PCR.

Minimal Inhibitory Concentration (MIC)

MIC in liquid medium

The liquid media non-amended (controls) or amended with different concentrations of metals prepared from stock solutions using a dilution factor of 1/2. Concentrations range of heavy metals was prepared (Table1).

The tubes containing a final volume of 10 ml of nutrient broth and metallic solutions were inoculated each with 100 µl of preculture of one night of each strain. Tubes were inoculated at 37°C for 24h.

MIC in solid medium

Each plate of Trypticase Soy Agar (TSA), were inoculated with 100 µl of suspensions from precultures of one night, supplemented with decreased concentrations of each metal. Therefore was incubated at 37°C for 24h.

Table 1 Concentrations range of heavy metal salts

Heavy metal salts	Metal concentrations
$HgCl_2$ (Hg)	0.002 – 10 mM
$CdCl_2.H_2O$ (Cd)	0.78 – 25 mM
$AgNO_3$ (Ag)	0.313 – 10 mM
$CuSO_4.5H_2O$ (Cu)	4.75 – 38 mM
$K_2Cr_2O_7$ (C	1.25 – 10 mM

Antibiotic susceptibility and resistance

Antibiotic susceptibility test was determined using disc diffusion method. The turbidity of the bacteria suspension has been adjusted to 0.5 Mcfarland. 100ul of each strain was inoculated using sterile swabs. Therefore, antibiotic-impregnated discs were placed onto the medium constituted with Muller-Hinton (MH) agar and incubated at 37°C for 24h. The following antibiotics were tested: Chloramphenicol (30µg), Naldixic Acid (30 µg), Gentamicin (10 µg), Sulfamid (200 µg), Ciprofloxacin (5 µg), Amoxicillin (30 µg), Tremethorpin (5 µg), Tetracycline (30 µg), Vancomycin (30 µg), Penicillin (10 µg), Novobiocin (5 µg), Piperacillin (30 µg), Amikacin (30 µg). The inhibition zone diameters were measured for each antibiotic test. Therefore, strains were classified as resistant (R), Intermediate (I) and Sensible (S).

Growth Kinetics

Growth kinetics of strains in the presence of heavy metals was performed in 250ml Erlenmeyer flasks containing 100 ml of nutrient broth supplemented with 0.2 mM concentration of metal salts (Cd^{2+}, Cr^{6+}, Ag^{+} and Cu^{2+}), were inoculated with 1ml of overnight culture. The incubation was carried out at 37°C in a rotary shaker at 110 rpm. The absorbance was measured at 600 nm using a spectrophotometer.

Effect of pH on bacterial growth

To determine the optimum pH of bacteria in the presence of cadmium, bacterial strains were cultured and adjusted to different values (ranging from 4 to 11) using a solution of HCl (1 M) or NaOH (1 M). The incubation was carried out at 37°C in a rotary shaker at 110 rpm. The absorbance was measured at 600 nm using a spectrophotometer.

Extraction

For oxidative stress monitoring, strains (*B. cepacia and B. subtilis*) were cultured in MH broth in the presence of $CdCl_2$ at different concentrations. After growth, bacterial cells were centrifuged at (8000×g; 10min). Cultures were centrifuged (8000×g; 10min) when they growth. Cells were harvested and washed three times with 20mM Tris Hcl buffer (pH 7.5) and re-centrifuged under the same conditions. Therefore, they were re-suspended in 50mM Tris Hcl containing (1mM Ethylenediaminetetraacetic acid (EDTA), 10mM β mercaptoethanol, 1mM phenylmethylsulfonyl fluoride (PMSF) and 1% (v/v) glycerol). Thereafter, they were disrupted by sonication in the ice bath for 5min. Cellular debris were removed by centrifugation (15000×g; 45min). The obtained supernatant considered as crude enzymatic extract.

Catalse (CAT)

CAT activity was assayed conforming to [12]for example, methanol, ethanol, formic acid, phenols, with the consumption of 1 mol of peroxide (peroxide activity, 7.5 mM of H_2O_2 was added to the reaction mixture containing enzymatic extract and 50 mM of phosphate buffer (pH7). The CAT activity was calculated by measuring the decomposition of H_2O_2 using 240 nm.

Superoxide dismutase (SOD)

SOD activity was assayed conforming to [13] the reaction mixture contains 5 mM EDTA, 2.5 mM $MnCl_2$, 0.27 mM NADH, 3.9 mM 2-mercaptoethanol in 50 mMof phosphate buffer (pH 7) and 50 µl of enzymatic extract. The activity triggered by the addition of 0.2 mM of NADH. Absorbance was measured at 340nm.

Statistical analysis

Data were expressed as mean of standard deviation, the statistical analyses were performed by ANOVA multiple comparison test Tukey's using R STUDIO version R i386 3.5.1 ($p < 0.05$).

Results and discussion

MIC in solid medium

The determination of resistance values for our strains to heavy metals tested highlights the quality of these strains both through the high minimum inhibitory concentration values obtained and the number of metal ions to which they are resistant (Table2). The order of metals toxicity was found to be in order of Hg > Ag > Cd > Cr = Cu.

Obtained MICs values in solid medium were higher than those in liquid medium assuredly, because of diffusion conditions, complexation and bioavailability between the metals and medium. For Cr and Cu were the most tolerated metals by the strains this was due to their low solubility and diffusion and their strong adhesion to the organic components unspecified in the medium by complexation and adsorption, which leads to a decrease in the level of inhibition. [14] Reported that the medium composition has an effect on the availability of metals, for example, the interactions of Cu to the undefined organic components, such as peptone reduce the activity of metal. Cd was less strongly adsorbed than other metals, due to its mobility and more bioavailability. On the other hand Hg, despite its strong complexation and its low diffusion [15,16]or as annual treatments from 1971 through 1974 in amounts totaling from 50 to 800 t/ha. Surface soil samples were obtained from the plots in 1977 and the soil solutions were analyzed for Zn, Cd, Cu, Ni, Pb, Mn, Fe, Ca, Mg, Al, Na, K, PO4, SO., Cl, pH, and organic C. The metal and ligand species in the soil solutions were calculated with the computer program GEOCHEM. Application of sludge increased soil solution concentrations of

Zn, Mn, and Cd, while Cu, Ni, and Pb were present in most solutions at levels below the detection limits of the analytical methods used. Cadmium, zinc, and manganese in the soil solution appeared to exist predominately as the free ion. Complexation by inorganic and organic ligands ranged from 9 to 37%, 3 to 22%, and 3 to 31% of the total soluble Cd, Zn, and Mn, respectively, depending on the model used to represent metal interaction with soluble organic C. Cadmium was complexedwith organic ligands to a greater extent than were zinc or manganese. Sulfate, phosphate, and chloride formed inorganic complexes with Cd, Zn, and Mn, but they constituted < 10% of the total metal present. Speciationof Zn and Cd into free metal ion and complexed forms was essentially the same in the soil solution, regardless of metal concentrations in solution and rate or frequency of sludge application. The solubility and DTPA extractability of Cd were increased to a greater extent by a single application of sludge than by multiple, annual additions.Bacteria were isolated from different naturally polluted environments. Metal-resistant bacteria were selected and minimal inhibitory concentrations of heavy metals (MICs, seemed to be the most toxic metal with a large zone of inhibition around each disc (Fig 1). [16,17]Bacteria were isolated from different naturally polluted environments. Metal-resistant bacteria were selected and minimal inhibitory concentrations of heavy metals (MICs have also reported similar observations the mercury was the most toxic metal with the lowest MIC. The only advantage offered by the test of toxicity in solid media is that the bioavailability of the metals is similar to that in sewage sludge and contaminated soils [16]. Bacteria were isolated from different naturally polluted environments. Metal-resistant bacteria were selected and minimal inhibitory concentrations of heavy metals (MICs, where the ions could be adsorbed on organic matter, iron hydroxide phases, aluminum and manganese [18].

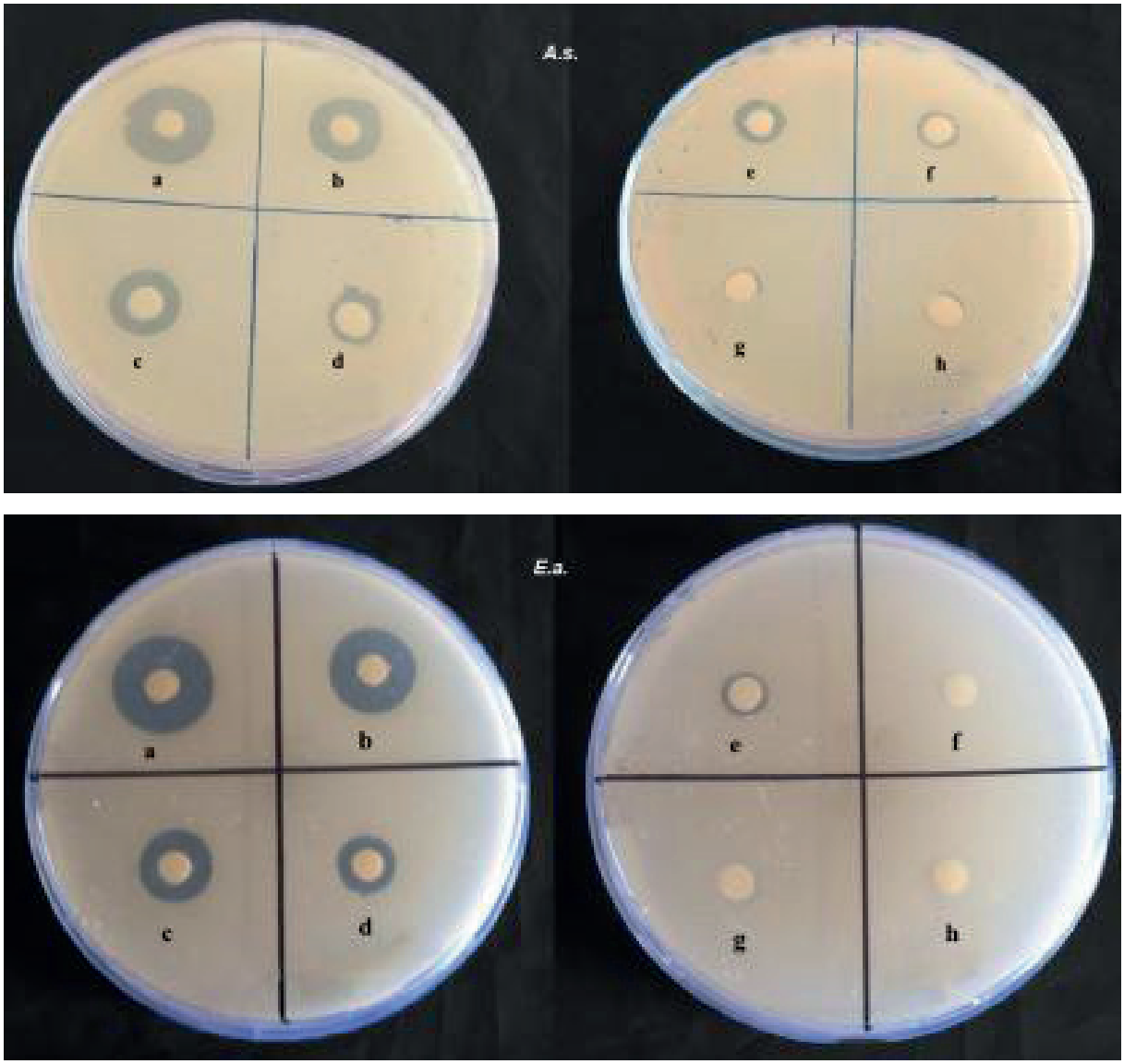


Fig. 1 Effect of $HgCl_2$ on *Aeromonas salmonicida (A.s.)* and *Exiguobacterium aurantiacum (E.a.)* at different concentrations 10, 5, 2.5, 1.25, 0.625, 0.312, 0.156 and 0.078mM.

MIC in liquid medium

According to the results obtained (Table 2), metals toxicity in isolated strains *B. subtilis, A. salmonicida and E. aurantiacum* proved to be Hg > Ag > Cr > Cd > Cu and Hg > Ag > Cd > Cr > Cu for *B. cepacia.*

Cu appeared to be the least toxic metal. Therefore, the concentration 9.5 mM was the most tolerated in *B. subtilis*, *A. salmonicida* and *E. aurantiacum*, while *B. cepacia* was inhibited by the concentration 4.75mM. Cd and Cr showed a moderate effect on all strains, with MIC reached 6.25 mM of $CdCl_2$ on *B. cepacia* and 2.5 mM of $K_2Cr_2O_7$ on all strains. Meanwhile, Ag showed relatively high toxic effect with MIC values from 0.3 to 0.6 mM on *A. salmonicida* and *B. subtilis*. Hg considered to be the most toxic tested metal with a tolerance of 0.156 mM on *B. subtilis.* It was realized that Hg showed toxicity for both Gram-positive and Gram-negative bacteria. The inorganic forms of Hg are more toxic than methylmercury and dimethylmercury. In this sense, the strains were unable to grow at Hg concentrations higher than 0.156 mM.

MICs in liquid media showed a great evaluation of metal toxicity in polluted environments [16]. Bacteria were isolated from different naturally polluted environments. Metal-resistant bacteria were selected and minimal inhibitory concentrations of heavy metals (MICs. Thus, strains were resistant to different tested metals, *B. subtilis* presented the highest resistance, which were comparable with those mentioned previously in the literature [16,19,20]India. The tolerance has even proven to be superior to what was usually found in the literature and the high tolerance of certain metals could be due to continuous exposure of lagoon. The Anthropogenic activities have induced the microbial population to develop a natural resistance to heavy metals. Many publication revealed that the absence of a metal in an environment noticeably reduce the degree of microbial resistance [21,22] by contrast the strains exposed to the heavy metals for a long time will adapt in their environment polluted by developing various resistance mechanisms [23]The pollution of the environment with toxic heavy metals is spreading throughout the world along with industrial progress. Microorganisms and microbial products can be highly efficient bioaccumulators of soluble and particulate forms of metals especially dilute external solutions. Microbe related technologies may provide an alternative or addition to conventional method of metal removal or metal recovery. The present study deals with isolation, identification and characterization of heavy metal resistant bacteria was isolated from sewage water collected in and around Madurai district, South India. Initially, a total of 300 isolates were screened from sewage water. The four isolates were selected based on high level of heavy metal and antibiotic resistances. On the basis of morphological, biochemical, 16S rDNA gene sequencing and phylogeny analysis revealed that, the isolates were authentically identified as Proteus vulgaris (BC1. These mechanisms include the production of siderophores [24–26] and active transport (efflux pumps) [27].

Table 2 Minimum inhibitory concentration (mM) of heavy metals

Strains	Medium	Metals				
		Hg	Cd	Cr	Ag	Cu
B. subtilis	L	0.156	6.25	2.5	0.625	9.5
	S	0.156	12.5	ND	2.5	ND
A. salmonicida	L	0.0195	3.12	2.5	0.313	9.5
	S	0.312	12.5	ND	2.5	ND
B. cepacia	L	0.078	1.56	2.5	0.313	4.5
	S	0.625	12.5	ND	2.5	ND
E. aurantiacum	L	0.039	1.56	2.5	0.313	9.5
	S	0.625	6.25	ND	2.5	ND

L: liquid, S: solid, ND: not determined.

Antibiotic resistance:

Antibiotic resistance test results are presented in (Table 3). These results are obtained by measuring the inhibition diameter. All strains were resistance against all tested antibiotics, except chloramphenicol and amikacin. [28] Have reported that antibiotic resistance was frequently linked with metal resistance due to the transportation of plasmids and transposons encoding which were genetically associated.

Several reports indicate that the mechanisms of antibiotic-resistant by bacteria might be associated with tolerance to metals. The genes responsible for resistance to heavy metals and antibiotics are often genetically linked to the same plasmid (co-resistance), which makes it possible for bacteria under stressed conditions will adapt faster by the spread of resistance factors and natural selection.

Table 3 Results of antibiotic resistance

Strains	Antibiotics													
	SSS	GN	CIP	AMX	NA	TMP	TE	VA	C	P	NV	AM	PIP	
B.subtilis	R		I	R	R	I	R	R	R	S	R	R	S	I
A.salmonicida	R		I	R	R	S	R	I	R	S	R	R	S	I
B. cepacia	I		I	R	R	I	R	R	R	S	R	R	S	I
E. aurantiacum	R		I	R	R	I	R	I	R	S	R	R	S	R

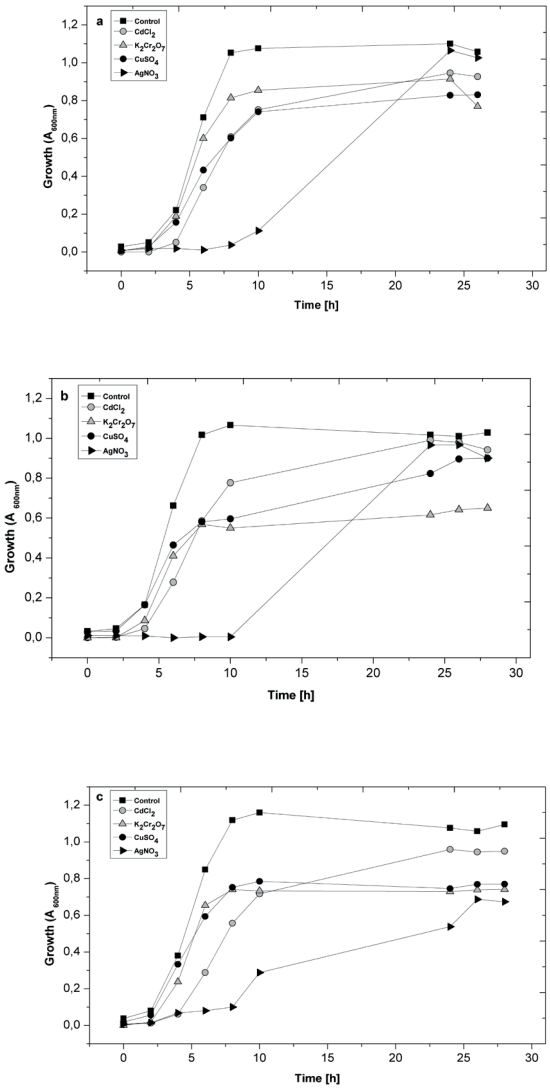
Note: degree of susceptibility: S = sensitive (≥ 21 mm); I = intermediate (16–20 mm); R = resistant (≤ 15 mm).

AMX: Amoxicillin, GN: Gentamicin, TMP: Tremethoprin, SSS: Sulfamid, VA: Vancomycin, C: Chloramphenicol, P: Penicillin, NV:Novobiocin, NA: Nalidixic Acid, PIP: Piperacillin, TE: Tétracyclin, AM: Amikacin.

Growth kinetics

Different isolated strains were grown in the presence of 0.2 mM of $CdCl_2$, $CuSO_4$, $AgNO_3$, and $K_2Cr_2O_7$. Whereas, kinetics varied in function of strains and tested metals. In the presence of silver, the general profile of the growth curves remained the same with lag phases duration (2-10h) followed by whether an important growth or no growth such as *E. aurantiacum*, *A. salmonicida* showed a stationary and decline phase after 24h of growth. Whereas *B. subtilis* showed an early decline phase characterized by a loss of the stationary phase. All strains were able to grow in the presence of copper and chrome, with a short lag phase similar to that of the control. The highest growth rates were observed in *B. subtilis* and *B. cepacia*, these maximum growth rates were in order of (0.085 h^{-1} instead 0.171h^{-1}; 0.078 h^{-1} instead 0,135h^{-1}) in the presence of copper and (0.107h^{-1} instead 0.171h^{-1}; 0.043h^{-1} instead 0.135h^{-1}) in the presence of chromium. After 24 h the decline phase occurred on *B. subtilis*. In the presence of cadmium, the growth curves of bacteria were similar to that of the control with lag phases between (2-4h) and after 24h the stationary phase occurred (Fig 2).

Growth kinetics provided information on the behavior of strains, which highlighted the ability of our strains to survive and grow under different conditions such as the presence of metals. It was difficult to make a precise and meaningful comparison between our results and those in the literature, due to culture medium, temperature and heavy metal concentrations. The most commonly effects mentioned were lag phase duration [20,21,29–31]which was isolated from the rhizosphere of Elsholtzia splendens obtained from the heavy-metal-contaminated soil in the north-central region of the Zhejiang province of China, has been studied for tolerance to copper (Cu, growth rates [32–35]. The use of microorganisms to remove heavy metals from industrial effluent is an area of extensive research and development. Attempts have been made to isolate and characterize metal-resistant microorganisms from treated oil mill industry effluent wastewater samples. The metal-resistant organisms that showed values of minimum inhibitory concentration towards metals (Cd, Cr, Ni and Pb, suggesting inhibition of bacterial metabolism and early decline phase [29,36,37]. The growth kinetics of bacteria is a vital to understanding how bacteria adapt to external constraints. The example that we give is that of the effect of $AgNO_3$ concentration on the growth of strains has inhibited cell proliferation and extended the lag phase. It has been assumed that the duration of the lag phase gives an indication of the rate of adaptation of strains in the presence of inhibitors. Our strains are able to adapt and resume growth after a long lag phase (except *E. aurantiacum*). This may be caused by external stresses on cells, which may require a longer adaptation period, during which they develop biochemical, physiological and/or genetical mechanisms which affect the speciation and mobility of the metal [38,39].



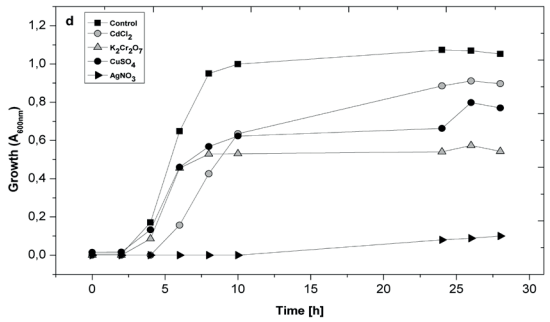


Fig. 2 Growth kinetics of *Bacillus subtilis* (a)*, Aeromonas salmonicida* (b), *Burkholderia cepacia* (c)*,* and *Exiguobacterium aurantiacum* (d) in the presence of Cd, Cr, Cu, Ag and control.

Effect of pH on bacterial growth

The effect of pH on the growth of strains under cadmium stress is shown in (Fig 3). The evaluation of the effect of this physicochemical factor showed that the growth of strains, in the presence of cadmium, to be affected by variations in pH. Indeed, the maximum growth has occurred in a pH range (7-9). However, in an acidic medium (pH 5), was observed less growth. This suggests that the growth of these bacterial strains is affected by acidity in the culture medium. The optimum growth and Cd reduction by *B.subtilis*, *B. cepacia and A. salmonicida* was at pH 9, while that of *E. aurantiacum* was optimal at pH 7.

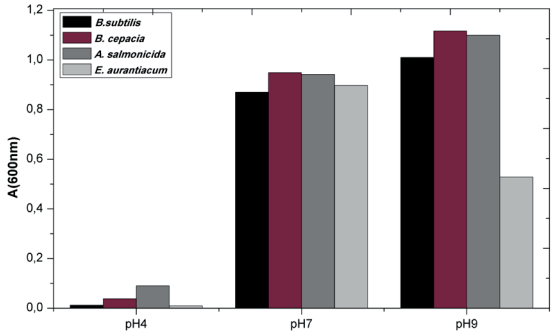


Fig. 3 Effect of pH on growth of *Bacillus subtilis, Burkholderia cepacia, Aeromonas salmonicida* and *Exiguobacterium aurantiacum* with cadmium.

Antioxidant enzyme under cadmium stress

Results showed the presence of catalase activity at different Cd concentration. Nevertheless, this activity was different depending on the strain. In the presence of cadmium, *B. cepacia* showed a progressive increase in catalase activity 10.09 U/mg and 25.53 U/mg for 0.5 mM and 1mM of Cd respectively, compared to control 7,68 U/mg. In contrast, this activity significantly reduced when used 2 mM, it was 14.44 U/mg. This decrease could be due to an excessive intracellular production of H_2O_2, for which catalase activity was inhibited. On *B. subtilis* the catalase activity gradually increased with the increase of metal concentration whereas, superoxide dismutase showed an activity similar to that of catalase (Fig 4). The incubation of *B. subtilis* with 0.5mM of Cd resulted in a slight increase in SOD activity compared to control (0.32 U/mg ; 0.25 U/mg), beyond 0.5 mM it increased progressively and significantly with 0.49 U/mg and 0.71 U/mg for 1 mM and 2 mM of Cd respectively. For *B. cepacia*, the SOD activity increased up to 0.57 U/mg and 0.87 U/mg for 0.5 and 1mM of Cd, whereas it decreased to 0.72 U/mg when used 2mM.

The results of antioxidant enzyme assay were correlated with the MIC of Cd in tested strains (6.25 mM for *B. subtilis* and 1.56 mM for *B. cepacia*), which highlighted the potential role of these antioxidants in bacterial resistance toward Cd.

The first line of defense antioxidant against free radicals or reactive species is the induction of SOD activity. [40] Reported that increased SOD activity was proportionally increased with the increasing of Cd concentration in *B. subtilis.* In our investigation too, the SOD activity was increased with increasing Cd concentration in *B. subtilis*. It increased in *Pseudomonas aeroginosa* after it was exposed to UV-C radiations [41].

Our Strains exhibited CAT and SOD activities, these enzymatic activities increased in the presence of Cd. This result possibly indicated an induction intracellular mechanism of H_2O_2 formation by SOD and the implication of CAT in the reduction. [42]during growth, on the production of biomass, total protein, catalase, glutathione-S transferase, glutathione peroxidase, peroxidase, polyphosphate, acid and alkaline phosphatases, ultrastructure and the ability to remove this metal from Aspergillus niger, obtained from caatinga soil, were evaluated. All parameters tested were influenced by the concentration of metal in the culture medium. The presence of metal induced high levels of antioxidant enzymes, including lipid peroxidation, thereby revealing the appearance of an oxidative stress response. The variation in polyphosphate levels indicates the participation of the polymer in response to stress induced by copper. The activities of the phosphatases were positively influenced by growing them in the presence of copper. Ultrastructure changes in the cell surface, electron density, thickness, and septation were visualized by exposing cells to increasingly larger concentrations of metal. The isolate was able to remove the agent from the growth medium, while maintaining its physiological functions. The metal removed from the cultures exposed to 0.5mM, 1mM and 2mM copper exhibited percentages of removal equivalent to 75.78%, 66.04% and 33.51%. The results indicate that the isolate was able to grow in high concentrations of copper, activates mechanisms for adaptation and tolerance in the presence of metal, and is highly efficient at removing the agent. Such data are fundamental if a better understanding is to be reached of the cellular and molecular abilities of native isolates, which can be used to develop bioprocesses in environmental and industrial areas. Have also observed that in both SOD and CAT activity increased in *A. niger* after it was exposed to $CuSO_4$. Thereafter, higher concentrations of metal salts leaded to overproduction of ROS with great damage, since antioxidant could not protect cellular components, cell dies [13].

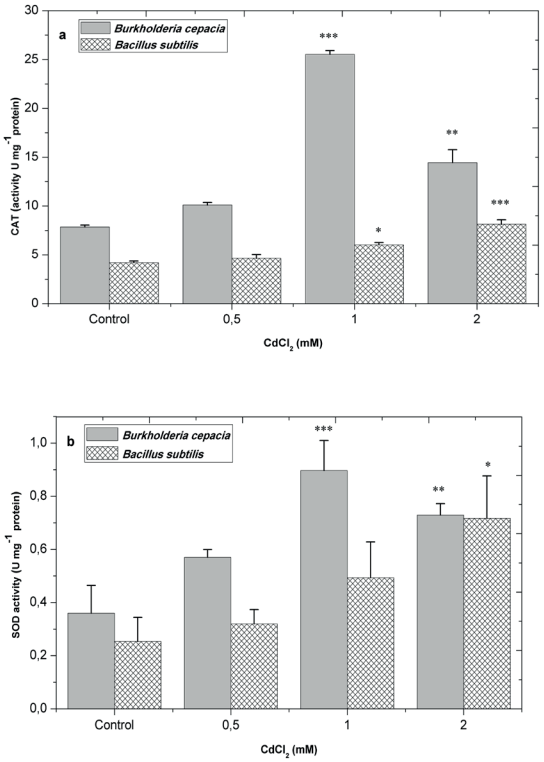


Fig. 4 Effect of different concentration 0.5, 1 and 2mM of $CdCl_2$ on the activity of enzymes CAT (a) and SOD (b) content of *B. subtilis* and *B. cepacia*. Data are expressed as mean ± standard deviation (n = 3).
***Significantly different from the control value at $p < 0.001$
** Significantly different from the control value at $p < 0.01$
* Significantly different from the control value at $p < 0.05$

CONCLUSION

Our paper is mainly focused on the impact of metals on growth and the antioxidant defence mechanism against oxidative stress induced by cadmium. The determination of the minimum inhibitory concentrations showed that bacterial strains are highly resistant to certain heavy metals. These results are in perfect accordance with those obtained in the study of growth kinetics and of antioxidant enzyme. Besides the resistance to heavy metals, strains also exhibit high resistance to certain antibiotics.

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