A Resistance Study of *Pseudomonas aeruginosa* to Heavy Metals

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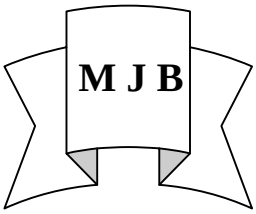

Abstract

The prefect example for microorganisms which resist heavy metals is *Pseudomonas aeruginosa* , it has a good ability to resist and accumulate different metal ions , This article studied the resistance ability of *P. aeruginosa* against different concentrations of the following metals compounds : (HgCl,MgSo4,Zn2O3,MgCO3,Na2SO4,C10H20O,EDTA,NiSO4,CuCl2 and CdCl2), and describing the role of these metals to influence the production of bacterial pigments .

الخلاصة

تعتمد مقاومة المعادن الثقيلة على كفاءة النوع الميكروبي من جهة وعلى أنواع المعادن وتراكيز تلك المعادن في البيئة من جهة أخرى ، ويمكن اعتبار بكتريا *Pseudomonas aeruginosa* المثال الأفضل لمقاومة المعادن في البيئة ، حيث أن هذه البكتريا تمتلك قدرة فريدة على مقاومة عدد كبير من المعادن بما تمتلكه من آليات قادرة بواسطتها من استهلاك تلك المعادن أو تجميعها بشكل غير مؤذي لها أو عن طريق انتاج انزيمات خاصة تقاوم بها تلك المعادن .

بينت هذه الدراسة قدرة تلك البكتريا على مقاومة تراكيز مختلفة من المعادن التالية :
(HgCl,MgSo4,Zn2O3,MgCO3,Na2SO4,C10H20O,EDTA,NiSO4,CuCl2,CdCl2).
كما أظهرت الدراسة أن هناك علاقة بين وجود تلك المعادن و إنتاج البكتريا للصبغات حيث لاحظت الدراسة أن تلك البكتريا تغير من قابلياتها الفسلجية في إنتاجها للصبغات بوجود المعادن الثقيلة في البيئة التي تعيش فيها .

Introduction

Pseudomonas aeruginosa has a very simple nutritional requirements. It possesses the metabolic versatility and often observed "growing in distilled water" which is evidence of its minimal nutritional needs(1). Organic growth factors not required and it can use more than seventy-five organic compounds for growth(2). It is tolerant to a wide variety of physical conditions, including temperature, and resistant to high concentrations of salts, dyes, weak antiseptics, many commonly used antibiotic and most of heavy metals (1).

When the bacteria faces a high concentration of any heavy metal which is accumulated by unspecific system ,the specific heavy metal ion is transported into the cytoplasm in spite of its high concentration , because these unspecific transporters are constitutively expressed .Thus , the gate cannot be closed(2) .

This " open gate " is the first reason why heavy metal ions are toxic to a lot of microorganisms (2). Inside the cell , especially heavy metal cations with high atomic numbers tend to bind to SH groups , e.g. Hg^{+2},Cd^{+2} and Ag^{+}

Other heavy metal cations may interact with physiological ions such as $Cd^{+2}$with Zn^{+2} , Ca^{+2}, Ni^{+2}, Co^{+2} with Fe^{+2} and $Zn^{+2}$with Mg^{+2} , thereby inhibiting the function of the respective physiological cation . Heavy metal cations may bind to glutathione , the resulting bis-glutationocomplexes tend to react wi 1 molecular oxygen to oxidized bis-glutathione GS-GS (3). Since the oxidized bis-glutathione has to be reduced again in a NADPH-dependent reaction and the metal cations immediately catches another two glutathione molecules , heavy metal cations cause a considerable oxidative stress(4) .

All these ways and may be others are the reasons of heavy metals toxification , while In the case of *P. aeruginosa* , this bacteria have three possible mechanisms for a heavy metals resistance system .

Firstly, the accumulation of the specified ion can be diminished , not by interference with uptake but by active extrusion of the heavy metal ion from the cells , this mechanism is specific only *Pseudomonas spp.* (4) .

Secondly , cations especially the "Sulfur lovers" can be segregated in to complex compounds by thiol- containing molecules and then ejected from the cell .

Thirdly , some metal ions may be reduced to a less toxic oxidative state by the complex enzymes and special oxidation mechanisms in the cells .

Finally , for many metals , resistance and homoeostasis is a combination of two or three of the mentioned basic mechanisms that is the case which *P. aeruginosa* success (4).

P. aeruginosa produce an extracellular compound with yellowish green fluorescence, called Pyoverdin, which functions as a byproduct.

The production of **Pyoverdin,** formerly called **fluorescein**, is concomitant with the production of another byproduct, **Pyochelin** (5).

Pseudomonas aeruginosa produce other types of soluble pigments, the blue pigment **pyocyanin** . (1) demonstrated that , the production of pyocyanin pigment abundantly in media of low-iron content

and it have a functions in iron metabolism of bacterium .

Materials and Methods

P. aeruginosa isolated from wastewater in Basrah and diagnosed according to (6). Nutrient agar media used as a stage to growth bacteria with heavy metal. Different of heavy metal concentrations were prepared by dissolving of : HgCl,MgSO4,Zn2O3,MgCO3,C10H20O, EDTA,NiSO4,CuCl2,CdCl2 in deionizer water to have a certain concentrations .

Concentrations of heavy metals :

(0.02M,0.05M,0.1M,0.15M,0.2M) for each heavy metals, prepared by using of molarities value according to (6) .

Demonstration resistant of bacteria to heavy metals:

By using of filter paper disk technique, filter papers saturated with heavy metal solution for 30 minutes (6).

Test the alteration of bacterial ability to produce pigments :

To investigate the role of heavy metal in pigment production from bacteria, 12 tubes were used .

Ten tubes , each tubes have 0.2M of each heavy metals and determinant amount(0.1 ml of *P. aeruginosa* at 18 h.) of bacterial culture, then by spectrophotometer, the color of media was measured after incubation at 18,24,48h. respectively .

a control tube containing bacterial culture with out heavy metals was incubated ,then by spectrophotometer, the color of media was measured after incubation at 18,24,48h. respectively .

A first control tube was contained only nutrient broth .

There are 12 tube classified as a following :

- One tube as a control containing Nutrient broth .
- One tube has a broth culture media of *P. aeruginosa* incubated during 18,24,48 h.
- Ten tubes , each tube has broth culture media of *P. aeruginosa* with one of tenth heavy metals(subject of study)
- Spectrophotometer was used to detect the alteration of pigment production in broth media .

Results and Discussion

1-Bacterial resistance to heavy metals :

Table (1) shows that the bacteria *P. aeruginosa* was resisted most concentration of the heavy metal discs .

In the case of HgCl ,the results were referred to resistance of bacteria to four of fifth of concentrations used in study , the Minimal Inhibitory Concentration(MIC) of HgCl was 0.3 M , and the inhibition zone in the concentration 0.4M was 6 *mm* . In the case of CdCl2 , the (MIC) was 0.2M , and the inhibition zones were 11,7,7 *mm* for 0.2,0.3,0.4 M respectively .

In all other case , the bacteria was appeared a good ability to resist other heavy metals except the concentration 0.4M of CuCl2 was appeared about 7 *mm* as inhibition zone ,we can see all these result in table (1)

From all the obtained results , it was concluded that *P. aeruginosa* have one or more mechanisms to resist these heavy metals . (4) have been demonstrated that the bacteria have three mechanisms for a heavy metal resistance system , these mechanisms were referenced in the introduction.

In the case of Hg^+ ,*P. aeruginosa* is able to reduce Hg^+ to the metal and this metal dose not remain inside the cell with the potential to extrusion of the heavy metal ion from cell according to (3).

The researchers have been demonstrated that the bacteria have an ability to detoxified Hg^+, Mg^+,Zn^+ , Cu^+ by reduction, and the prevent toxic effects of these metal by transported into the cell with specific uptake system (7) .

Most type of bacteria may be inhibited by increasing the concentration of MgSO4 , Na2SO4 , similar results were obtained with, Zn2O3 and NiSO4 because of there toxification with increasing concentrations (8) , but in the case of *P. aeruginosa*, the synthesis of polysaccharide by *P. aeruginosa* may require MgSO4 and Na2SO4 for full expression ,and stimulation of polysaccharide synthesis by MgSO4 and Na2SO4 was not limited in this bacteria, other salts Zn2O3 , NiSO4 have high affinity in the metabolism of cell .(2) referred to importance of these salts in variety of enzymes and DNA-binding protein such Zn^+, and Ni is very important in the CorA system in bacteria

More than one reports referred to a physiological importance of these salts (MgSo4, Zn2O3 , MgCO3 ,NiSO4,CuCl2, Na2SO4) for *P. aeruginosa* and its activities, such these results obtained by (9, 10 , 11 , 2). Accordingly , the results lead to suggest that the metals (C10H20O and EDTA,MgSO4,MgCO3,Zn2O3,NiSO4,Na 2SO4) have no effect on the bacteria , and that mean the bacteria have ability to resist these mater by later mechanisms .

2-The alteration of bacterial ability to produce pigment

By absorption spectra were obtained by using of PERKIN ELMER MODEL 124 spectrophotometer .The absorbance of media contained bacteria with heavy metal was calculated in 600 nm after three incubation times 18 ,24 ,48 h., and compared with control containing broth culture media without heavy metal also after three times . The Table (2) and figure(1) have been reported that these results :

- Some of heavy metals have a good ability to induce bacteria to produce the pigment (green pigment Pyoverdin) such of these heavy metals, NiSO4, MgSO4, MgCO3, Zn2O3, Na2SO4 .- In the Case of NiSO4, when the results were compared with the control (with out heavy metals) ,three time of incubation lead to increase the absorbance values, after 18h. the absorbance was increased from 0.054µm to 0.33µm ,and after 24h.and 48h., the results were increased reaching to 0.4 to 0.45 µm respectively .

According to these results , all metals have a good ability to increase pigment production during incubation times . (12) were referred to the inverse relationship between that two pigments Pyocyanin and Pyoverdin production from *P. aeruginosa* , while (13) was studied the production of Pyocyanin , and showed that the production of pyocyanin pigment was increased in media of low-iron content .But accordingly to the results of study , the Pyoverdin (fluorescent pigment) was increased in production during incubation times , that suggest the relation between this pigment and bacterial metabolism . (3) referred that the presence of HgCl with culture media of *P. aeruginosa* reached to increase bacterial resistance .

In the case of CdCl2 ,the results referred to decrease absorbance value during incubation times ,that is very clear result when we know the concentration 0.2M of CdCl2 have an inhibition effect to bacteria by (11 *mm* as inhibition zone)

An active form of iron – Pyoverdin was studied as a toxic materials more than Iron-free Pyoverdin. These activities ,iron binding, and the stimulation of bacterial iron transport indicated that Pyoverdin can function as a resistance agent for P. aeruginosa . The function of iron-Pyoverdin may be related to the pathogenicity of this bacterium because Pyoverdin stimulated growth not only in iron –efficient culture medium but also in defined medium containing transferring and human serum or plasma .efficiency (13) .

According to the results, when some heavy metal found in media with these bacteria, the production of Pyoverdin pigment was increased and continue increased during the time by bacterial mechanisms for accumulate of these metals ,these mechanisms referenced in introduction by (5 ,1)

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Table (1) : The inhibition zones of heavy metal discs against *P.aeruginosa*

Heavy metal	Concentration of filter paper				
	0.4M	0.3M	0.2M	0.1M	0.04M
$HgCl_2$	6 *mm*	-ve	-ve	-ve	-ve
$MgSo_4$	-ve	-ve	-ve	-ve	-ve
Zn_2O_3	-ve	-ve	-ve	-ve	-ve
$MgCO_3$	-ve	-ve	-ve	-ve	-ve
$C_{10}H_{20}O$	-ve	-ve	-ve	-ve	-ve
EDTA	-ve	-ve	-ve	-ve	-ve
$NiSO_4$	-ve	-ve	-ve	-ve	-ve
$CuCl_2$	7mm	-ve	-ve	-ve	-ve
Na_2SO_4	-ve	-ve	-ve	-ve	-ve
$CdCl_2$	7 *mm*	7 *mm*	11 *mm*	-ve	-ve

Table (2) : The absorbance of pigments in broth media of *P. aeruginosa* with heavy metal at three times .

0.2 M	Value of spectrophotometer		
	18 h.	24 h.	48 h.
HgCl2	0.089	0.09	0.43
MgSO4	0.063	0.12	0.23
Zn2O3	0.10	0.14	0.144
MgCO3	0.21	0.32	0.4
C10H20O	0.35	0.35	0.023
EDTA	0.2	0.26	0.3
NiSO4	0.33	0.40	0.45
CuCl2	0.12	0.089	0.01
Na2SO4	0.26	0.269	0.35
CdCl2	0.05	0.02	0.003
With out heavy metal	0.054	0.176	0.02

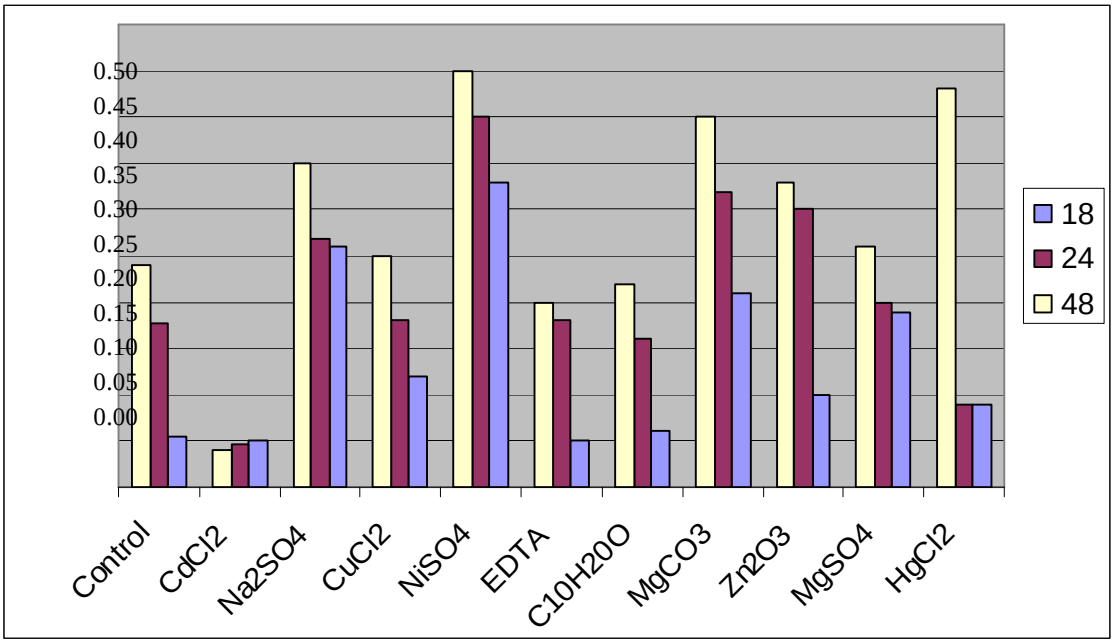

Figure 1 : show the absorbance of pigments in broth media of ***P .aeruginosa***