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Identification and Biochemical Assay of Chromium Resistance Bacteria From Cotton Industry Effluent : Research

Seema Dwivedi
Associate Professor,
Deptt.of School of Biotechnology,
Gautam Buddha University,
Greater Noida

Mohd Nawaz Khan
Student
M. Tech, Biotechnology
Gautam Buddha University
Greater Noida

Abstract

Chromium is a toxoid metalloid which is found in both natural and combined form in a number of ways. The toxic action of Chromium hexavalent form is due to its tendency get incorporated easily into cellular membranes, and cell membrane damages caused by oxidative stress induced by Chromium hexavalent form have been extensively reported, both in eukaryotes and prokaryotes, with effects such as loss of membrane stability or inhibition of the electron transport chain mechanism .The polluted water sample was collected from the Team Krian industrial site , Kasna, Greater Noida. Chromium homogeneous solution was prepared by dissolving the potassium dichromate in 1 ml of eppendorf and LB agar plates were made on which different (increasing order) concentration of chromium and 100µl sample was plated in order for the investigation of chromium, resistance bacteria species colonies which was further streaked and incubated and then different biochemical test like gram staining, catalase test, oxidase test and carbohydrate test were carried out in order to find the chromium resistance bacterial species and its characteristics features.

Keywords: Chromium, Resistance Bacteria, Industrial Effluent, Gram Staining, Catalase Test, Oxidase Test.

Introduction

Chromium is a transition element of group VIA on the periodic table. Elemental form, it is a hard, white, and brittle metal with a high melting point (2000°C). It is a naturally occurring element with atomic number 24. The element belongs to the group of transition metals. It is naturally present in the environment, it is widely found in rocks, animal, plants and soil, and is the 7th most abundant element on Earth's crust. Naturally, chromium is found in the comined state or simply in compound form such as in the form of ores , for example chromite, [1] [2]. Chromium is an important nutrients for the biological development and growth of microorganisms. The trivalent chromium is found in traces and it play a vital role in providing carbohydrate to the mammals and yeasts and maintains an optimal carbohydrate concentration [3].

It has also been reported that the trivalent species of chromium plays a vital role in formation of tertiary structure of proteins and in maintaining conformation of DNA and RNA [4] [5]. The hexavalent chromium species causes different types of health risks to humans such it can cause allergies, irritations, eczema, ulceration, nasal and skin problems, perforation of eardrum, respiratory disorders and lung cancer [6]. Hexavalent chromium when gets aggregated in the placenta it causes severe damage to the foetus [7]. The toxic property of hexavalent chromium is because of its tendency by which it can get easily incorporated in cell membranes and causes damages to its by the phenomenon of oxidative stress and this feature have been noticed both in the case of prokaryotic and eukaryotic species which effects the stability of the membrane potential and also effects the the chain of electron transport [8]. Hexavalent chromium gets incorporated in the cell by the use of transport system (sulfate transport system) and the microorganism have ability to use sulfate [9] [10] [11].Hexavalent chromium induces into cells, chemical reactions occurs with the intracellular reducing agents such as glutathione which produces intermediates having short life span [12] [13] [14].Chromium pentavalent is converted to chromium hexavalent with the help of oxidation reaction in the cytoplasm and it also generates some ROS(reactive oxygen species)which get bound to the DNA and protein complex. Chromium have capability to get associated to the several cellular material which changes its physical functions [15] [16]. The products that are formed by the reaction of

chromium hexavalent causes damages to cell organelles, proteins and nucleic acids [20]. Chromium hexavalent is a hazardous chemical form for biological systems as it can cause mutation, cancer and teratogenic problems. Chromium hexavalent have tendency to cause oxidative stress in cells, which damages its DNA [17] [18]. The dangerous effects of chromium hexavalent can cause life threatening risks to human health and so considering it the hexavalent chromium species has been categorized in the class A pollutant list by US Environmental Protection Agency (USEPA) [19] [20] [21] [22] [23] [24] [25].

Materials and Methods

Effluent Sample

The effluent sample was collected from the cotton textile industrial site TEAM KRIAN, D-11-16, EPIP, Kasna, Greater Noida for the identification of chromium resistance bacterial species.

Requirements

Sample, LB Agar, 70% ethanol, Potassium Dichromate, Hydrogen Peroxide solution, Gordon Mcleod reagent – Oxidase test, Himedia Carbohydrate kit, Himedia Gram stain kit, Petri plates, Slides, Laminar Air Flow, Incubator, Microscope, Vortex, Heating plate, Spectrophotometer, Pipettes, Autoclaved water, Autoclaved tips, BOD bottles, Conical flasks, Distilled water, Whatman filter paper, Parafilm , Gloves

Preparation of Potassium Dichromate Solution and Chromium Plates

1. 0.015 grams of potassium dichromate was taken in eppendorf and 1ml of autoclaved water was added to it and a orange color solution was formed which was further vortex for 5-10 minutes to obtain a homogeneous solution.

Fig -5 Potassium Dichromate Solution

2. 4 grams of LB agar was weighed and dissolved in 100 ml of distilled water in conical flask and was autoclaved.
3. The LB agar was kept on heating plate for 5-10 minutes to revive it into solution state.
4. 50µg/ml chromium concentration was taken for the 100ml LB agar and the V_1 was simply calculated by the formula $C_1V_1 = C_2V_2$ (Stock - 15 µg/ml).
5. 333 µl (50µg/ml chromium concentration) of chromium was added to the conical flask and four petri plates were prepared containing 25 ml solution.
6. 100 µl of cotton industry sample was poured to each petri plates.
7. The plates were incubated at 37^0 C in incubator for 12-16 hours.
8. The colonies were observed.
9. Similarly the same procedure was followed for 100µg/ml – 0.8 mg/ml chromium concentration (Stock - 15 µg/ml) and the incubation was carried out at 37^0 C in incubator for 12-16 hours and the colonies was observed and fewer colonies was reported at higher chromium concentration and the concentration at which the colonies got diminished it was due to the excess chromium toxicity , so the concentration before the chromium toxicity was taken because it contains some chromium resistant colonies.
10. The chromium resistant colonies were obtained at 0.8mg/ml chromium concentration.
11. The above resistant colonies were further streaked on LB agar plate and kept for 12 – 16 hrs incubation for ambient growth.

Biochemical Assay of Chromium Ressitant Colonies

Gram Staining

Himedia Gram Stain Kit Was Used.

Procedure

1. Dropped a drop of water on the slide to which chromium resistance colonies wad added and the smear was prepared by the heat fixation process.
2. Crystal violet was gently poured on the slide and it was left for 1minutes.
3. The slide was tilted at a certain angle and was washed with distilled water gently.
4. The slide was now gently flooded with decolorizing agent such as acetone or alcohol and was left for 15-20 seconds.
5. The slide was now counter stained with safranin and was left for 30 seconds to 1 minute.
6. The slide was gently washed with tap or distilled water and the slide was dried with absorbent paper.
7. The slide was viewed with the help of microscope.

Catalase Test

$$2H_2O_2 \xrightarrow{\textbf{Catalase}} 2H_2O + O_2$$ (Formation of gas bubbles)

Procedure

1. Sterile inoculating loop was used to transfer the chromium resistant bacterial colony from the chromium plates to the slide.
2. A drop of H_2O_2 was poured on the slide and was observed for the formation of bubbles.

Oxidase Test

Procedure

1. A drop of Gordon Mcleod reagent was poured on the whatman filter paper.
2. Sterile loop was used to transfer the chromium resistant bacterial colony from the chromium plate to whatman filter paper and was observed for bluish or purple color.

Carbohydrate Test

Kit Contents

KB009- Part A

1. Lactose,
2. Xylose,
3. Maltose,

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4. Fructose,
5. Dextrose,
6. Galactose,
7. Raffinose,
8. Trehalose,
9. Melibiose,
10. Sucrose,
11. L- Arabinose,
12. Mannose.

KB009- Part B

1. Inulin,
2. Sodium Gluconate,
3. Glycerol,
4. Salicin,
5. Dulcitol,
6. Inositol,
7. Sorbitol,
8. Mannitol,
9. Adonitol,
10. Arabitol,
11. Erythritol,
12. α methyl- D- glucoside.
13. The numbers on the kit indicates the different sugar.

Procedure

1. A single chromium resistance colony was isolated from the chromium plates and was inoculated in the 4 ml of LB broth.
2. The incubation was carried out at 37 ^{0} C for 4 - 5 hours till the inoculum O.D reached approximately 0.5 at 620nm.
3. The kit was opened under aseptic conditions and the sealing foil was removed.
4. The numbered wells were inoculated with 50µl of above inoculum both Part A and Part B.
5. The Part A and Part B kit was incubated at 37^{0}C for 18 – 24 hours.

Results & Discussion

1. Incubation Results of Chromium Plates

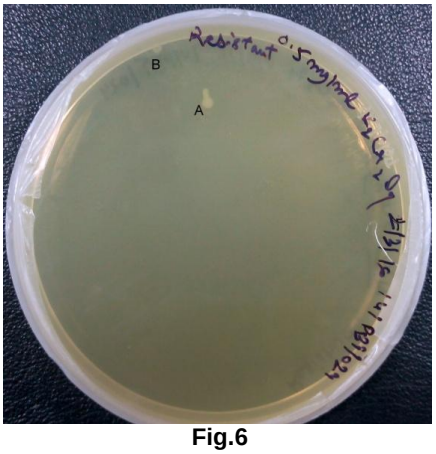

Fig.6

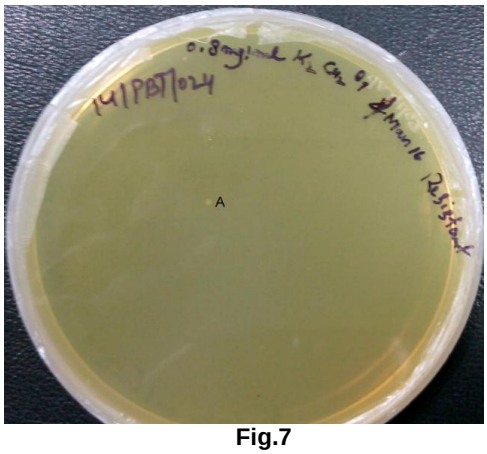

Fig.7

Fig.6 - A & B representing total 3 bacterial colonies resistant to chromium after 12 - 16 hours of incubation at 37^0 C and concentration of chromium was 0.5mg/ml. Fig.7 - A representing only single bacterial colony which is highly resistant to chromium after 12 - 16 hours of incubation 37^0 C and concentration of chromium was 0.8mg/ml and which was further streaked for experimental analysis

2. Streaked Plates

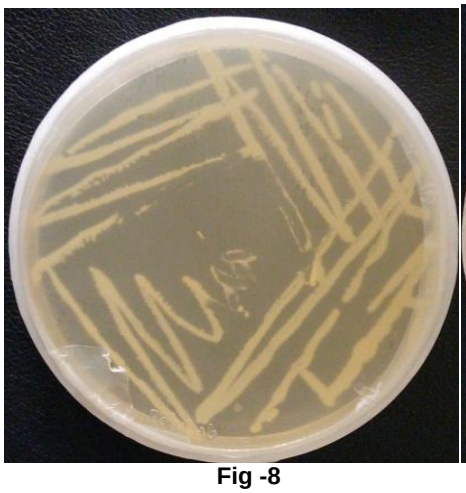

Fig -8

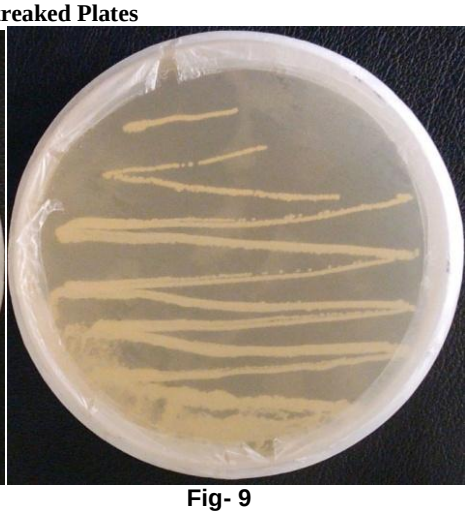

Fig- 9

Fig. 8 and Fig.9 representing the streaked chromium resistant bacterial colony Concentration of chromium was 0.8mg/ml.

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Gram Staining Results

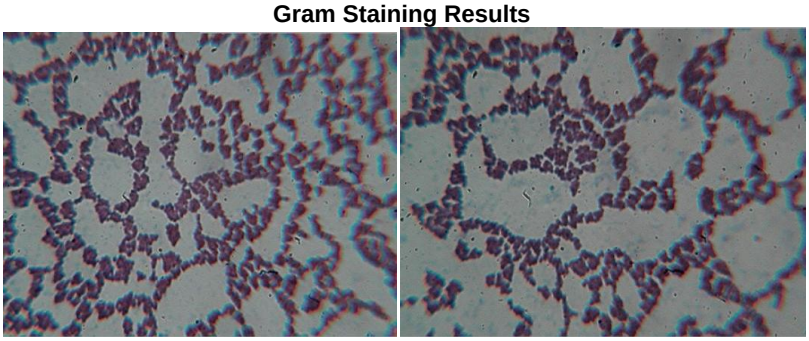

Fig.8 **Fig.9**

Fig.8 & Fig.9 representing gram positive bacteria as the cell wall stains purple and coccus shaped and grape like structure was reported so the bacteria was Staphylococcus which was resistant to chromium

3.Catalase Result

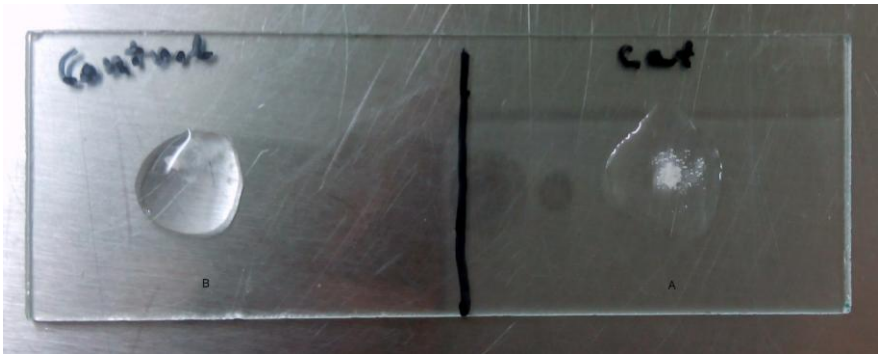

Fig.10 - B represents control and A represents catalase positive test as immediate bubble formation occured, so the staphylococcus was catalase positive and could be aerobe or facultative aerobe

Oxidase Result

Fig. 11 Oxidase result for the chromium resistant colony (concentration 0.8 mg/ml) was negative as at A region there is no bluish color formation so staphylococcus is oxidase negative.

Hi Media Carbo Kit Result

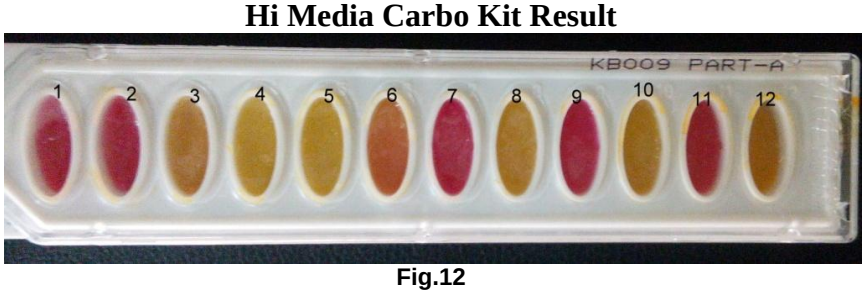

Fig.12

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Fig.13

Fig.12 and Fig.13 represents Part A and Part B Himedia kit and the numbers indicates different sugars.

Pink/orange/Red

Represents negative carbohydrate utilization

Yellow/Dark Yellow

Represents positive carbohydrate utilization

KB009- Part A

1. Lactose,
2. Xylose,
3. Maltose,
4. Fructose,
5. Dextrose,
6. Galactose,
7. Raffinose,
8. Trehalose,
9. Melibiose,
10. Sucrose,
11. L- Arabinose,
12. Mannose.

12 KB009- Part B

1. Inulin,
2. Sodium Gluconate,
3. Glycerol,
4. Salicin,
5. Dulcitol,
6. Inositol,
7. Sorbitol,
8. Mannitol,
9. Adonitol,
10. Arabitol,
11. Erythritol,
12. α methyl- D- glucoside.

Table Shows The Carbhohydrate Utilization Test Results

S.no	Sugar test	Positive result (+)	Negative Result (-)
1	Lactose		**-**
2	Xylose		**-**
3	Maltose		**-**
4	Fructose	**+**	
5	Dextrose	**+**	
6	Galactose		**-**
7	Raffinose		**-**
8	Trehalose		**-**
9	Melibiose		**-**
10	Sucrose	**+**	
11	Arabinose		**-**
12	Mannose		**-**
13	Inulin		**-**
14	Sodium Gluconate		**-**
15	Glycerol	**+**	
16	Salicin		**-**
17	Dulcitol		**-**
18	Inositol		**-**
19	Sorbitol		**-**
20	Mannitol	**+**	
21	Adonitol		**-**
22	Arabitol		**-**
23	Erythtritol		**-**
24	α methyl- D-glucoside		**-**

Discussion

The results of the gram staining , catalase test and oxidase test confirmed that the chromium resistant bacteria was staphylococcus but to know the species name we matched the details of carbohydrate test of sugars like mannitol, sucrose, lactose, arabinose, raffinose, trehalose, maltose (main sugars for the bacterial species identification) with the technical details of Hi media Staph kit. The results of the above mentioned sugars where cross checked with 48 bacterial species whose information was given in the Hi Staph kit KB004 (Fig – 14) and finally found the 100% match with the *Staphylococcus capitis* .

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So the Chromium resistance bacterium which was found in the cotton industrial effluent was Staphylococcus capitis.

Identification Index of various *Staphylococcus* species

Tests	Voges Proskauer's	Alkaline phosphatase	ONPG	Urease	Arginine utilization	Mannitol	Sucrose	Lactose	Arabinose	Raffinose	Trehalose	Maltose
S. aureus subsp. *aureus*	+	+	-	+W	+W	+	+	+	-	-	+	+
S. epidermidis	+	+	-	+	+W	-	+	V	-	-	-	+
S. haemolyticus	V	-	-	-	+	V	+	V	-	-	+	+
S. lugdunensis	+	-	-	V	-	-	+	+	-	-	+	+
S. saprophyticus	+	-	V	+	-W	V	+	V	-	-	+	+
S. schleiferi subsp. *coagulans*	+	+	nd	+	+	V	V	V	-	-	-	-
S. schleiferi subsp. *schleiferi*	+	+	V	-	+	-	-	-	-	-	V	-
S. arlettae	-	+	V	-	-	+	+	+	+	+	+	+
S. auricularis	V	-	V	-	V	-	V	-	-	-	+	+
S. capitis subsp. *capitis*	V	-	-	-	V	+	+	-	-	-	-	-
S. capitis subsp. *ureolyticus*	V	-	-	+	+	+	+	V	-	-	-	+
S. caprae	+	+	-	+	+	V	-	+	-	-	+	V
S. cohnii subsp. *cohnii*	V	-	-	-	-	V	-	-	-	-	+	V
S. cohnii subsp. *urealyticum*	V	+W	+	+	-W	V	-	+	-	-	+	V
S. hominis	V	-	-	+	V	-	+	V	-	-	+	V
S. pasteuri	V	-	-	+	V	V	+	V	-	-	+	V
S. simulans	-W	+	+	+	+	+	-	-	-	-	V	-W
S. warneri	+	-	-	+	V	V	+	+	-	-	+	+
S. xylosus	V	V	+	+	-	V	+	V	-	-	+	+
S. casseolyticus	-	-	-	-	V	-	V	+	-	nd	V	+
S. carnosus	+	+	+	-	+	+	-	V	-	-	V	-
S. chromogenes	-	+	-	V	+	+	+	+	-	-	+	V
S. delphini	-	-	nd	+	+	+	+	+	-	nd	-	+
S. equorum	-	+	V	+	-	+	+	V	+	-	+	V
S. felis	-	+	+	+	+	V	V	+	-	-	+	-
S. gallinarum	-	+	-W	+	-	+	+	V	+	+	+	+
S. hyicus	-	+	-	V	+	-	+	+	-	-	+	-
S. intermedius	-	+	V	+	V	V	+	V	-	-	+	+
S. kloosii	V	+	V	V	-	+	-	V	V	-W	+	V
S. lentus	-	+W	-	-	-	+	+	V	V	+	+	V
S. muscae	-	+	-	-	-	-	+	-	-	-	+	-
S. piscifermentans	-	+	V	+	+	V	V	V	-	-	+	V
S. sciuri	-	+W	-	-	-	+	+	-W	V	-	+	V
S. vitulus	-	-	-	-	-	+	+	-	-	-	V	-
S. hominis subsp. *novobiosepticus*	V	-	-	+	-	-	+	V	-	-	-	+
S. saprophyticus subsp. *bovis*	V	-	V	+	-	+	+	-	-	-	+	+
S. succinus	-	+	nd	+	-	nd	nd	+	nd	V	+	nd
S. carnosus subsp. *utilis*	nd	-	-	-	+	-	-	-	-	-	V	-
S. condimenti	nd	+	+	+	+	+	+W	+	-	-	-	-
S. lutrae	-	+	+	+	-	V	nd	+	nd	nd	+	-
S. sciuri subsp. *carnaticus*	-	V	-	-	-	+	+	V	V	-	+	V
S. sciuri subsp. *rodentium*	-	V	-	-	-	+	+	-	V	-	+	V
S. fleurettii	V	V	-	-	-	nd	+	-	V	-	+	+

Fig -14 Hi Staph Kit technical detail , Himedia KB004
Source : himedialabs.com/TD/KB004.pdf

Conclusion

Result of the present investigation reveals the chromium toxicity and bacterial species tolerant property in the cotton industry effluent. Various concentration of chromium plates were made and colonies resistant to chromium was observed and at 0.8 mg/ml chromium concentration only single colony was found whose further experimental analysis was done by gram staining, catalase test, oxidase test, Hi media carbohydrate test kit and finally came up with the conclusion that the bacterial species which was tolerant to the chromium in cotton industry effluent was Staphylococcus capitis.

The future prospects of the present research work could be used in making a sensor. The identified bacterial species protein could be isolated and research work could be conducted in immobilizing the protein on a material which is non reactive to this protein and for this one has to understand the reaction mechanism of this protein with different materials and a chromium responding fluorescence dye could be mixed with the protein and then finally it could be immobilized on a non reactive material as soon as this sensor would be dipped in the effluent the material would fluorescence and the chromium concentration could be measured by the MS spectra.

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