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ISOLATION AND BIOCHEMICAL CHARACTERIZATION OF CHROME RESISTANT BACTERIA FROM SOIL SAMPLES OF MUSLIM BAGH MINES

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Abstract:

In the biosphere, chromium is widespread heavy metal occurring in trivalent chromium (Cr^{3+}) and hexavalent chromium (Cr^{6+}) forms. Trivalent chromium has been extensively used in different industries. Hexavalent chromium is an environmental pollutant, toxic, carcinogenic and mutagenic metal whereas trivalent form is less soluble in water and even an essential nutrient. The distinctive capability of different isolated bacterial species, to reduce hexavalent chromium into trivalent form is going to be used in bioremediation. The present study was aimed to isolate and identify chromium resistant bacteria species from chromium mines. Random samples (n= 3) were taken and biochemical test were applied to identify chromium resistant bacterial species. Four bacterial species, belonged to genus Bacillus (iso-four, seven, eight, nine) were identified as B.pumilus, B.circulans, B.subtilis and B.sphaericus respectively. Isolates belonged to genus enterobacter (iso-one and iso-five) were identified as salmonella enteric and E.arogenes respectively. Isolate belonged to genus arthrobacter (Iso-three) was identified as A. creatinolyticus. Isolate belonged to genus pseudomonas (iso-two) was identified as p.fluorescene. Isolate belonged to genus staphylococcus; (iso-six) was identified as S.aureus, while iso-ten belonged to micrococcusluteus. All the isolates showed ability to tolerate chromium but iso-eight B.subtilis showed highest resistance against chromium. Furthermore, these bacterial species alter hexavalent Cr into trivalent Cr. This ability is being used on commercial scale to remove or minimize chromium pollutants.

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1. INTRODUCTION:

High level of all metal concentrations in the environment may have negative impacts because they can be stored in many foods and food chains [1-2, 27].Chromium is one of the most common heavy metal in the environment [14]. The range of average concentrations of chromium differs from 7 to 150 ppm and the type of the soil's mother rock affects its content and distribution in the soil [23]. Chromium is one of the highest poisonous heavy metals settled into the environment through many industrial practices has been reported as a severe health issue [9]. Hexavalent chromium has highest oxidative state with very stable oxidizing characteristics [6]. Hexavalent form is more toxic than trivalent. The hexavalent chromium is considered as severe pollutant whereas trivalent chromium does not possess serious concerns [19]. The contamination of soil by heavy metals is often permanent and may kill or reduce the microbial populations as well as the exposure to metals lead to the establishment of a resistant microbial population [25].

In a variety of industrial applications, common use of hexavalent chromium to clean up contaminated area in bioremediation, which uses the metabolic potential of microorganisms to eliminate poisonous metals [8]. Studies have been done to evaluate the transformation of hexavalent chromium bacterial species in to trivalent chromium bacterial species. Many bacteria have ability to transformCr^{6+}, into less mobile and less toxic formCr^{3+}, [5, 9, 15].

2.MATERIALS AND METHODS:

The soil samples were collected (0-15cm depth) from different locations of chromite mines of Muslim bagh district Kila Saifullah using aseptic techniques in sterilized jars. Soil samples were kept at4^{o}Cuntil examined. Soil samples were diluted, spread plate method and streak plate method were used for culturing, and isolated bacterial colonies were observed on the bases of the colony morphology on agar. The standards for colony morphology of isolated bacterial colonies were based on colony form, edge, surface, elevation and colony color. For identification of chromium resistant bacterial strains, a chromium stressed media was prepared [26]. The progress of the isolate under diverse chromium concentrations were measured after 24 hours of incubation at 37^{o}C at incubator (Binder, Germany). Morphologically different colonies of isolates were characterized by using standard bacteriological techniques as gram staining and spore staining. Biochemical tests as catalase, eosin methylene blue, oxidase test, citrate test, MacConkey agar test and sulphate indole motility test were performed.

3. RESULTS:

Among processed samples, ten isolates were selected and further purified on the basis of colony morphologies. These isolates were labeled from iso-one to iso-ten. These isolates were observed and analyzed for staining and biochemical analysis. Pure isolates were selected on the basis of five colony morphological features as mentioned in Cappuccino and Sherman (2005) laboratory manual. These five morphological features were whole colony, edge of colony, colony surface, elevation, color of colony in Table 1.

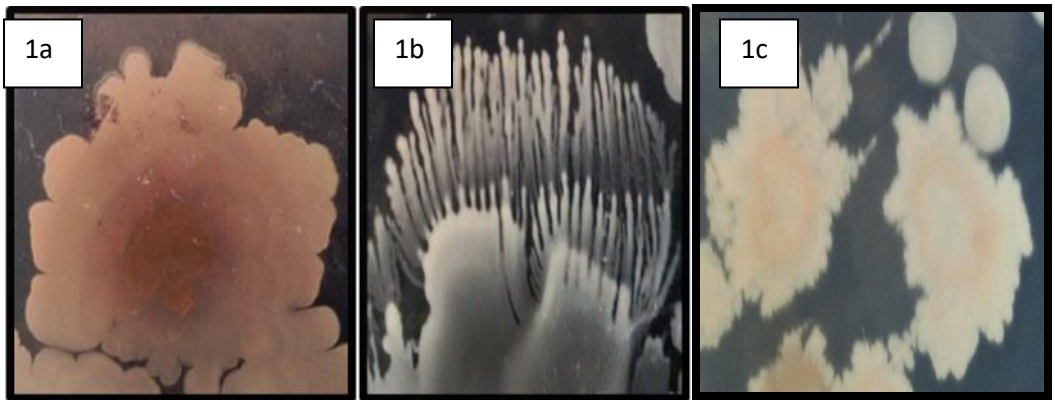
Table 1: Colony Morphology

Isolates	Whole Colony	Edge	Surface	Elevation	Colony Colour
Iso-1	Irregular	Undulate	Wrinkled	Umbonate	Light pink
Iso-2	Filamentous	Filamentous	Smooth	Umbonate	White
Iso-3	Irregular	Lobate	Wrinkled	Umbonate	Light pink
Iso-4	Circular	Entire	Glistening	Pulvinate	Light pink
Iso- 5	Rhizoids	Smooth	Smooth	Umbonate	Cream white
Iso -6	Circular	Entire	Smooth	Pulvinate	Pink
Iso -7	Rhizoids	Undulate	Smooth	Umbonate	Cream white
Iso -8	Rhizoids	Lobate	Smooth	Umbonate	Off white
Iso -10	Irregular	Undulate	Smooth	Umbonate	White

Bacteria were classified by direct examination with the light microscope (Leica). From the isolates under study, iso-one, two, three, five, six and ten were round (cocci) in shape shown in Fig.1a, b, c, d, e and f among which iso-six was in cluster form (staphylococcus) and remaining were in pair form (diplococci), while iso-four, seven, eight, nine were (bacilli) rod shaped and among which iso-nine was in chain form (*Streptobacilli*) shown in Fig. 2g, h, I and j [Table 2].It was found that six isolates were gram negative and four were gram positive. The gram negative isolates were iso-one, two, three, five, six, ten, while gram positive isolates were iso-four, seven, eight and nine shown in Table 3. In spore staining it was found that all isolates were spore forming shown in Table 4. Among samples, 90% showed catalase positive.50% showed eosin methylene blue positive. Citrate utilization test was performed on all the isolates and were observed for growth of bacterial colony and also the color change, 10% showed positive. All isolates were observed to be negative for oxidase, 60% isolates showed negative result for MacConkey test in Table 5. 90% showed sulphur positive result while 10% were negative, 80% isolates showed indole negative and 20% isolates showed positive as well as for motility test 70% isolates showed positive result. The observed resistance against chromium is as, iso-one *salmonella enterica* 10%, iso-two *p.fluorescen* 15%, iso-three *A.creatinolyticus* 15%, iso-four *B.pumilus* 20%, iso-five *E.arogenes* 15%, iso-six *S.aureus* 10%, iso-seven *B.circulans* 30%, iso-eight *B.subtilis* 100%, iso-nine *B.sphaericus* 0% and iso-ten *micrococcus luteus* 5% in Fig.3

Table 2: Bacterial Morphology

Isolates	Shape	Arrangement
Iso-1	Round	*Diplococcic*
Iso-2	Long Round	*Coccobacilli*
Iso-3	Round	*Diplococcic*
Iso-4	Rod	*Bacilli*
Iso-5	Round	*Cocci*
Iso-6	Round	*Staphylococcus*
Iso-7	Rod	*Bacilli*
Iso-8	Rod	*Bacilli*
Iso-9	Rod	*Streptobacilli*
Iso-10	Round chain	*Cocci*



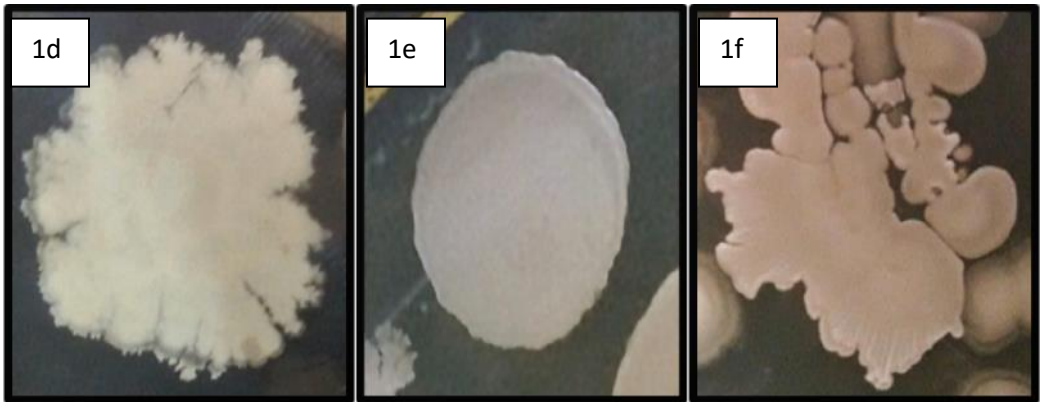
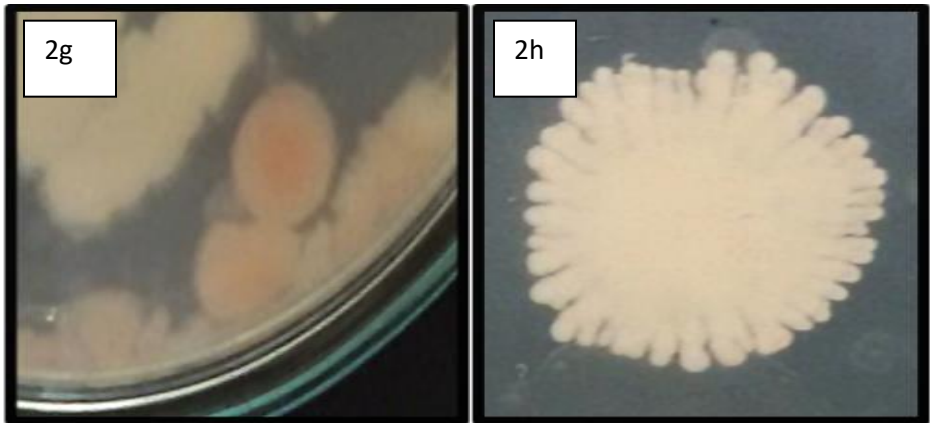


Fig. 1: a, b, c, d, e and f.
iso-1, iso-2, iso-3, iso-5, iso-6 and iso-10 respectively

Table 2: Bacterial Morphology

Isolates	Shape	Arrangement
Iso-1	Round	*Diplococcic*
Iso-2	Long Round	*Coccobacilli*
Iso-3	Round	*Diplococcic*
Iso-4	Rod	*Bacilli*
Iso-5	Round	*Cocci*
Iso-6	Round	*Staphylococcus*
Iso-7	Rod	*Bacilli*
Iso-8	Rod	*Bacilli*
Iso-9	Rod	*Streptobacilli*
Iso-10	Round chain	*Cocci*



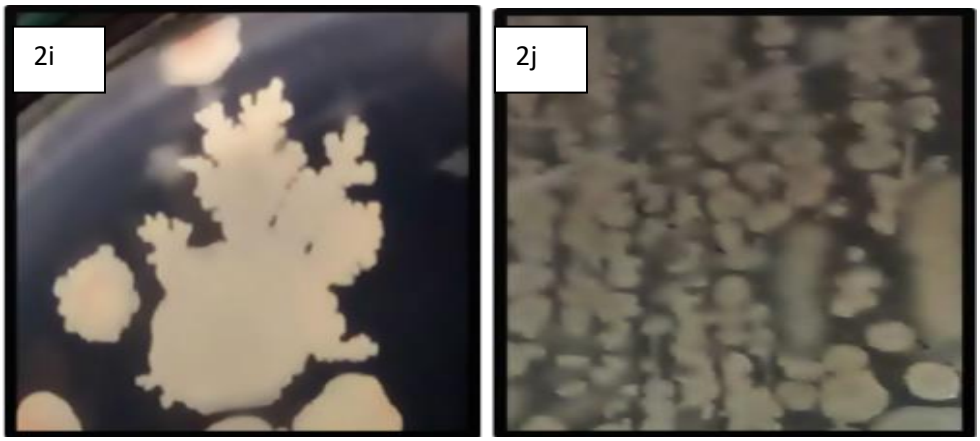


Fig. 2: g, h, i and j
iso-4, iso-7, iso-8 and iso-9 respectively

Table 3: Gram staining results of Isolates

Isolates	Iso-1	Iso-2	Iso-3	Iso-4	Iso-5	Iso-6	Iso-7	Iso-8	Iso-9	Iso-10
Gram Negative/Positive	-	+	-	+	-	+	-	-	+	-

Table 4: Spore staining results of Isolates

Isolates	Free spore	Vegetative cells	Location of spore
Iso-1	+	_	Central
Iso-2	+	_	Sub-terminal
Iso-3	+	_	Central
Iso-4	+	_	Central
Iso-5	+	_	Central
Iso-6	+	_	Sub-terminal
Iso-7	+	_	Central
Iso-8	+	_	Sub-terminal
Iso-9	+	_	Central
Iso-10	+	_	Central

Table 5: Biochemical tests

Isolates	Iso-1	Iso-2	Iso-3	Iso-4	Iso-5	Iso-6	Iso-7	Iso-8	Iso -9	Iso-10
Catalase	+	+	+	+	+	-	+	+	+	+
EMB test	+	+	+	-	+	-	+	-	-	-
Citrate utilization test	+	-	-	-	-	-	-	-	-	-
Oxidase test	-	-	-	-	-	-	-	-	-	-
MacConkey test	-	+	-	-	+	+	-	-	-	+
Sulphur	-	-	-	-	+	-	-	-	-	-
Indole	-	-	-	-	+	-	-	-	+	-
Motility	+	+	+	+	-	+	+	-	+	-

4. DISCUSSION:

In this study iso-one identified as *Salmonella enteric* with 75% biochemical similarity. These observations are compared with morphological characteristics of *Salmonella enterica* which is defined in Bergey's Manual [13]. *Salmonella enteric* has been documented as pathogen responsible for health risk [3]. Iso-two placed close to *Pseudomonas fluorescens*, four isolates as iso-four, seven, eight and nine identified which belonged to genus *Bacillus*, and iso-six places close to Staphylococcus aureus. Effective chromium reduction in different *Bacillus specie* had been reported [4, 10, 22, 21, 24] while different researchers also reported chromium resistance in gram negative bacteria such as *pseudomonas species* [17, 18, 21, 22] and staphylococcus species [12,26]. Iso-three placed close to *Arthrobacter creatinolyticus*. These observations are homogenous with morphological characteristics of *Arthrobacter creatinolyticus* defined in Bergey's Manual. Results obtained from biochemical analysis of isolates, iso-five identified as *Entrobacterarogenes* with 75% biochemical similarity. These observations are homogenous with morphological characteristics of *Entrobacterarogenes* with defined in Bergey's Manual [13]. *Entrobacter*aerogenes occurs in soil, sewage, water, dairy products, and the feces of animals and humans. It produces infections and bacteria in humans [16]. Results obtained from biochemical analysis of isolates, iso-ten identified as *Micrococcus luteus* with 60% biochemical similarity. These observations showed similarity with morphological characteristics of *Micrococcus luteus* which is also defined in Bergey's Manual [13]. These physical and biochemical features for isolates, iso-ten placed close to *Micrococcus luteus. Micrococcus luteus* has an unusual ability to tolerate and to use very toxic organic molecules as carbon sources, and metals but it showed less tolerance against chromium though it can be used in the degradation of metals such as zinc, lead and nickel. It has been sequenced because these features are important for potential applications in bioremediation and biotechnology [20]. All the bacterial isolates mentioned above were examined against chromium (potassium dichromate) at different levels. For that purpose five different concentrations were selected from low to quite high concentration. Then the bacterial growth was evaluated to on that diverse concentration, so iso-eight demonstrated the highest Cr(VI) tolerant ability, with 64µg/ml Cr(VI) being removed within 24 h [11]. However, iso-nine showed the lowest tolerance on nutrient media plate (64µg/ml Cr (VI).

5. CONCLUSION:

In this study it was concluded that selective bacteria can grow successfully in chromium stressed medium. Microorganisms which reside in heavy metal polluted soil, usually adopt resistance against those metals by shifting their intrinsic structural, physiological and biochemical properties. Therefore, the survival of microbes in polluted soil is prone to show higher resistance to heavy metals. In the present study it was also concluded that biochemical test can efficiently identify and characterize bacterial specie. Among those ten isolated bacterial species, iso-eight *Bacillus subtilis* showed highest resistance against potassium dichromate (source of chromium) and remaining were showed lowest resistance while iso-nine showed 0% resistance against chromium.

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