Isolation and Characterization of Chromium Resistant Bacteria from Marine Soil

A project submitted

by

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This work is committed to my family for their adoration and unswerving support.

Certification Statement

This is to certify that this project titled "Isolation and Characterization of Chromium Resistant bacteria from Marine Soil" is submitted for the partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (Hons.) from the Department of Pharmacy, BRAC University constitutes my own work under the supervision of Md. Samiul Alam Rajib, Senior Lecturer, Department of Pharmacy, BRAC University and this project is the result of the author's original research and has not previously been submitted for a degree or diploma in any university. To the best of my knowledge and belief, the project contains no material previously published or written by another person except where due reference is made in the project itself.

Signed

_____

Countersigned by the supervisor

_____

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Abstract

Hexavalent Chromium is carcinogenic, highly toxic and mobile in nature. It is considered as an ecological contamination which is utilized as a part of the majority of the commercial industries and is discharged without treatment. Hexavalent form of Chromium can be reduced into trivalent form of Chromium which is water insoluble. The trivalent form is less toxic due to a decrease in bioavailability and they can be used in bioremediation process. Samples were gathered from the Sitakunda Ship Breaking Yard, seaside locales of Bangladesh, for isolation, characterization and identification of the bacteria which have the potential to diminish the cancer-causing Chromium (VI) to Chromium (III). Nutrient agar medium was used which was supplemented with Chromium (VI) as potassium chromate. By purification on nutrient agar plates, containing different concentrations of Chromium, the isolate was obtained. Further the sample was studied. The isolated strain was examined for Chromium (VI) diminishment capacity in development subordinate way. It was found that the isolate was resistant to Cr (VI) as well as has the reducing activity. The isolated strain was also examined for antibiotic resistance profile and the result was, the isolate showed mild to moderate resistant to antibiotic discs. Furthermore, Minimum Inhibitory Concentration was also determined for the isolated strain.

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LIST OF ACRONYMS

BLAST	Basic Local Alignment Search Tool
Cr	Chromium
DPCZ	Diphenyl Carbazide
EPA	Environmental Protection Agency
ETP	Effluent treatment plant
IDLH	Immediately Dangerous to Life and Health
Kg	Kilogram
MCL	Maximum contaminant level
mg	Milligram
MHA	Mueller Hinton Agar
MIC	Minimum Inhibitory Concentration
mL	Milliliter
mM	Millimolar
NADH	Nicotinamide adenine dinucleotide
NB	Nutrient broth
NIOSH	National Institute for Occupational Safety and Health
nm	Nanometer
O.D.	Optical density
OSHA	Occupation safety and health administration

PEL	Permissible exposure limit
ppm	Parts per million
RPM	Rotation per minute
rRNA	Ribosomal ribonucleic acid
SCHER	Scientific Committee on Health and Environmental Risks
WHO	World Health Organization
ZI	Zone of Inhibition
µM	Micro molar

Chapter 1: Introduction

1.1 Background

Chromium has different oxidation states ranges from +2 to +6. Among them, the hexavalent form causes cancer. It is also mutagenic which is extensively used in the leather tanning, electroplating, textile dyeing (McGrath & Smith, 1990). Thus hexavalent form of Cr is lethal for the species of human and further entities (Thacker & Madamwar, 2005). Inside the environment, Cr is widely distributed in the nature and frequently occurs in the Earth's crust by holding 21st position (Chandra & Kulshreshtha, 2016). Chromium is found in plants, animals, rocks, soils, volcanic dust and gases. If not regulated properly, the transfer of chromium containing effluents can cause contamination of water, soil and aquatic sediments and this is how these heavy metal chromium can enter into our body and can cause deadly diseases like cancer.

Most of the contaminated sites over the world is treated by utilizing the abiotic process. Abiotic process is implemented by using Dig-and-treat or pump-and-treat methods which requires precipitation or immobilization step. Biological reduction is the best method for treating wastes containing hexavalent form of chromium. We have recently found that, continuous low bioreactors and fixed film bioreactors are used for the reduction of hexavalent chromium. This type of reduction is done biologically. Carbon sources are used as electron contributors in these bioreactors.

Bioremediation technology is also utilized for the treatment of Cr^{6+} by physically and chemically. Electrolytic reduction, ion exchange, electro-coagulation, membrane filtration, reverse osmosis, adsorption and liquid-liquid extraction. They are used to reduce the hexavalent chromium. But these techniques have some drawbacks which includes: High cost, low productivity, the generation of poisonous sludge or various wastes that needs to be discard. But heavy metal bioremediation process is inexpensive and environment-friendly and therefore, bioremediation technology is the most advantageous above all those method.

Our current study helps us to assess that, the bacteria strains which are chromium resistant are used as the main treatment of intoxication of chromium by hexavalent Chromium lessening into trivalent Chromium before Cr(VI) interacts with the human body or by identifying chromium reductase enzyme containing bacteria so that this ChR enzyme and other related enzymes can act as one of the potential operator of chemotherapy (Nandi et al., 2016).

1.2 Methodology

The isolated culture was identified by 16s rDNA sequencing. To find out a considerable microbial strain that will show resistant to hexavalent chromium and also can reduce the hexavalent chromium was the crucial part of the present study which is found abundantly in the medium. The methods were utilized to analyze the reduction profile that we can distinguish to put a specific bacterial strain. Chromium supplement nutrient broth media were used to led the laboratory analyses. A thorough isolation process was performed again and again unless the unaltered colonies of bacteria were distinguished morphologically and then, Chromium reducing capability of the isolated strains were assessed.

1.3 Aims and Objectives

This research's purpose is for evaluating the Chromium resistant bacteria's availability for bioremediation of mutagenic and carcinogenic heavy metal chromium and also to assess the predictable source of chromium reductase enzyme. Following tests were carry out in order to accomplish the objectives-

- Evaluation of the performance of the isolated bacteria in chromium contaminated environment.
- Investigation of Minimum Inhibitory Concentration of chromium to evaluate the tolerance of the isolated bacteria
- Investigation of the antibiotic resistance profile of the bacterial isolate.

Chapter 2: Literature review

2.1 Introduction

Chromium has been utilized to produce steel and alloys that are distinctive (Rifkin, Gwinn, & Bouwer, 2004). Chromium acts as a moving mineral and the appropriation of it are vast into environment. The position of twenty-one is possessed inside the list of the most ordinarily happening components into Earth's outside layer (Chandra & Kulshreshtha,2016). A few positions of oxidation are demonstrated by Chromium that spreads as +2 towards +6. Cr(III) and Cr(VI) is most regularly found in the earth (Chandra & Kulshreshtha,2016). Chromium is utilized for leather tanning, electroplating, dying, preserving meal. In obstinate blocks Chromium is utilized as stain, high thermal heaters, penetrating muds, prohibition of erosion or rust, chemical for replicated and cloths (Rifkin et al.,2004). Pollution of Chromium are happening into atmosphere because of arrival of untreated manufacturing drainage containing Chromium mixes into the common asset like sediment, water, air and soil. It is happening not only in underdeveloped countries but also in developed countries (Thacker & Madamwar,2005). For appropriate glucose digestion, nucleic acid adjustment and incitement of catalyst framework, Chromium is considered as a basic micronutrient yet it is dangerous at raised level. Hexavalent chromium is poisonous and mutagenic to people and different life forms (Thacker & Madamwar,2005). The versatility of hexavalent Chromium is additionally noteworthy than trivalent Chromium in aquatic systems (Rifkin et al.,2004). When that goes inside human body, that transports through plasma film promptly and composites that are situated intracellularly are oxidized by that. It is cancer-causing for human at the time it is breathed in. Furthermore, various systemic impact can happen because of indigestion and also because of dermal contact, dermatitis can happen. (Thacker & Madamwar,2005). Conversely, the oxidative potential of trivalent Chromium is not the same as hexavalent Chromium. Cr(III) is originated into un-melted formula mostly. Furthermore, it barely go by plasma film (Thacker & Madamwar, 2005).

2.2 Chemistry

Cr is an odorless, silvery gray and hard metal. In dilute hydrochloric acid and sulfuric acid, they get solubilized though they are insoluble in alkali and strong alkalis (Merck,1989). The atomic number of Chromium is 24 and it is symbolized as Cr. It is brittle and highly polished. The boiling point of Chromium is 2672°c and the melting point is 1907°c ("Chemical properties of Chromium - Health effects of Chromium – Environmental effects of Chromium," 1998-2016). Cr has different colored compounds. Therefore, it has derived from the Greek word "chroma" (Horn,2013). Chromium is utilized to produce alloys and stainless steel because the solidity and imperviousness towards erosion and corrode turns Cr an extremely valuable element (Rifkin et al.,2004).

Regardless the way that Cr is an essential trace mineral for adjustment of nucleic acid, metabolism of glucose and stimulus framework of chemical, lifted levels of Chromium is poisonous, however its deficiency may cause disease (Thacker & Madamwar,2005). Cr(VI) is deadly and mutagenic to living creatures and it causes lung cancer in individuals and also respiratory tract and skin diseases (Thacker & Madamwar,2005). This poison gigantically impacts the biosphere. Therefore, the natural reduction of Chromium is significantly important.

Table 2.1: Chromium's widespread data ("American Elements," 2016; "Chromium," 2013)

Emblem	Cr
Term	Chromium
Class	Transition metal
Group, period, block	6, 4, d
Atomic number	24
Atomic weight	51.996
Atomic radius	128pm
Appearance	Silver-grey

Electronic configuration	[Ar] $3d^5$ $4s^1$
Thermal conductivity	93.9 $W.m^{-1}. K^{-1}$
Oxidation state	-2, -1, 1, 2, 3, 4, 5, 6
Crystal structure	Body-centered cubic

2.3 Occurrence and Sources of Chromium compounds

Cr is typically trendy element originated in soil, creatures, vegetation and rocks. Though the trivalent (Chromium III) and hexavalent (Chromium VI) states are most basic naturally, Chromium exists in various oxidation states. Chromium is known not distinctive compound and natural reactions in characteristic structures. In the situations like sea-going and geologic, diminishment of Cr(VI) and oxidation of Cr(III) can occur. Chromium (VI) may change into Chromium (III) after reacting with dust particles or distinctive substances in the atmosphere (Chandra & Kulshreshtha, 2016).

Hexavalent Chromium occurs in uncommon minerals and may be really occurring in groundwater. From human activities, the hexavalent Chromium is fully introduced in this earth (Chandra & Kulshreshtha, 2016).

For instance, consolidated through United States Environmental Protection Agency (US EPA), fundamental Cr are originated into soil, biota, liquid and air through the groups of 0.1- 6.0 µg/L fresh water, 0.2-50 µg/L sea water and 1.0 to 2,000 mg/kg dirt (usually forty mg per kg dirt) (EPA, 2010). In polluted areas, fixation of Cr may become greater, such as, equal to thirty µg/L into the new water (ATSDR, 2000a).

In South Africa, Ferric Chromite, $FeCr_2O_4$ is fundamentally found which is the most mined nineral. The chromite metal store addresses around 72% of the earths perceived sources in South Africa. Distinctive countries with vulnerable stocks of mineral consolidate Brazil, India, Zimbabwe, Finland, Kazakhstan and Philippines.

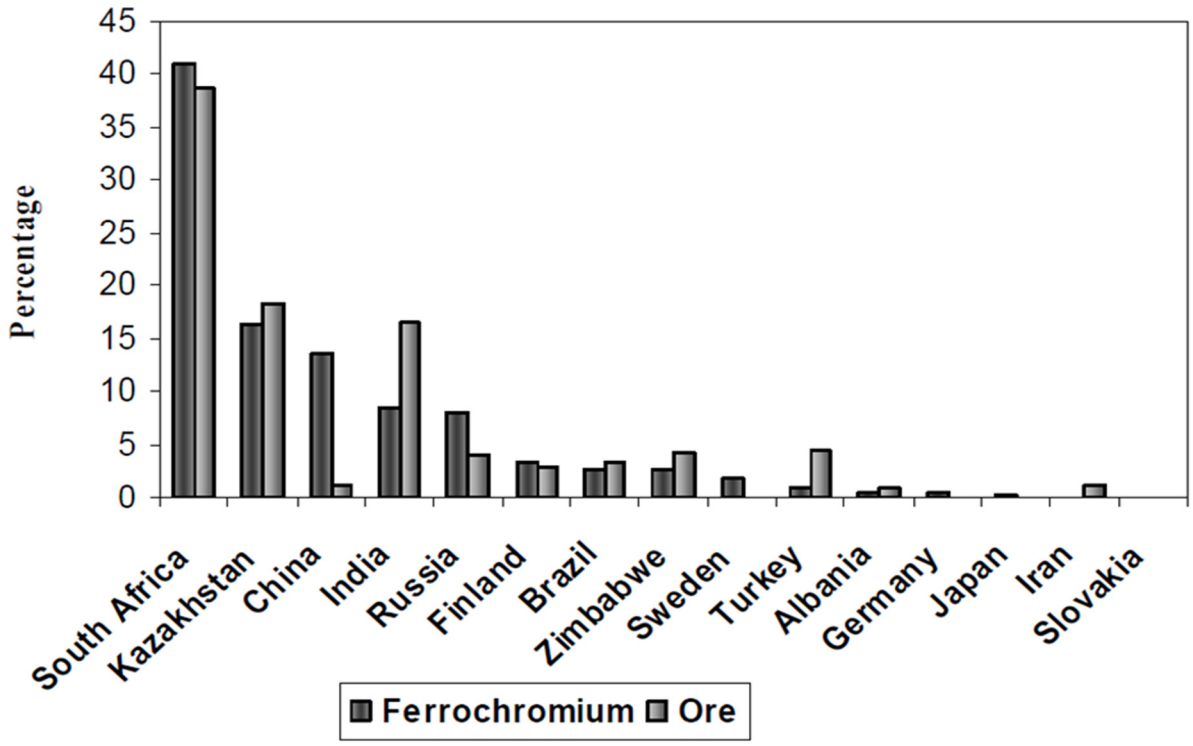

Figure 2.1: Ore of chromite and Ferrochromium fraction created globally (Papp, 2006).

2.4 Chromium utilization

Electroplating, leather tanning, preservatives for wood, pigment and dyes manufacturing, chemical synthesis, corrosion inhibitors and refractory production are some common uses of hexavalent Chromium compounds (EPA, 2010). Compounds of hexavalent Chromium are additionally found in various purchaser things such as, leather tanning utilizing chromic sulfate or wood treatment utilizing copper dichromate.

2.5 Regulation regarding level of Chromium

Assortment of unfriendly impact happens in people, animals and plants because of nearness of hexavalent chromium in the surface of water more prominent than standard point of confinement. Subsequently, strict controls have been constrained by various organization. As showed by the World Health Organization (WHO) drinking water regulations, the best sensible purpose of repression is 0.05mg/L & 2mg/L separately for both Cr (VI) and other types of chromium (Gupta & Rastogi, 2009). According to the Act of the nontoxic intake

liquid, Chromium's extreme toxin point is proved to be 0.1 mg/L. Safe concentration of the Cr into intake liquid is proved to be 0.1mg/L. If Chromium concentration crosses 50 ppm in the dyes, then it will be toxic. In the leather tanning, maximum acceptable level of Chromium is 2.75% by weight. According to Occupational Safety and Health Administration (OSHA), for the posting of chromic acid and chromate, 0.1 mg/m^3 is the PEL (Permissible Exposure Limit) to Cr (VI). IDLH (Immediately Dangerous to Life and Health) point of Cr(VI) is $15mg/m^3$ specified by the National Institute for Occupational Safety and Health (NIOSH). For the acid of chromic, chromates & chromyl chloride listing, proposed disclosure border is kept up-to 0.001 $mg/m^{3.}$

2.6 Toxicity of Chromium and the effect of it towards health

2.6.1 Chromium toxicity

Individuals who are unprotected from hexavalent Chromium are influenced through sensitivities, ulceration, dermatitis, nasal & skin disorders, lung cancer, infection of lungs, aggravations and wounding of the membrane of ear (Poopal & Laxman, 2009). Likewise, hexavalent Chromium affirmations capacity for accumulating into placenta, damaging the enlargement of fetal (Poopal & Laxman, 2009). Pollution of hexavalent Chromium into the environment alters structures of the dirt microbes and reducing bacterial advancement and also similar reactions of enzymes, with a resulting dirt persistence natural substance and storing up of hexavalent Chromium (Shi, Becker, Bischoff, Turco & Konokpa, 2002). Toxic action of the hexavalent chromium has been a direct result of the ability of it for easily invading membrane of cell and the adverse effect on plasma membrane is brought by oxidative force prompted by hexavalent Chromium which has been extensively seen both in eukaryotic and prokaryotic cells, with effects, such as, diminish in the cell layer unification or deterrent of the transportation sequence of the electron (Codd, Rillon, Levina & Lay, 2001; Francisco, Moreno & Vasconcelos, 2010). Likewise, hexavalent Chromium invade the cell of body by using the transportation system of sulfate of the layer of cell in which sulfate can be used (Ohtake, Cerventes & Silver, 1987; Otha, Glasworthy & Pardee, 1971). Cell membrane are resistant towards trivalent Chromium and thus Cr(III) is about one thousandth of toxic nature of the hexavalent Chromium (Polti, Amoroso & Abate, 2010). By observing all kinds of

examinations, we can possibly say, Chromium shows biological effect through hexavalent Chromium, that is exceptionally deadly towards maximum living things. Trivalent Chromium is normally safe for living being (Katz & Salem, 1993; Wong & Trevors, 1988).

2.6.2 Effects on health

Scientific Committee on Health and Environmental Risks (SCHER) build the examination of it according to the information assembled into the later reviews & assessments, such as complex of the mineral hexavalent Cr (WHO/IPCS, 2013). Summary of Toxicology for the Cr (ATSDR, 2012), establishment of one allusion measurements response connection to the malignancy creating behavior of Cr(VI) (RAC,2013), Cr contained into bottled liquid (WHO,2003), synthetics into the puppets (RIVM,2008), hexavalent Chromium mixtures (IARC,2012), judgement based upon science onto threats towards common prosperity related to Chromium's closeness into foods and drinkable liquid (EFSA,2014) & the aim towards Cr(VI) on public health in fresh water (OEHHA, 2011). Late written work was guided with specific focus on fresh reachable verification on potential carcinogenic effects of hexavalent Chromium. SCHER focused on a very basic level of information on prosperity effects taking after oral performance and then saw that diverse salts of Chromium were used to control hexavalent Chromium in the studies of animals and furthermore in the in-vitro analyzes. These combine zinc, calcium, strontium, di ammonium, dipotassium and disodium salts which contains unique solvency degrees. In the epidemiological reviews, co-appearance of trivalent Chromium and hexavalent Chromium occurred.

2.6.2.1 Kinetics

Extension of archetypal had been done to the individuals verbally exhibited for hexavalent Cr. Therefore, information obtained through renowned literatures & studies for toxicokinetics for total Chromium in individuals was used. According to the model on the process of pH-subordinate, combined second order gave affair depiction on predictable toxicokinetics of Chromium with the current data on people exposed to Chromium. pH, travel time and volume of gastric lumen and the formation of gastric juice were recognized as basic hotspot for human changeability and for this, data is lacking in order to additionally make assumptions created

in the models of PBPK (Physiologically-Based Pharmacokinetic) and to allow upgraded health hazard evaluation (Kirman et al., 2013). Hexavalent Chromium is exceptionally open in organic frameworks and can rapidly be reduced to trivalent Chromium which is less instantly held and more safe for human beings than hexavalent Chromium. In people there is an extensive between and intra-particular fluctuation with respect to maintenance of hexavalent Chromium since the change depends on centralizations of both hexavalent Chromium and the nearby reducing experts and moreover on the pH and gastric substance. Specially acidic circumstances with high common substance propel the decline of hexavalent Chromium to trivalent Chromium. Some ascorbate and thiols are the central natural reducer of hexavalent Chromium, demonstrating to over eighty percent of the assimilation framework of it (Zhitkovich, 2011). Hexavalent Cr known to be chromate fundamentally proceeds subsequently the sulfate & phosphate and that is immediately occupied through each organs and the cell totally over our physique through methods for the transportation of sulfate (Costa, 1997). It differs from trivalent Chromium that is non-permeable for our body cell. Former take-up are directed through competition among reduction of hexavalent Cr extracellularly and fast ingestion of it intracellularly. Amount of Chromium are recently discreetly raised next to the introduction towards hexavalent Chromium (Costa, 1997; Thomann et al., 1994; Witmer et al., 1989; Collins et al., 2011; Witt et al., 2013). For mice and rats which are orally exhibited to Chromium, physiologically-based kinetic models were created (Kirman et al., 2012; Schlosser, 2014).

2.6.2.2 Mode of action

Confirmation support validates that hexavalent Cr^{6+} can perform by genotoxic & mutagenic mechanism. Similarly, hexavalent Cr is seemed for decontrolling the growth of cell (IARC, 2012). Hexavalent Cr instantly transfers plasma layer by the transportation of sulfate. Inherited wounds join the complexes of DNA and Cr, complexes of DNA, complexes between DNA, Chromium and DNA, DNA between crosslinks, complexes between protein, Chromium and DNA, abasicsites, protein and DNA crosslinks, DNA intra-strand crosslinks, DNA strand breaks and oxidized bases (Wise, Holmes & Wise, 2008). Inside a cell, trivalent Cr is able to attach with DNA inciting transmutation & genomic variability as observed by in-

vitro contemplates in human and bacterial cells (Quievryn et al., 2003). From the reactive oxygen species, hexavalent Cr can also do damage of DNA. The carcinogenic and mutagenic activities of Chromium are extremely intricate. Chromium (III) goes inside the cell inadequately and is less unsafe for human body. Conversely, if Chromium (III) is made inside cell by Chromium (VI) reduction, then it can go into the cell promptly. Chromium (III) may bring about capture of the polymerase of DNA by creating cross-linking with DNA. ROS is created whenever hexavalent Chromium's lessening occurs and thus Chromium (III) is formed. By nearness of the Hydrogen Peroxide, trivalent Chromium itself makes the reactive oxygen species level ascend inside the cells that creates 8-hydroxy deoxyguanosine in the DNA. Trivalent Chromium inside the cell can likewise can tie outside the plasma membrane so that ROS can be produced that enters into cell making damage to the cell (Hadjiliadis, 2012). Fixation of low hexavalent Chromium in-vitro cause persistent origination of mitogen-activated kinases of protein ERK-1, JNK, p38 and ERK-2 (Chuang and Yang, 2001; Kim and Yurkow, 1996) & mutagenic elucidation modules c-Jun, ATF-2 and NF_KB's phosphorylation (Samet et al.,1998; Ye et al., 1995). Because of the elucidation modules and kinases of protein establish crucial negotiator inside the inflammatory processes and the growth of tumor, effects upon cell motion transduction which decontrol cell advancement are moreover non-strange by virtue of hexavalent Chromium, despite the straight genotoxic frameworks included (Hartwig, 2007, 2010). Hexavalent Chromium showed genotoxicity *in-vivo* by all groups of compounds in the rodents, which were treated with great amount of hexavalent Chromium (ATSDR, 2000b; ATSDR, 2008; OEHHA, 2011). Study in animal planned to develop upon 90 days and expand review of NTP, inflammation observed into little tracts of assimilation of the rats which had been verbally uncovered towards carcinogenic estimation of hexavalent Cr. That is obliged for becoming result of the oxidative harm & acclaimed which it can be proceed previously tumor organization and reformative hyperplasia (Thompson et al., 2013). Whole analysis of the microarray of genome of duodenal epithelial sample perceived changes in the genes essential in oxidative pressure response, lipid digestion system and control of cell cycle (Kopec et al., 2012). Damage of DNA in the lymphocytes has occurred because of the exposure during inhalation (IARC,2012).

2.6.2.3 Effects in Animals

Particular reviews kept an eye on the extraordinary lethality of hexavalent Chromium. In diagram, acute oral value of LD_{50} were exhibited to compounds of hexavalent Chromium in the rats moved something like 13 mg/kg & 29 mg/kg b.w depending upon the complex oversaw & on sexual role of rat (Gad etal.,1986). Main effects found into mouse when the mixes of Chromium introduced orally at medium range and they decreased in the weight of the body and also changed in the parameters of hematology and immunity. The mice and rats were revealed for quite a while to sodium dichromate controlled in bottled water was used to preserve through consistently utilization levels for non-carcinogenic impacts. Presentation of rats through internal breath achieved respiratory irritation and rearrangement of neutrophil (Cohn et al., 1998). Distinctive reviews exhibited that Chromium mixes incited sicknesses in trial animals after the introduction in several pathways such as oral route, intra-muscular, intra-pleural, inhalation, intra-tracheal, intraperitoneal, intravenous infusion and subcutaneous (ATSDR, 2008). Cancers occurred generally at the site of association. Inward breath expanded the possibility of lung carcinoma in the rats and mice (Glaser et al., 1986; Glaser et al., 1988; Nettesheim et al., 1971). Sarcomas occurred locally through the infusion of some Cr containing substances such as lead chromate, strontium chromate, calcium chromate and zinc chromate through respiratory tract. UV-actuated skin carcinoma and the effects of tumor were showed after the potassium chromate was taken orally (Davidson et al., 2004).

2.6.2.4 Human effects

For the individuals, maximum records upon impacts have been found from detailed occasions of circumstantial introduction towards the high estimations through inward breath from industrial disclosure. Generally, workers are disclosed in chromate era, electroplating and the formation of pigments with Chromium who are exhibited to Chromium compounds.

Ulcers and rashes occurs after the skin get exposed with the compounds of hexavalent Chromium. Dermal introduction to hexavalent Chromium compounds has also been associated with negatively vulnerable contact dermatitis. Using a fix test, 2µg concentration was necessary to draw out a positive hypertensive skin reaction. The power of the effects of

Chromium in the comprehensive group has assessed to be something close to 0.5% to 1.7% into the investigation into a couple of European countries (Peltonen & Fraki, 1983; Hartwig, 2007; Hartwig, 2010). Sharpening properties of hexavalent Cr have not been inclined to through SCHER into the decision. Inhalation into the occupationally revealed experts incited effects in the nasal routes, such as, septal orifice and nasal mucosal ulceration. Alteration in the lung limit parameters were also viewed. Disclosure was assessed considering the presentation time allotment (described as the interval in the middle of when a worker was enrolled and the time signs were at first recognized). Furthermore, on mean and center yearly hexavalent Chromium fixations disposed to be familiar about the position of occupation help at the time the side impacts at first happened (Finley, Proctor & Paustenbach, 1992; Lindberg & Hedenstierna, 1983). Hexavalent Chromium has seemed to achieve damage of DNA (micronuclei, chromosomal abnormalities, DNA-protein crosslinks, trades of sister chromatids and DNA strand breaks) inside the authorities lymphocytes (WHO/IPCS,2013). All reviews on human were not demonstrated dependable outcomes. They were obliged in a couple of perspectives: generally, the amount of demonstration to hexavalent Chromium were not identified and revealed and closed assemblies were evaluated regularly considering several operational duties. A bit of the reviews used assemblies that had insufficient measurable energy to reliably assess cytogenetic alteration in workers.

Concerning effects of hexavalent Chromium on sinus diseases and nasal infections, epidemiological affirmation remains indicative yet unverifiable (IARC,2012). A connection among the growth of abdominal tract and presentation of hexavalent Cr into the unadulterated intake liquid are represented at China's one of the polluted territory (Zhang & Li, 1997). There are critical results concerning the review result, especially into assessment of disclosure (Brandt-Rauf, 2006; Beaumont et al., 2008 & Smith, 2008). A contextual review had not disclose some development for the progress in digestive tube into employees because they were uncovered generally during breathing (Gatto et al., 2010). However, separate inquiries were slight and clarification was hindered through lacking of necessary exposure assessments and nonappearance of data upon smoking, dietary factors, financial status and alcohol use.

2.7 Carcinogenesis induced by Chromium

Compounds of Cr(VI) is considered as dominant carcinogenic agent and it has seemed as a DNA damaging agent including the cross-linking of protein and DNA in various tissues and cells. However, hexavalent Chromium does not attach to proteins or DNA in the open cell structures (Fornace et al., 1981; Koster, 1985). Hexavalent Chromium is immediately transfers into cell by the sulfate anion transport structure if it subsists as oxyanion at biological pH (Jenette, 1981; Arslan, 1987). Hexavalent Chromium is diminished into its most steady structure, trivalent Chromium by the cell redox system and then it is acknowledged into the cell (Conett, 1983; Nieboer, 1988). Trivalent Chromium binds with DNA and furthermore with proteins in without cell systems and has great affection for other natural ligands (Tsapakos, 1983; Earley, 1965). In any case, trivalent Chromium is deficiently used up inside the cell and is believed to be non-carcinogenic (deFlora, 1985). In the midst of intracellular diminishing of hexavalent Cr into trivalent Cr, species which response are made (Conett, 1983; Mattagajasingh, 1995; 1997) and in this way, carcinogenic processes begins by altering the structure of DNA (Kawanishi, 1986). Reduction of Chromate creates Hydroxyl (-OH) ions which is responsible for the cross-linking between DNA and protein and also are deliberated as a crucial agent in the malignancy of chromate (Shi, 1990; Margolis et al., 1988; Gajewski, 1990). Hydroxyl ions are produced from trivalent Chromium by the redox processes and therefore, trivalent Chromium is also considered as carcinogenic (Sugden, 1992) and Chromium V is responsible for the damage of DNA (Kortenkamp et al., 1989). Hence, chromate- incited protein-DNA buildings are entangled in the carcinogenic nature of chromate, the system of their improvement, amalgamation and natural significance are not exactly known. It is suggested that, DNA-protein cross-linking may hamper the structure of chromatin and normal direction of the expression of gene (Bedinger et al., 1983). This, along these lines, could expect a section in cancer- causing into the deletion of bases of DNA can be arise to fruition in the time of portions of reproducing DNA are secured under the structure of protein and DNA (Briggs, 1988). Rise of trouble or gene inactivation are arise by deletions of tumor suppressor genes which ultimately stimulate cancers (Bouck & Benjamin, 1989). In addition, amongst characteristic control on the expression of gene and proteins, reversibly coordinate with specific DNA progressions (Stein, 1979). DNA and boorish protein's cross-linkage

might agitate protein's natural bearing and DNA collaboration, achieving veritable inherited results, fusing intrusion in or modification of the expression of gene. Thusly, it is vital to recognize of the proteins that appreciate chromate- impelled protein and DNA structures and the method for their coordinated effort with the DNA. Recognizing confirmation of proteins cross-connected with DNA may in like manner help with our perception of structure of chromatin and protein association with the realistic presentation of the protein round DNA.

2.8 Conventional methods for remediation of chromium toxicity

Particular normal frameworks for declining of hexavalent Chromium from the stream of waste water combines physical & blend systems, like, precipitation, treatment electrochemically, molecule exchange, lessening chemically, vanishing recovery, filtration, film development and adsorption (Ahluwalia & Goyal, 2007; Al-Sou'od, 2012).

2.8.1 Electro chemical precipitation

Electrostatic potential is used by this technique to build the removal of liberal metal from dirtied squander water over the standard industrial precipitation methodology (Kurniawana, Chana, Loa & Babelb, 2006). In ppm (parts per million), generally this strategy is the ideal perceived method for emptying hurtful overpowering level of metals from the water. ECP method is used to remove hexavalent Cr from effluent liquid of electroplating (Polprasert & Kongsricharoern, 1995). Utilizing this framework hexavalent Chromium fixation might be expelled from 3860mg per liter upto 0.2mg per liter

Notwithstanding the fact that, this method has been shrewd the sufficiency of it & affected by the lower amount of pH & vicinity of different salts. This framework needs different chemicals improvement that at last fasten time of the irregular state liquid sludge of content & this conversation is price certifiable. Precipitation by molecule exchange, disulphide or lime does not contain any specificity. At the lower level of obsession, it lacks the withdrawal of metal elements.

2.8.2 Ion Exchange

Ion exchange methodology is changing into a striking framework that has turned out to be much assumption at the late of the starting from the all other physicochemical strategies for the discharge of Chromium from discarded water. Ion exchange is an entity framework and in this framework, elements of a particular type diversity categories are released from unsolvable trade material through the elements of an auxiliary type in strategy. Chromium's course of action pass in over one side of the underweight portion, then goes through the bed of resin, finally Chromium has been removed. The slice is discharged for clearing the amassed solids when the farthest point of the resin is depleted and then it is recovered. Normally, the utilized cross areas are manufactured common particle trade pitches and they are used as particle trade.

Synthetic Dowex 2-X4 particle trade pitch was utilized to inspect the consumption of hexavalent Chromium from the discarded water of realistic plating (Sapari, Idris & Hamid, 1996). An undeniably key anion fluid in the hydroxide assembly was utilized as a piece of areas as the exchanger of anion. Examination distinguished about 100% reimbursement of hexavalent Chromium. Additional examination was done on the premeditated resin of particle trade and on Ambersep 132 for recouping chromic acid synthetic plating strategy through a four-stage method of particle trade (Lin & Kiang, 2003).

An obstacle for molecular exchange framework for the clearance of Cr is because the molecule exchange fluids are to an incredible particular grade. Resin which have been picked should be skillful to particularly ousting the metallic pollutant. Moreover, deficient elimination of Cr can happen and the apparatus of particle trade may be exorbitant. Rigorous arrangement of metals cannot be accomplished by it because different organics and dense materials of wastewater effortlessly spoils the arrangement. In like manner, particle trade is mostly brittle to the pH and it is disregardful.

2.8.3 Biosorption

Biosorption of Chromium by using the strategy of water is diffidently another procedure that has indicated remarkably comforting in the discharge of pollutions from fluid waste. Adsorbent solids procured from simplicity cultivating squanders can utilize for the viable

discharge and recovery of Chromium form the streams of effluent water. To some point biosorrption is a difficult method which is exaggerated by some variables. Complexation, adsorption- complexation on holes and surface, micro precipitation, major development of metal hydroxide on surface and bio surface adsorption, chemisorption and exchange of ion are the methods needed for biosorption (Gardea-Torresdey, Rosa & Peralta-Videa, 2004). This system experiences biosorption's few amount of energy & lower limit in adsorption.

2.8.4 Adsorption utilizing actuated carbon

It has been seen that, to adsorb Chromium, actuated carbon are more proficient which is got from different unrefined materials such as shells of coconut and nut, saw dirt and so on (Mohan & Pittman, 2006). GAC type of filtrate Aquatic Air Mud Pollute 400 from the solution of aqueous is utilized for the clearance of hexavalent Chromium (Demirbasa, Kobyab, Senturkb & Ozkana, 2004). Decay in the adsorbents size of elements produces the surface zone for metallic adsorption. It then achieves greater decline benefit on hexavalent Chromium. Moreover, at the high temperature, adsorption of hexavalent Chromium was very optimistic. From the nuts of *Terminalia arjuna,* a small number of endorsed carbon were organized and initiated zinc which were used chemically that showed most convincing expulsion of Chromium at the pH of 1.0 (Mohanty et al., 2005). Enacted carbon was utilized which were passed on through Sutcliffe carbon beginning from a firewood of bituminous for the adsorption of hexavalent Chromium (Natale et al., 2007). Adsorption was constrained for the endorsed carbon solidly relies on solution's saltiness and pH. The vital insufficiency of the technique depends on its steady salvage and in the life of adsorbent.

2.8.5 Membrane filtration

Procedure of filtration by membrane has become a significant thought for the treatment of effluent water. Utilization of aquatic hydrolytic force is deliberated here to separate by semipermeable film. Membranes of different types such as aquatic membrane, inorganic membrane and polymeric membrane is used for the clearing of hexavalent Chromium. (Pugazhenthi et al.,2005) organized maintained non- interpenetrating transformed radical-filtration film of carbon through the nitration of gas phase applying amination and NOx by

hydrazine hydrate development. To separate hexavalent Chromium from the preparation of liquid, the membrane was applied. Portion examination of the corrosive strategy of chromic was completed by utilizing 96% discharge of basic membrane, 84% discharge of nitrated carbon membrane and 88% discharge of aminated membrane. Absorption of film provender solution and alteration of pH was occurred for the ejection of hexavalent Cr through the utilization of diverse polyamide films of nano-filtration compounds (Muthukrishnan & Guha, 2008). Two types of membranes such as high dismissal film (NFI) and low dismissal film (NFII) were utilized for the inspection.

Augmentation of the pH of provender solution rise Chromium's discharge rate. Great vivacity, poor discharge of metal, ingestion of chemical and dangerous overflow or effluent generation which needs interchange are the snags which are bona fide problems of this framework insulated from being economically extravagant.

High measure of vitality is utilized ceaselessly, reductant utilization which is noxious and expensive are the downside of this strategy which is used to treat hexavalent Chromium polluted water of ground and dirt.

These strategies are exorbitant and now and again the auxiliary squanders needs appropriate management. The best option to treat Cr is bioremediation which is economical and safe to nature (Kamaludeen, Arunkumar, Avudainayagam & Ramasamy, 2003).

Development of *in situ* bio-reduction is related with dodge the inadequacy of physical and chemical system. A few analysts have documented straight metabolic diminishing of hexavalent Chromium by microbes life forms. As lessening hexavalent Chromium groups were secluded from polluted territories and moreover unpolluted areas of hexavalent Chromium, bioremediation of hexavalent Chromium emanates an imprint of becoming inevitable. After the diminishment of bacteria, it can be seen that, structures of hexavalent Chromium are altered into static unsolvable and hydroxide of Chromium. Starting now and into the foreseeable future, this advancement may relate at ground areas to immobilize Chromium underneath the surface of earth.

2.9 Metals and Microorganisms

2.9.1 Metal resistance mechanism into Bacteria

In environment, metallic inevitable technique has accomplished the boundless presence of metal's resistance in microbe. In the bases of inborn and biochemical, microorganism's resistance of metal is diverse and can be transposon, ended chromosomally or plasmid alongside different potentials. Five types of segments about microbe's resistance to metal (Rouch et al., 1995) are given below:

1. Metal disposal by permeability deterrent.
2. Metal eradication from human body's cell through active transportation.
3. Metal's physical isolation intracellularly through protein restriction to shield it from damaging metal-fragile cell component.
4. Separation extracellularly
5. Metal decontamination in which metal is exaggeratedly attuned to reduce a small amount of energy.

2.9.2 Metal sensitive cellular components

Metal particles are proficient to lessen or fabricate impetus action or modify specificity of composite by impelling modification in catalyst or by securing the catalyst in specific amenabilities or by modeling making ties with the dynamic and other imperative regions in the transport framework and chemicals, consequently their capacity is avoided.

Structure of DNA can be particularly damage by the particles of metal through transporting cross connection elements or DNA facts elements can be affected obliquely by lessening the dedication of DNA amalgamation (Beyersman, 1994)

Regardless of the way that a broad assortment of the parts of cell are likely concentrations foe metal-instigated harm, a topic of this section is vital for urgent cell limit, such as, DNA utilization for repetition. Passing of cell will happen in light of inactivation because of induction of metal through metal compassions so as to the concentration of a particular metal risings, their task will be inactivated if the focus extents to dangerous level. Thusly, dependent upon the meeting of metal, cell should carry a couple of techniques for affirmation for at least

one target destinations so that they can survive. Amount of central parts will be more remarkable if the gathering of metal is more significant that needs security. For example, *E.coli* fundamental protein origination may be kept away from by solitary gene alteration through the generation of prolonged resistance of metal (Lutkenhaus, 1977).

2.9.3 Uptake system of metal and resistance

In cytoplasm, those cells are located that contain basic fragments of cells that are greatly subtle to metal, the mechanism of imperviousness is chosen through the amount of take-up framework so that metal can pass inside cell. Lipid portion of the layer of cell is extraordinarily resistant to the cations of metal (Anderson, 1978). Metal, hence, for the most part goes through the membrane where resistant sites are less. For example, in the event of E.coli, transport structures of phosphate or magnesium are permeable to Arsenate and Cobalt (Silver et al., 1975).

2.9.4 Metal as a biological requirement

Different considerable metals have been imperative for maintaining crucial microbial cell's metabolic actions. Most classes of microbe need nickel, copper and iron and some of the classes need cobalt, molybdenum and tungsten. Cell will be less damaged by the metals when appeared differently in relation to metal with non-optimistic metabolic exercises, as legitimate frameworks will happen to cell that will adjust by the small capacities in nearby fixations.

2.9.5 Gene cassette versus chromosome-mutation-determined resistance

Innate imperviousness of metal's introduction into the harmless infinitesimal living beings can be managed through the availability in the close-by populace of a accomplished cartridge of gene that decides a conferred part of imperviousness. By developmental choice they are recommended to be grasped to done the imperviousness fruitful. Moreover, it can be borne on plasmid, chromosome or transposon. Two last contenders can propel trade of the associated cartridge of resistance among the microbes. Open chromosomal transformation will give less

imperviousness than the cartridge mediated resistance. Greater population will give greater probability of a huge behavior of cartridge genetic segment being accessible.

2.10 Mechanism of Chromosome resistance in Bacteria

Decontaminating activities of free radical, healing of the harming of DNA, systems associated with the homeostasis of iron or sulfur are the strategies that microbes use for the resistance of chromosome (Morais et al., 2011; Ramirez-Diaz et al., 2008). Hazardous metal-contaminated circumstances can be subsist by various bacteria by making frameworks for avoiding harmfulness of metals such as take-up of adsorption, methylation of DNA, efflux of metal and biologically conversion of metal through decreasing by enzymes for making little convenient and damaging assemblies or by creating edifices with H_2S like metabolites (Camargo et al., 2005; Pei et al., 2009; Soni et al., 2012). Diminishment of hexavalent Chromium by bacteria to trivalent Chromium is mainly perilous from biological remediation's perspective that may be counted as an additional system of the resistance of chromate (Cervantes et al., 2001). A collection of microbes which are impervious to Chromium with greater reducing potential of hexavalent Chromium are *Deinococcus, Enterobacter, Pseudomonas, Thermus, Eschericia, Agrobacterium, Bacillus, Shewanella* and diverse species (Ohtake et al., 1987). Strains that are impervious and non-impervious to chromate have the ability to decrease the concentration of chromate but at the extent concentration of Chromium, the growth of future will be stuck completely (Bopp & Ehrlich, 1988). By this way, the property of bacteria, that is mainly beneficial towards the potent method for bioremediation, connects great imperviousness or strength by the ability to lessen hexavalent Chromium to trivalent Chromium (Dhal et al., 2013). A couple of microbes showing resistance and decreasing exercises of hexavalent Chromium are withdrawn and recognized from chromate debased condition and furthermore in unpolluted natural frameworks (Schmieman et al., 1998; Turick et al., 1996; Wang & Shen, 1995). CRB (Chromium reducing bacteria) are those bacteria which can lessen the amount of hexavalent Chromium. Gram-optimistic microbe is one type of CRB and it shows immense imperviousness to the poisonousness of hexavalent Chromium at abstemiously extent fixations but those bacteria shows a smaller amount of resistance towards hexavalent Chromium which are gram-negative (Coleman, 1988). Microbes which originate in the

spoiled condition of metal are really harmless for those metals. Microscopic organisms gathered from the mine dirt of chromite are protected to hexavalent Chromium nearby other generous elements (Das et al., 2013). Decrease of chromate and imperviousness are non inter-connected and every microscopic organisms that are impervious to hexavalent Chromium can not lessen hexavalent Chromium to trivalent Chromium. Along these lines, both diminishment and imperviousness of Chromium are microorganism's autonomous assets (Bopp & Ehrlich, 1988; Silver, 1997). Hexavalent Chromium lessening by microbes may happen 'straightforwardly' through utilization of catalysts or 'in a roundabout way', where metabolic deciding items catalyzes deterioration of hexavalent Chromium such as, iron's HS and Fe(II) and also sulfate-lessening microorganisms (Hwang et al., 2002). Microbes use characteristic segments of resistance so that it can overcome the destructiveness of hexavalent Chromium in ground which fuse the decreased take-up of hexavalent Chromium, lessening of hexavalent Chromium extracellularly, diminishment of hexavalent Chromium intracellularly through cleansing by ROS (reactive oxygen species), healing of DNA by enzymes, Hexavalent Chromium efflux outside cell and delving of ROS are depicted in the figure 2.2

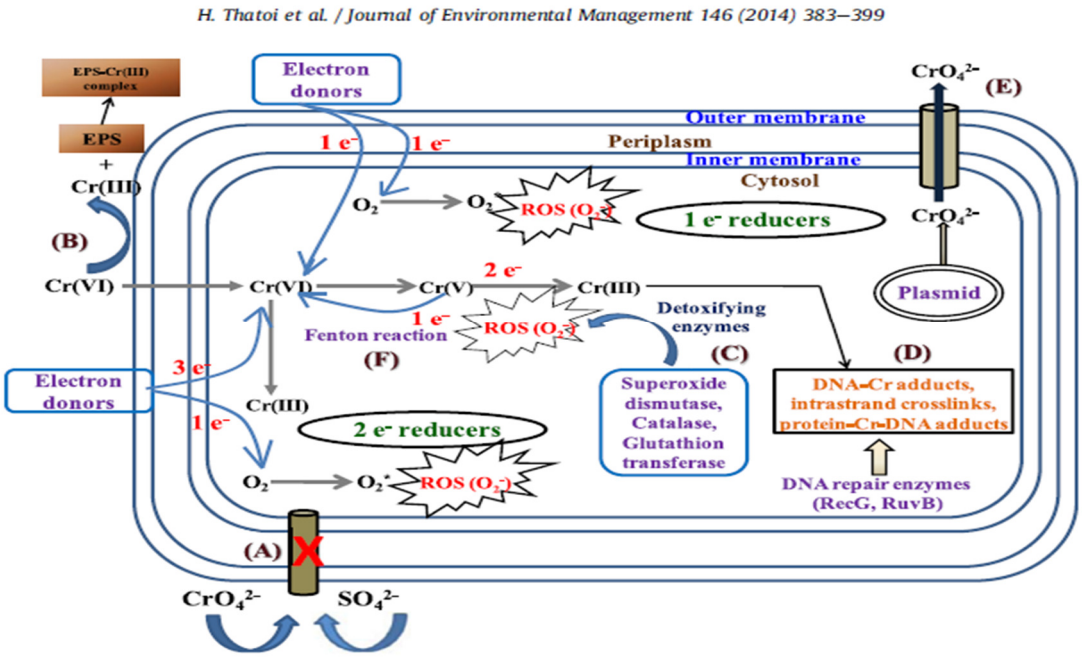

Fig 2.2 Resistance of chromate mechanisms into the cells of bacteria. A: Transformation in the chromosome-encrypted take-up transporter of sulfate, B: Lessening of hexavalent Chromium extracellularly into trivalent Chromium, C: Lessening of hexavalent Chromium

intracellularly to trivalent Chromium through chromate reductase, D: Utility of the healing method of SOS in diminishing oxidative pressure, E: Chromate efflux from cytoplasm, F: ROS foraging enzyme activity to diminish oxidative pressure (Thatoi et al., 2014).

(A) Reduced uptake of Cr (VI)

Decreased take-up of hexavalent Chromium such as take-up passageway of sulfate and through the homeostasis of iron or sulfur are the capable protective frameworks in contrast to the destructive effects of hexavalent Chromium. Particles of chromate has fundamental brings after through the particles of tetrahedral sulfate (Figure 2.2) and then it is permeable to the membrane of cell through the SO_4^{2-} transportation passageway, by the help of the non-specific anionic carriers such as PO_4^{3-} and SO_4^{2-} (Wenbo et al., 2000). If mutation occurs in the microbe's sulfate take-up passageway which is encrypted by chromosome, then diminishment occurs in the chromate movement (Ramirez-Diaz et al., 2008). Bacteria which exist in the spoiled condition of metal encounter expedient change to make imperviousness of hexavalent Chromium which stimulates reduced take-up of hexavalent Chromium through sulfate transportation passageway. Exposed living beings may acquire the opportunity to be harsh by alteration or through inherited information solidification that encrypts the imperviousness (Kummerer, 2004).

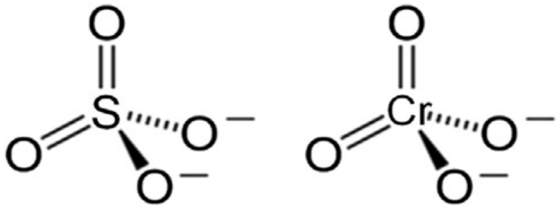

Fig 2.3: Resemblance among structures of the ions of sulfate and chromate

(B) Extracellular Cr (VI) reduction:

An additional framework of resistance is the lessening of hexavalent Chromium into trivalent Chromium extracellularly straggled through attachment of its with functional group on the

cell exterior of bacteria (Ngwenya & Chirwa, 2011). Attachment of the diminished trivalent Chromium to the cell exterior of bacteria supports straightforward removal of it from degraded condition. Portions of peptidoglycan are the extraordinary trivalent Chromium binder and they exist into the partitions of cell of these life forms (Hoyle & Beveridge, 1983). It can be seen that a couple sorts of microorganisms have the ability of adsorption that boost the removal of the species of metal from the solution of water. Normally these abilities go for the movement of responsive functional groups such as phosphate, amine, carboxyl, sulfhydryl and hydroxyl group upon the partition of cell exterior of microorganism (Parmar et al., 2000). Thusly, cell becomes impermeable for hexavalent Chromium at the time of its extracellular lessening occurs.

(C) Detoxifying enzymes of ROS or Cr (VI) reduction intracellularly:

In the midst of the depletion of hexavalent Chromium into trivalent Chromium a transient, exceptionally responsive transitional radical of hexavalent Chromium is generated that undergoes sequences of redox. By this way, pentavalent Chromium goes for oxidization and turns again into hexavalent Chromium. Thus dioxygen gets the electron of it and ROS (reactive oxygen species) are formed. As a result, oxidative pressure is originated in the life forms. By this methodology, proteins situated in the bacteria are in like manner actuated through chromate into the shield in contrast to oxidative pressure provoking extra preparation of chromate's imperviousness (Ramirez-Diaz et al., 2008). In any case, oxidative pressure is developing on account of reactive oxygen species are cancelled into a huge amount through purifying enzymes such as, catalase, superoxide dismutase, glutathione transferase and so forth (Ackerley et al., 2004).

(D) DNA repair enzymes

One of the other armor of security made by hexavalent Chromium which is protection of the cells of bacteria through the healing enzymes of DNA of that DNA which is harmed. Hexavalent Chromium transfers into the cell of microbe that is speedily decreased into trivalent Chromium through the movement of various activity of non-catalyst or catalyst

which delivers reactive oxygen species (ROS). In this way that does damaging effects upon the cell's DNA and protein. Reactive oxygen species that is produced makes DNA harm, like alteration of base, disruption of one strand and disruption of two-fold strands. Such DNA damage may be healed by extraordinary healing mechanism of DNA such as, the reaction catalysts of SOS which are RuvB, RecA and RecG (Hu et al., 2005). For example, Hexavalent Chromium into E.coli has not been known as the healing system of SOS which from the oxidative pressure defends DNA (Llagostera et al., 1986). Moreover, helicases of DNA such as, RuvB, RecG, sections of healing framework of recombinant DNA are seemed to appreciate the response to the harm of DNA acquired through chromate into Pseudomonas aeruginosa (Miranda et al., 2005). Hexavalent Chromium diminishment of cell is performing technique, making pentavalent or tetravalent Chromium like intermediates of redox that is active and constant trivalent Chromium enclosing adducts of DNA and Chromium that is the utmost abundant kind of destruction of DNA which is responsible for alterations and also disruptions of chromosome (Zhitkovich, 2011).

(E) Efflux of Chromium from cell

From cytoplasm of the cell, transporters interfere with the efflux of chromate particles which is encrypted by specific genes that is stomached of plasmid, is also a mechanism of imperviousness originate in microorganism. In every way, chromate efflux is a successful and broad resistance system and it inhibits poisonous particle's accumulation in the cell of bacteria (Ramirez-Diaz et al., 2008). P. *aeruginosa* has the finest appreciated resistance mechanism of chromate. Superfamily of CHR of the particle carrier of chromate is connected with the protein of ChrA. CrhA is a protein of membrane that tends to repel and not absorb water, encrypted by pMOL28 and P. *aeruginosa*'s plasmids called pUM505 originated in *Cupriavidusmetallidurans* and previously from *Ralstoniametallidurans* and *Alcaligeneseutrophus* (Cervantes et al., 1990; Nies et al., 1990) which is appeared as incorporated in imperviousness of chromate through efflux segment of chromate (Ramirex-Diaz et al., 2008). Protein of chrA executes as chemi-osmotic propel system. From the periplasm or cytoplasm it then charges chromate so that external driven can occur through proton held method energy (Alvarez et al., 1999). Proteins of CHR of a few microorganisms

are incorporated into imperviousness of chromate through efflux framework of chromate (Ramirez-Diaz et al., 2008).

(F) Scavenging of ROS

Consequent of hexavalent Chromium for inflowing cell may be reduced into pentavalent Chromium. Donors of electrons such as NADPH or glucose donates the electrons towards pentavalent Chromium and thus it provokes the improvement of comparative unpredictable noxious moderate pentavalent Chromium. However, through the semi-tight instrument, reinstructs of chromate further decrease pentavalent Chromium into trivalent Chromium with a trade between two electrons, occasionally this reaction is not extraordinarily brisk. In this way a fragment of the intermediate of pentavalent Chromium is instantly reoxidized into hexavalent Chromium thusly delivering reactive oxygen species through one Fenton-like reaction. In the midst of this methodology radicals of hydroxyl are formed into the cell of bacteria (Shi & Dalal, 1994) and it is represented into underneath situation:

$Cr\ (V) + H_2O_2 \rightarrow Cr\ (VI) + -OH + OH$

Hydrogen peroxide is made when the radical of oxygen is created by the reduction of nuclear oxygen in the midst of deterioration technique through dismutation method. Reaction occurs between Cr (VI) and hydrogen peroxide and radicals of hydroxyl are created by one Fenton alike reaction. This progression resembles ferrous ion oxidation with hydrogen peroxide into the reaction of Fenton because hydroxyl ion production from the ferrous ion by Fenton reaction is supported colossally through the course of action of the complex of ferrous ion which have discharge districts for the synchronization of hydrogen peroxide.Table 2.2 Outlines the techniques of bacteria & they are associated with the resistance of chromate

Table 2.2: Methods of bacteria for the imperviousness of chromate

Enzyme/System	**Species**	**Function**	**Reference**
Transport			

ChrA transporter	*Pseudomonas Aeruginosa*	Efflux of cytoplasmic chromate	Alvarez et al. 1999
Cys operon products	*Shewanella Oneidensis*	Sulfate transport	Brown et al. 2006
TonB receptor, hemin Transporter	*S.oneidensis*	Iron Transport	Brown et al. 2006
Reduction			
Chromate Reductases	Diverse species	Reduction of Cr (VI) to Cr (III)	Cervantes et al. 2001
SOD, catalase	*Eschericia coli*	Combat of oxidative stress	Ackerley et al. 2004
Outer membrane proteins	*Caulobacter Crescentus*	General stress response	Hu et al. 2005
DNA repair			
RecG and RuvB DNA helicases	*Pseudomonas Aeruginosa*	Repair of DNA damage	Miranda et al. 2005
SO0368, UvrD and HrpA helicases	*Shewanella Oneidensis*	Repair of DNA damage	Chourey et al. 2006
Other Mechanisms			
Cys operon products	*S. oneidensis*	Sulfur metabolism	Brown et al. 2006
Adenylyl sulfate Kinase	*S.oneidensis*	Sulfur metabolism	Brown et al. 2006
Ferritin	*S.oneidensis*	Iron binding	Brown et al. 2006

2.11 Microbial reduction of hexavalent Chromium

From capacity of the investigation bioremediation have made into a totally promoted development over the span of the latest thirty years into various commercial countries. A productive arrange of bioremediation relies on upon the organization of the population of dirt microbes that is suitable to the pollutants catabolizing. Substantial metals indicate unsafe effects upon the organisms of soil and development of the bacteria of dirt, vital systems of bacteria and also the quantity reduction are inspired by them (Obbard et al., 2001). Masses of bacteria have been frequently recommended as a basic and sensitive indicator of the results of anthropology for the biology of dirt. Hexavalent Chromium is represented for carrying developments in the course of action of the dirt masses of bacteria and impacts problematic outcomes for the assimilation framework of the cell of bacteria at extent concentration. As the disclosure of essential life form suitable for hexavalent Chromium diminishment within 1970s, the mission for the anaerobic and extent-effect bacteria that decrease hexavalent Chromium have been vigorously looked for with different strains becoming segregated (Zhu et al., 2008).

2.11.1 Bioremediation of Chromium

Precipitation, interchange of ion, deterioration or oxidation, use of membrane, adsorption upon cinder, alum, activated coal kaolinite, evaporation and filtration of compound are the standard procedures to oust elements from spoiled locations (Barceloux & Barceloux, 1999; Otha, Galsworthy & Pardee, 1971). In any case, this technique's major portion need greater energy or significant measures of reagents that is chemical with possible making of auxiliary contamination (Jeyasingh & Ligy, 2005; Komori, Rivas, Toda & Ohtake, 1990). Regarding hexavalent Chromium ejection, customary systems fuse compound abatement occurs through adsorption, particle trade upon instituted carbon, slag, alum and kaolinite and also precipitation and most by far of these procedures need greater vitality and one considerable measure of concoction reagents (A. Ganguli & A. Tripathi, 2002). Furthermore, costly harmless exchange of destructive garbage, divided lessening of hexavalent Chromium, greater expense for the diminishment of hexavalent Chromium, especially to remove tolerably lower hexavalent Chromium junctions are not beneficial from productive point of view (Kratochvil, Pimentel & Volesky, 1998; Patterson, 1985).

Bioremediation addresses a creative development that uses bacteria's metabolic ability for ousting metals that are destructive with one particular ultimate objective to sanitize the contaminated areas. In-situ or ex-situ can be called as the frameworks of bioremediation that depends independently upon either the intervention is finished with sensible microscopic organisms particularly upon debased area or upon fragments of normal system such as, liquid, residues or dirt, ensuing so that it can be removed and transferred into proper services for the management (Pattanapipitpaisal & Reakyai, 2013). Bacteria that are impervious to hexavalent Chromium address an indispensable opportunity for being protected, comfortable and environmentally considerate procedures to reduce hexavalent Chromium into trivalent Chromium for possible uses of bio-reduction (Raspor et al., 2000). Then, the reduction of hexavalent Chromium into trivalent Chromium is one likely supportive technique to recover purposes that are corrupted through hexavalent Chromium (Polti, Amoroso & Abate 2010). Departure of hexavalent Chromium through bacteria is by and by supposed for being an intense choice of methodology to normal techniques and accommodating great concern for latent use into bio-reduction (Dey & Paul, 2013; A. Ganguli & A. Tripathi, 2002). The biological conversion of hexavalent Chromium into trivalent Chromium is measured as one of the choice technique to treat polluted squanders of hexavalent Chromium after inspecting that trivalent Chromium's insolvability energizes the ejection and precipitation of it (Cervantes et al., 2001; Ohtake, Cervantes & Silver, 1987). Lessening of hexavalent Chromium by bacteria is eco-obliging and fiscally shrewd and also capable to suggest a applied choice from all other methods of biotechnology (Ge, Zhou, Dong, Lu & Ge, 2013). Cr (VI) decrease through bacteria as purification method and probable bacteria which are impervious towards Chromium are fit for normal lessening of hexavalent Chromium to fewer convenient trivalent Chromium and the subsequent precipitation of it and it may address a reasonable procedure for hexavalent Chromium's contaminated areas purification (Jain, Amatullah, Alam & Mahmud, 2012).

2.11.1.1 Phycoremediation

Integrated in the progresses of biological reduction, phycoremediation uses bacteria that are photosynthetic as macro and micro algae, cyanobacteria to clear toxins as elements. Also, this

is crucial to fathom metal transportation which are adsorbed externally in association with metal amassed into cell, remembering the true objective to grasp the mind-blogging removal systems and for settling upon decisions of possibility of adsorbed elements recovery (Olguin & Sanchez-Galvan, 2012).

2.11.1.2 Biosorption and bioaccumulation

For the determinations of biological reduction, Chromium's bioaccumulation and biosorption are demonstrated. Based on this edge molds and yeasts are mostly investigated and the Chromium imperviousness system of picked bacteria have particular noteworthiness in the innovation of biological remediation. Chromium's framework for purification and lethality are amassed generally into fungi and yeasts. Thus few promising outcomes have created from that system (Poljsak, Pocsi, Raspor & Pesti, 2010). This limit existing into different regular bacteria, recognized for the capacity to tie with elements, may be affirm inside the gastrointestinal microscopic organisms of people. Species of microbe dwell through Lactobacillus class, tenant into different locales like the body of human and inside the developed food, have the ability to tie with elements, comprising hexavalent Chromium and thus purify these from different regions (Monachese, Burton & Reid, 2012).

2.11.1.3 Bio augmentation-assisted phyto-extraction

One biological reduction's strategy of defiled destinations through elements, comprising Cr, is addressed with biological amplification supported phyto-removal and inside that microorganism and fungi are associated through vegetation prepared for collecting elements were breaking dejected upon the introduce of one suggested as biologically processing for the method of biological reduction. Implementation of the biologically extension for maintaining the existence of bacteria was prescribed remembering the true objective for updating the connection between bacteria and plant and capability of this strategy (Lebeau, Braud & Jezequel, 2008).

2.11.1.4 Bio-mineralization

Bacteria alters the watery metal particles comprising Cr in unclear and crystalline precipitous through the technique of bio-mineralization. Bio-mineralization is a auspicious and fiscally perception system to decrease the pollution created by Chromium. An instance of the precipitation of arsenic was measured as one possible framework to biologically reduce arsenic deposit degraded through As (Focardi et al., 2010). Normally intervened control, alteration & noxious elements mineralization can address a fundamental perspective towards biological reduction (Cheng, Holman & Lin, 2012).

Chapter 3: Materials and Methods

3.1 Introduction

Ingredients used are portrayed through this unit & plots outline of the test for biological dilapidation of Cr (VI) by the collection method. It similarly provides a segregation review & interpretation of the strains of microorganism through oceanic liquid. Abilities of tasters of the liquid and dirt attained from the territory of Sitakunda were finely reported. Examinations were additionally done on the culture advancement & method considerations so that cell can be developed and also dilapidation energy of hexavalent Chromium.

3.2 Chemicals

Chemicals which are unadulterated and analytical mark were applied into every investigations comprising organization of media for advancement. Following chemicals have been applied into this investigation:

1) Extract of beef
2) Extract of yeast
3) Agar of nutrient
4) Broth of nutrient
5) Potassium chromate (K_2CrO_4)
6) Diphenyl Carbazide powder &
7) MOPS buffer
8) Mueller Hinton Agar (MHA)

3.3 Glassware and Apparatus

The devices and tools applied during the research are itemized beneath into Table 3.1

Table 3.1: Total number of tools utilized throughout the research and their role

Instruments	**Functions**
Vertical Autoclave	Sterilization
Analytical Balance	Measurement of weight
Laminar airflow	Aseptic atmosphere
pH meter	pH measurement

BOD incubator	Culture incubation
Water system	Stock solution preparation
UV-vis spectrophotometer	Growth of the cell measurement and degradation of hexavalent Chromium measurement
Centrifuge	Pellet collection and measurement of hexavalent Chromium
Water bath	Solubilization of media
Microscope	Observation of bacterial morphology
Shaker	To incubate the culture of bacteria at the temperature of room
Micro pipette	For withdrawing chemical and media's trace quantity

3.4 Collection of sample

Breaking commerce of ship is one of the emerge among maximum fiscally basic & creating wanders of Bangladesh's ocean side regions which shows an impressive measure of biological weaknesses. This commerce is orchestrated inside the oceanfront of Fauzdarhat, Sitakunda from Bhatiary upto Barwalia (Figure: 3.1). It concealments sixteen kilometer extend near the Bay of Bengal's seashore, about twenty kilometer of Chittagong's southwest (DNV, 2001). Forward- facing atmosphere of seashore is extremely powerful and normally grouped running with essential natural and maritime domains, like, woodland of mangrove, consistently and forever submerged marshlands and swamps of salt. Breaking of ship is one method for dismantling unobtainable management ship remembering the true objective for reutilizing piece elements. Bangladesh has been the forerunner into widespread boat's splitting (Ahmed et al., 2013). Mainly costly deadly ships such as ships that are freight and also have partition, tankers are halted by it (Frey, 2013). All the ships reclaimed towards Bangladesh are toxic and outdated ships have age about the years of twenty to thirty (Sarraf et al.,2010). All things

considered, a watercraft includes around the steel's ninety-five percent & risky ingredient's five percent (Khan et al., 2011). Sea is known as one energetic natural group from the time from when dismantling of ship occurs into seashore region, thusly the region of tidal and sub-tidal, isolated sea gradually acquires the chance for hosting the differing kind of wastes. Toxins of microbe, hydrocarbon of petroleum and generous elements are encompassed by the wastes generated through them (Reddy et al., 2005). Substantial elements are combines through oceanic boneless living being, join forces within particulates & adsorption occurs through residue. Everything might be deferred or soluble compound could be created for the storage of Cr (Reddy et el., 2005). All around, one ship which have somewhere five thousand to forty thousand ton of quantity is covered with the dye of ten to one hundred ton and it holds substantial element (Khan et al., 2011).Assessments of liquid and dirt with the deepness of zero to fifteen centimeter accumulated from different regions where the evidence has been found on the presence of Cr and destroyed spot of Sitakunda's Ship breakage Yard.

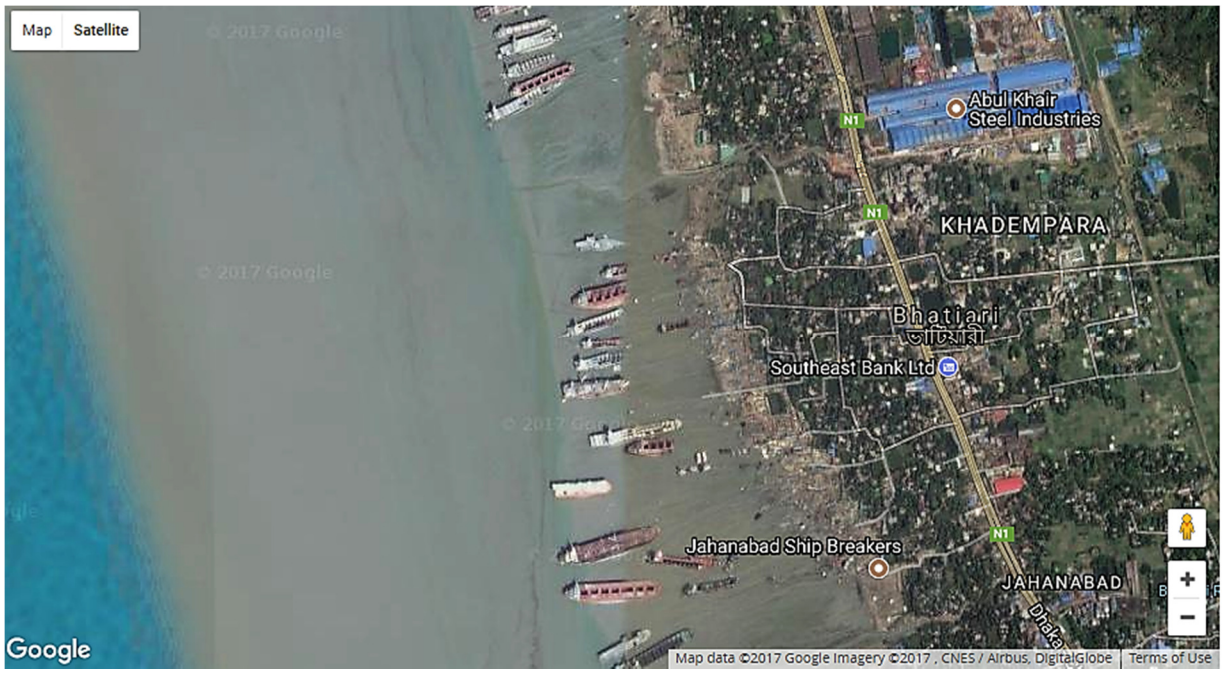

Fig 3.1: Ship breakage industry zone of Chittagong, Bangladesh (Google map, 2017).

3.5 Isolation and condition of the culture

Segregation of microorganisms from samples of the soil assembled had been finished by standard framework. For the withdrawal of microorganism that show imperviousness to Chromium, inoculation was done for one hundred micro-liter specimen of effluent liquid & one hundred micro-liter specimen of soil obtained from unspoiled sample (Into a tube, one gram of dirt had been occupied comprising nine milliliter of the liquid of saline for crushing the soil) through scattering framework into agar dish of nutrient towards media comprising two millimolar of hexavalent Chromium complemented as potassium chromate (K_2CrO_4) and then incubation was done. Some colony of bacteria had been observed taking after incubation for twenty-four hour at thirty-seven degree Celsius temperature. Establishment of the media of nutrient agar was accomplished through melting 2.8gram powder of nutrient agar into one hundred milliliter of H_2O. Sterilization was done for media at 121degree Celsius for forty-five minute with keeping fifteen Lb pressure. By then potassium chromate was incorporated into media and finally that media had been occupied throughout the dish for setting the arrangement of agar dish of nutrient. Bacterial colony that has been segregated contracted with toothpick which were sterilized and speckled upon the agar dish of nutrient on medium comprising two, three, four and five millimolar hexavalent Chromium. Once more it's incubation was done for twenty-four hours at thirty-seven degree Celsius. This system has been repeated with continuously greater focuses of six, seven, ten, fifteen, twenty, twenty-five, thirty, thirty-five, forty millimolar of hexavalent Chromium till MIC (minimum inhibitory concentration) of the restrain of bacteria has been procured. Enormous improvement & rapid debasement energy of hexavalent Chromium of specific species of bacteria inside the forty millimolar hexavalent Cr in the midst of the incubation of 24 hours at thirty-seven degree Celsius has been reflected as impervious to hexavalent Chromium. An introverted strain appropriate for emerging at such circumstance has been decided to do the advance examination. Segregation was done for eight solitary colonies obtained through different agar dishes of nutrient comprising characteristic Cr obsessions near the quarantine technique's termination and provided the label linking with their special center of Cr, such as, two millimolar of dirt for (3) and (4).

3.6 Chromium reduction profile of Chromium resistance bacteria

To investigate H_2O and effluent H_2O, test of Diphenyl Carbazide was done to estimate hexavalent Chromium was attuned through standard procedures (Greenberg et al., 1992) and likewise these approaches were documented into Turick et al., 1996. Arrangement was done for one curve that is standard for institutionalization of depletion chart of the microorganism that show imperviousness to Chromium.

3.6.1 Chemical preparation

3.6.1.1 10ml 3M H_2SO_4 preparation

At first, 8mL purified H_2O was poured into one falcon tube. After that, into falcon tube, 1670 µL conc. H_2SO_4 has been putted on droplet through droplet comprising 8mL purified H_2O. Next, solution's capacity was prepared to the equal of 10mL though putting on 330µL purified H_2O.

3.6.1.2 Diphenyl Carbazide preparation

0.025 diphenyl carbazide powder was added into one falcon tube. Then into falcon tube, 9.67mL acetone and afterward 3M sulphuric acid of 330µL were taken comprising diphenyl carbazaide powder. Mixing was finely done of that falcon tube to prepare uniform solution of DPCZ.

3.6.1.3 MOPS buffer preparation

At first, into 50mL H_2O, 0.1g sodium hydroxide has been taken to prepare 50mL 1N sodium hydroxide. Then, to prepare 20mM buffer of MOPS, MOPS powder of 334.88mg has been putted on with purified H_2O of 80mL. MOPA buffer's pH was attuned into 7 through the addition of adequate quantity of 1N sodium hydroxide inside the solution of buffer.

3.6.1.4 5mM 10mL K_2CrO_4 preparation

At first, for the solution preparation of 1M potassium chromate, 19.4g potassium chromate has been melted into 10mL purified H_2O. Then, filtration was done for this solution utilizing

membrane sieve containing 0.45micron size of the pore. At last, dilution was done for this solution upto 5mM and it was preserved so that it can be utilized in future.

3.6.2 Processes of experiment

3.6.2.1 Standard curve preparation

3.6.2.1.1 Sample preparation for reaction

Subsequent solutions of specimen of various strength have been arranged. Individual specimen's absolute volume was one milliliter.

Table 3.2: Sample preparation for standard curve

Concentration Final	**Quantity of 5mM K_2CrO_4 solution**	**Amount of NB added**	**Final volume to solution**
50µM	10µL	990µL	1ml
100µM	20µL	980µL	1ml
150µM	30µL	970µL	1ml
200µM	40µL	960µL	1ml
300µM	60µL	940µL	1ml
400µM	80µL	920µL	1ml
500µM	100µL	900µL	1ml
600µM	120µL	880µL	1ml

3.6.2.1.2 Reaction protocol for standard curve

Firstly, sample of 600 µL was added into one falcon tube. Then into the sample, 1.2ml 20mM buffer of MOPS, 99 µL 3M sulfuric acid, 981 µL purified H_2O and diphenyl carbazide of 120

µL were taken progressively and thus an uniform blend has been made. When the response occurs into the sample and solution forms, then the solution alters its shade into purple. Finally, the response solution's absorbance has been measured through UV-Visible spectrophotometer at 540nm.

3.6.2.2 Evaluation of reduction profile of selected isolates at room temperature

3.6.2.2.1 Procedure

- **Day 0:**

Broth of nutrient was made into two distinct conical flasks. Volume of each flask is 10mL. From that point forward, from stored culture, specimen was added and then into the broth medium of nutrient, inoculation was done. Control reflected that broth medium of nutrient where inoculation was not done. Finally, upon the rotating incubator, both of the 10mL conical flasks were given for incubation for 24hours.

- **Day 1:**

On following day, broth of nutrient of 25mL was made into two distinct conical flasks. To prepare 600µL potassium chromate (K_2CrO_4), 15µL K_2CrO_4 was putted on the broth of nutrient. Then 2mL solution of the culture was removed from that flask containing culture and at former day, it was in the incubator at the temperature of room. At next, for sample. positive control (E. coli) and negative control (blank), the solution of the culture's OD (optical density) has been recorded through UV-visible spectrophotometer at the wavelength of 600nm to observe the development of cell. Furthermore, calculation was done for the quantity needed from the 24hours culture for obtaining 0.2 optical density into test culture. After that, for withdrawing the needed quantity of 24hrs culture, utilization was done for one sterilized falcon tube for the sample, blank and E .coli. Then these were vortexed and after that for five minute, centrifugation was done for samples, blank and E.coli at the rpm of 4000. After the centrifugation of the needed quantity of the overnight pellets of culture was done, then these

have been carried and putted in 25ml broth of nutrient that were freshly organized and it were complemented with 600µL potassium chromate which contain hexavalent Chromium and moreover, it has been vortexed finely for obtaining culture's OD of 0.2. Afterward, at the temperature of room, culture that has been newly made, was given for incubation. Subsequently to 1.5hours, from 25ml conical flask of sample, positive and negative control, 2ml test cultures were withdrawn and optical density has been recorder at the wavelength of 600nm for observing the cell development. At next, these cultures of 2ml were vortexed and then for five minutes, centrifugation was done at the rpm of 4000 and thus bacteria were precipitated at the bottom of falcon tube and supernatant were obtained at the top of falcon tube and 600µL was withdrawn from the upper supernatant. Then the indistinguishable reaction procedure as stated into 3.6.2.1.2 section was driven and optical density was measured at the wavelength of 540nm. This methodology was reiterated following 3rd hour, 4.5th hour, 6th hour and after the growth of bacteria for 24hours (overnight), the final measurement was recorded. Into the v8.0 of OriginPro these data were set for obtaining the lessening profile.

3.7 Antibiotic resistance among Chromium resistant isolates:

3.7.1 Strain Culture preparation in Nutrient broth (NB)

For the sensitivity investigation of antibiotic, broth of nutrient was made for culturing strains. Into one conical flask, nutrient broth of 20ml was added and inoculation was done for the subsequent strains in the individual conical flasks comprising 20ml nutrient broth and incubation was done for overnight at 37 degree Celsius. Labeling was done into the conical flasks.

3.7.2 Inoculation of test plates

For the preparation of test plates, Mueller Hinton Agar (MHA) was utilized. Incubated strains of culture were withdrawn after overnight for their inoculation in the plates of MHA. One cotton swab which were sterilized was immersed in conical flask comprising the preparation of culture strain. Inoculation was done on MHA plate's dry exterior through moving the cotton swab upon whole sterile surface of the MHA. This method has been reiterated through moving

2 more periods, for each period, circling that plate about 60º for ensuring inoculum's uniform spread. Finally, the agar's rim was mopped.

For three to five minutes, the cover was opened, however, it could not left to be open after exceeding fifteen minutes so that no extra moisture of the surface can be absorb before giving antibiotic disks.

3.7.3 Application of Antibiotic discs

Antibiotic disc's fixed battery was distributed on MHA plate's surface which has been gone through inoculation. Individual discs were pushed down for ensuring of the thorough contact within the surface of agar. Following antibiotic discs were utilized in this test:

- ✓ Chloramphenicol (C:30 mg)
- ✓ Ciprofloxacin (Cip:5 mg)
- ✓ Gentamicin (CN: 10 mg)
- ✓ Ofloxacin (OF: 5 mg)
- ✓ Vancomycin (VA: 30mg)
- ✓ Sulphametronazol / Trimethoprim (SXT: 25 mg)
- ✓ Azithromycin (AZM:15mg)
- ✓ Neomycin (N: 30 mg)
- ✓ Ceftriaxone (CRO: 30 mg)
- ✓ Cefuroxime Sodium (CXM: 30 mg)
- ✓ Penicillin-G (P: 10 mg)

3.7.4 Incubation

Into the incubator, within fifteen minutes, the test plates were positioned at 37ºC for 24 hours after disc application has been done.

3.8 Determination of Minimum Inhibitory Concentration (MIC):

Into microbiology, MIC is an anti-bacterial minimum concentration which will prevent one bacteria's observable development after the incubation of 24hour. Chromate's minimum

inhibitory concentration for individual isolate was expressed through counting technique of colony. Inoculation was done of the plates comprising agar media of nutrient complemented with various concentrations of potassium chromate (K_2CrO_4) from 2mM – 30mM with 50µL culture of 24hours developed into broth media of nutrient. Then all of these plates was given for incubation at the temperature 37ºC for forty-eight hours. Finally, the bacterial development was measured through counting of the colony.

3.9 Identification of the isolate S_2:

Obtained sequence data file of S_2 isolate by 16s rDNA sequencing. Observed the sequence by Chromas tool and purified sequence data. Saved the file format as FASTA. BLAST (Basic Local Alignment Search Tool) was done of the query sequence with existing database from NCBI (National Center for Biotechnology Information). Bacterial strain was obtained on the basis of maximum similarity score.

Chapter 4: Results

4.1 Isolation data of Chromium resistance bacteria

Isolation was done for the individual colonies of two from the Agar dishes of nutrient with various concentrations of Cr. Labelling was done conferring toward distinctive concentrations of Cr of them such as S_2.

4.2 Chromium reduction profile of Chromium resistant bacteria

4.2.1 Standard Curve

Method stated into 3.6.2.1.2 was done to obtain the standard curve. After that, utilizing the value of absorbance, plotting was done of one graph using Microsoft Excel Software of 2016. Results which have been obtained are given below:

Table 4.1: Data of standard curve of hexavalent Chromium:

Concentration (µM)	Absorbance
50	0.294
100	0.624
150	0.907
200	1.214
300	1.675
400	2.117
500	2.587
600	2.875

From these data, one standard curve was found and that is given below:

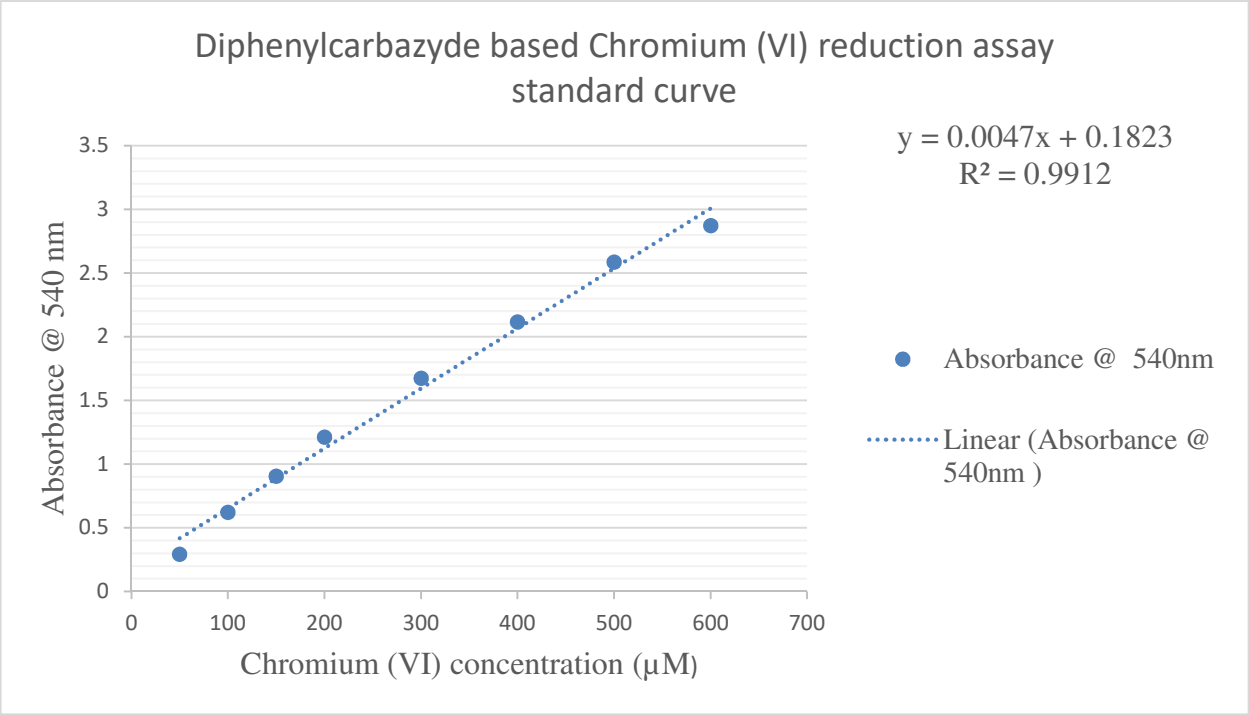

Figure 4.1: Standard curve of hexavalent Chromium

4.2.2 Reduction profile of Isolate: S_2

At various constraints, this test was done such as, altering the pH and temperature and the outcomes which was attained precise underneath:

Table 4.2: Isolate S_2: Chromium reduction profile Vs. Cell Growth at 25ºC, pH 7

Sample			Negative Control	
Time (Hours)	**Chromium concentration on (µM) at 540 nm**	**Bacterial concentration at 600 nm**	**Chromium concentration on (µM) at 540 nm**	**Bacterial concentration at 600 nm**
0	377.6666667	0.872	432.2765957	0.098
1.5	282.0638298	1.07	366.9574468	0.108

3.0	268.8723404	1.67	385.4680851	0.109
4.5	229.5815603	2.052	344.1914894	0.099
6.0	186.248227	2.135	385.4680851	0.098
24	86.17730496	2.771	386.106383	0.102

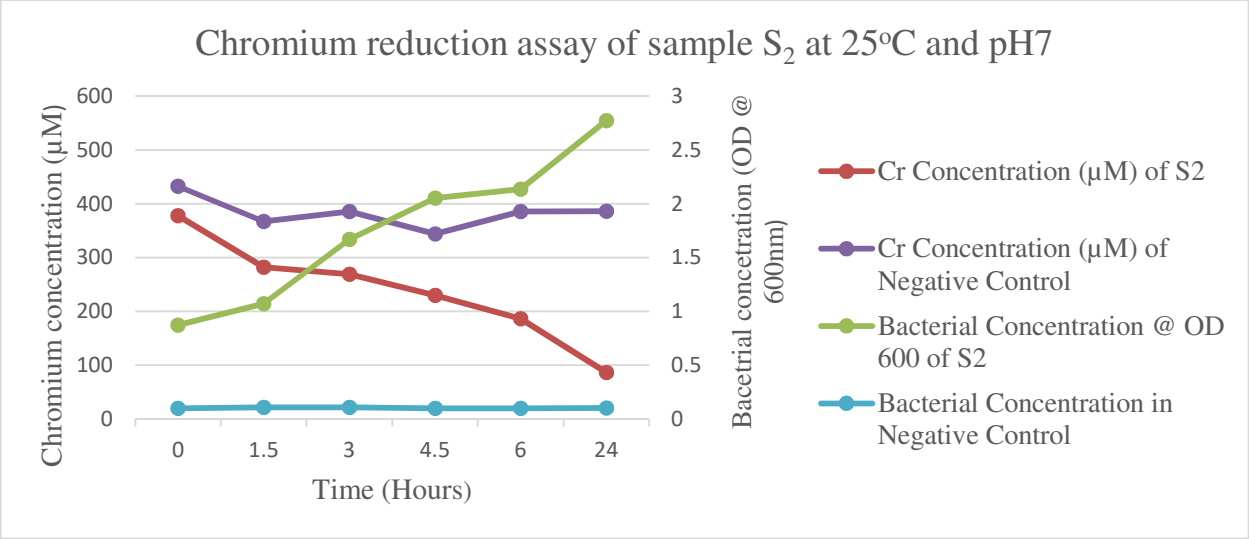

Figure 4.2: Chromium reduction Vs. Cell Growth in S_2 isolate at 25°C, pH 7

From the above **Figure 4.2** it was clear that in sample S_2 at 0hour, Chromium concentration was around 377.67 at 540nm. Then, a dramatic fall of the concentration of Chromium was seen after 1.5hour, which was 282.06 at 540nm. Chromium concentration continued to decrease in the following hours and lastly, a marked reduction of Chromium has been observed after 24hours, which was 86.177. Therefore, 77% reduction of Chromium in 24 hours was obtained at 540nm. Moreover, in sample S_2, a sharp growth of bacterial concentration was observed at 600nm from 0.872 to 2.771 in 24 hours. But no significant reduction of chromium concentration or bacterial growth was obtained in the negative control. Hence, it is observable that, sample S_2 is not only resistant towards Chromium, but also proficient of lessening the number of Chromium at 25°C, pH 7.

Table 4.3: Isolate - S_2: Chromium reduction profile Vs. Cell Growth at 25ºC, pH 5.5

Sample			Negative Control	
Time (Hours)	**Chromium concentration on (µM) at 540 nm**	**Bacterial concentration at 600 nm**	**Chromium concentration on (µM) at 540 nm**	**Bacterial concentration at 600 nm**
0	219.4397163	0.599	432.2765957	0.098
1.5	144.1914894	0.678	366.9574468	0.108
3.0	107.9503546	0.682	385.4680851	0.109
4.5	106.7446809	0.693	344.1914894	0.099
6.0	69.36879433	0.721	385.4680851	0.098
24	22.63120567	0.707	386.106383	0.102

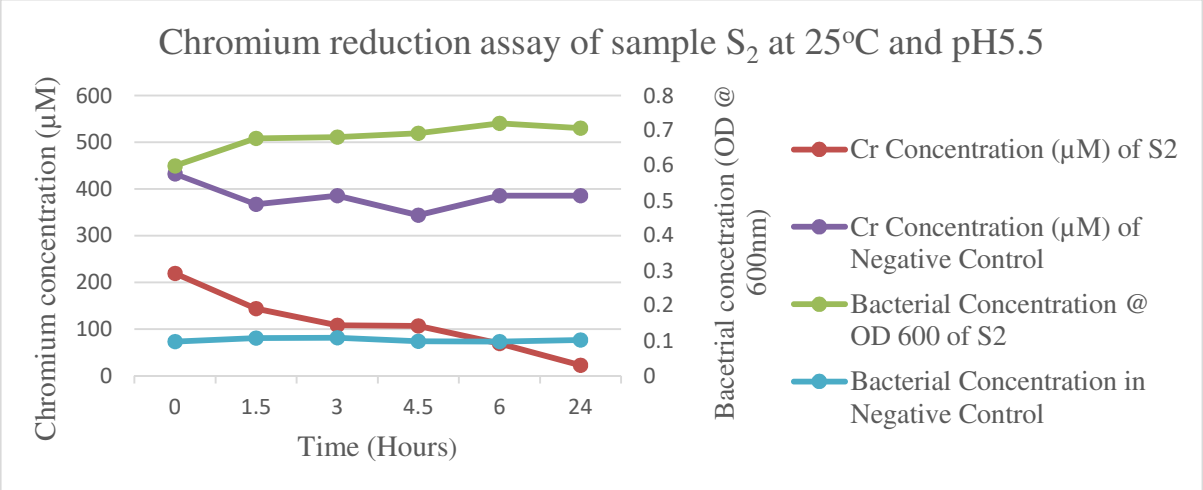

Figure 4.3: Chromium reduction Vs. Cell Growth in S_2 isolate at 25ºC, pH 5.5

From the above **Figure 4.3** it was clear that, in sample S_2 at 0hour, Chromium concentration was around 219.44 at 540nm. Then, gradually, the decrease in Chromium concentration was seen in the following hours at 540nm. Finally, a marked reduction of the concentration of

Chromium was found after 24 hours which was 22.63. Therefore, in 24 hours, 89% reduction of Chromium was obtained at 540nm. Moreover, minimal growth of bacterial concentration was observed in sample S_2 from 0.599 to 0.707 in 24 hours at 600nm. But no significant bacterial growth or Chromium reduction was obtained in the negative control. Therefore, it is observable that, sample S_2 can reduce the number of Chromium and also shows minimal resistant to Chromium at 25ºC, pH 5.5.

Table 4.4: Isolate-S_2: Chromium reduction profile Vs. Cell Growth at 25ºC, pH 8.5

Sample			**Negative Control**	
Time (Hours)	**Chromium concentration on (µM) at 540 nm**	**Bacterial concentration at 600 nm**	**Chromium concentration on (µM) at 540 nm**	**Bacterial concentration at 600 nm**
0	223.7659574	0.735	432.2765957	0.098
1.5	185.1134752	0.803	366.9574468	0.108
3.0	160.5744681	1.087	385.4680851	0.109
4.5	132.9148936	1.528	344.1914894	0.099
6.0	98.09219858	1.789	385.4680851	0.098
24	31.70921986	2.665	386.106383	0.102

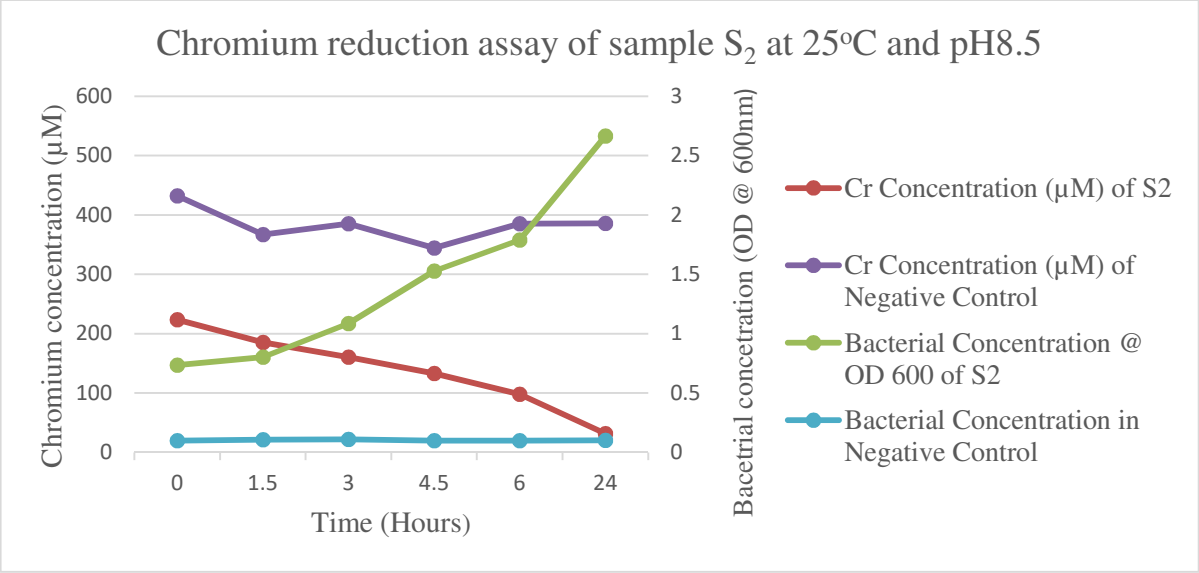

Figure 4.4: Chromium reduction Vs. Cell Growth in S_2 isolate at 25ºC, pH 8.5

From the above **Figure 4.4** is has been observed that, at 0hour, concentration of Chromium was approximately 223.76 at 540nm in sample S_2. After that, concentration of Chromium was rapidly declining and after 6hour, Chromium concentration in that sample was 98.09 at 540nm. At last, significant drop of Chromium concentration was found after 24 hours which was 31.709. Hence, 86% reduction of Chromium was seen for sample S_2 at 540nm. Meanwhile, a sharp growth of bacterial concentration of S_2 was recorded at 600nm from 0.735 to 2.665. Yet, no noteworthy reduction of the concentration of Chromium or growth of bacteria was obtained in negative control. As a result, it can be said that, sample S_2 is both resistant towards Chromium and capable of reducing Chromium concentration at 25ºC, pH 8.5.

Table 4.5: Isolate-S_2: Chromium reduction profile Vs. Cell Growth at 37ºC, pH 7

Sample			**Negative Control**	
Time (Hours)	**Chromium concentration on (µM) at 540 nm**	**Bacterial concentration at 600 nm**	**Chromium concentration on (µM) at 540 nm**	**Bacterial concentration at 600 nm**
0	376.177305	0.871	286.5319149	0.138

1.5	355.1843972	0.904	286.106383	0.149
3.0	330.929078	1.426	344.8297872	0.102
4.5	295.822695	2.102	322.4893617	0.1
6.0	244.5460993	2.333	345.0425532	0.098
24	47.95035461	2.728	314.1914894	0.131

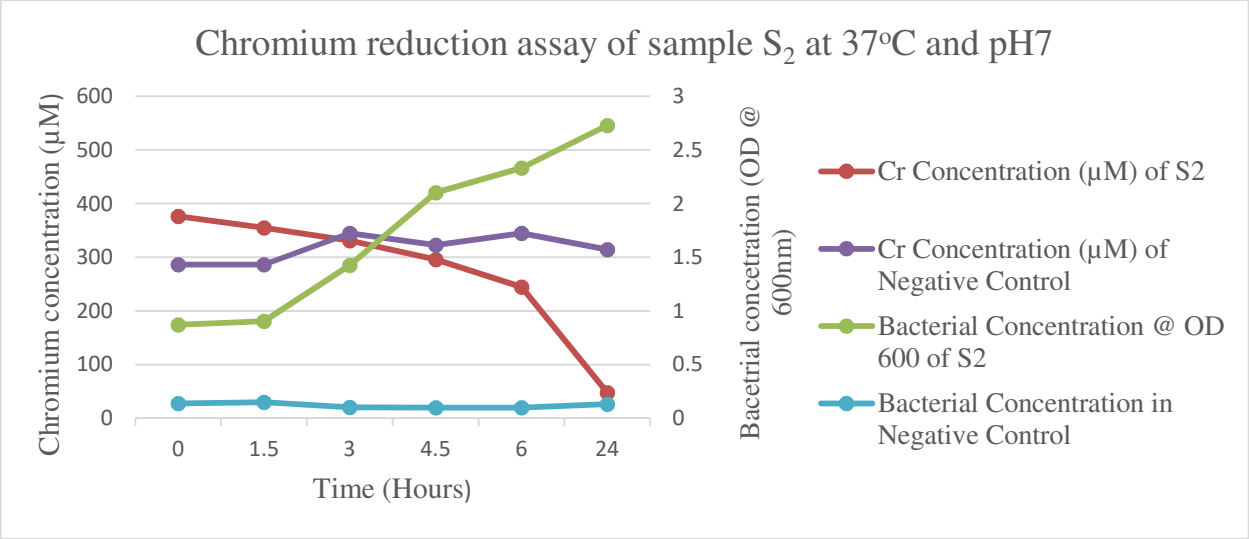

Figure 4.5: Chromium reduction Vs. Cell Growth in S_2 isolate at 37ºC, pH 7

From the above **Figure 4.5** it was clear that, at 0hour, Chromium concentration was around 376.18 at 540nm in the sample of S_2. Concentration of Chromium was gradually decreasing until 6hour, which was 244.55. But a dramatic fall down of Chromium concentration was observed after 24 hours which was approximately 47.95. Thus 87% reduction of Chromium was obtained between 24 hours at 540nm. In addition, a rapid growth of bacterial concentration of S_2 was seen at 600nm from 0.871 to 2.728. But no significant reduction of Chromium concentration or bacterial growth was obtained in the negative control. Therefore, it can be said that, sample S_2 shows remarkable resistance to Chromium and it can also decrease Chromium number.

Table 4.6: Isolate-S_2: Chromium reduction profile Vs. Cell Growth at 37ºC, pH 5.5

Sample			Negative Control	
Time (Hours)	**Chromium concentration on (µM) at 540 nm**	**Bacterial concentration at 600 nm**	**Chromium concentration on (µM) at 540 nm**	**Bacterial concentration at 600 nm**
0	358.8723404	0.113	286.5319149	0.138
1.5	298.8014184	0.118	286.106383	0.149
3.0	274.1205674	0.184	344.8297872	0.102
4.5	263.2695035	0.195	322.4893617	0.1
6.0	224.4042553	0.339	345.0425532	0.098
24	17.5248227	2.69	314.1914894	0.131

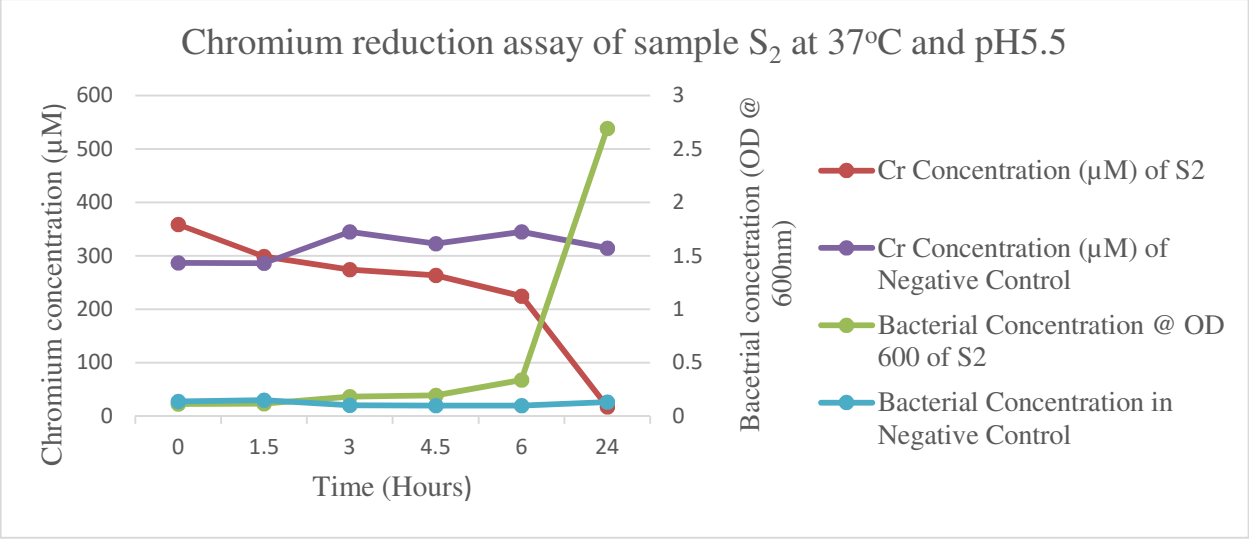

Figure 4.6: Chromium reduction Vs. Cell Growth in S_2 isolate at 37ºC, pH 5.5

From the about **Figure 4.6** it can be seen that, at 0hour in sample S_2, concentration of Chromium was around 358.87 at 540nm. Then, a sharp decrease of Chromium concentration was seen after 1.5hour, which was 298.81 of that sample. After 24 hours, Chromium concentration was dramatically reduced into 17.53. Thus, 95% reduction of Chromium concentration was done by S_2 isolate at 540nm. Meanwhile, bacterial growth was observed gradually from 0.113 to 2.69 in sample S_2 at 600nm. However, no significant bacterial growth or reduction of Chromium was seen in the negative control. Therefore, it has been clear that, sample S_2 has the capability to show noteworthy resistance to Chromium and to reduce the concentration of Chromium at 37ºC, pH 5.5.

Table 4.7: Isolate-S_2: Chromium reduction profile Vs. Cell Growth at 37ºC, pH 8.5

Sample			**Negative Control**	
Time (Hours)	**Chromium concentration on (µM) at 540 nm**	**Bacterial concentration at 600 nm**	**Chromium concentration on (µM) at 540 nm**	**Bacterial concentration at 600 nm**
0	291.9219858	1.188	286.5319149	0.138
1.5	264.2624113	1.285	286.106383	0.149
3.0	233.6950355	1.465	344.8297872	0.102
4.5	201.2836879	1.921	322.4893617	0.1
6.0	173.6241135	2.219	345.0425532	0.098
24	65.18439716	2.987	314.1914894	0.131

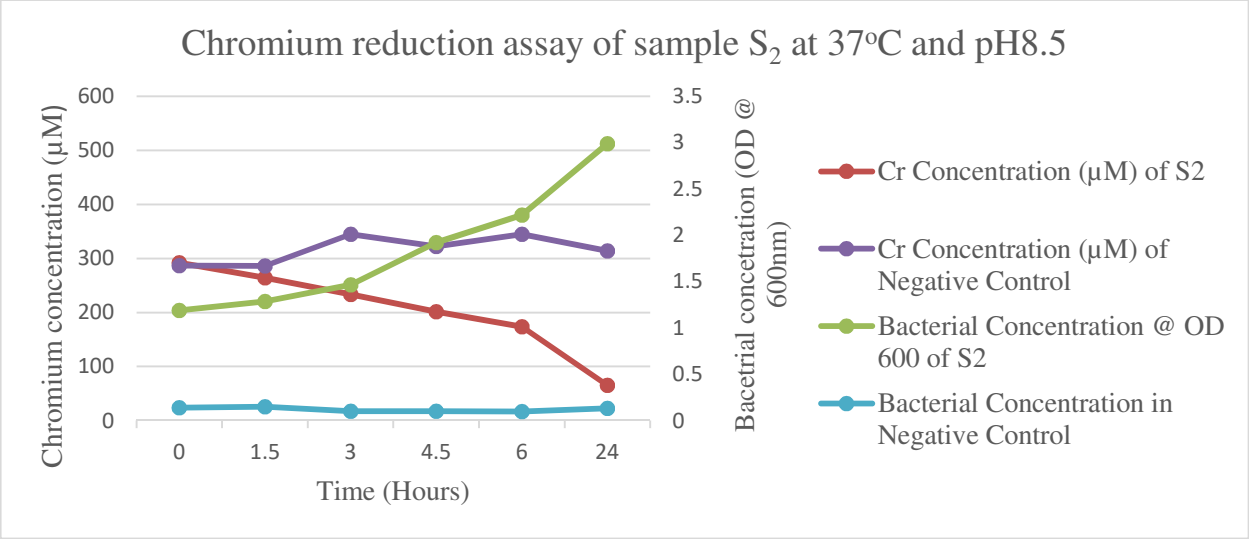

Figure 4.7: Chromium reduction Vs. Cell Growth in S_2 isolate at 37ºC, pH 8.5

From the above **Figure 4.7** it was clear that, in sample S_2 at 0hour, Chromium concentration was 291.92 at 540nm. The concentration of Chromium of that sample was gradually decreasing until 6hour, which was 173.63 and then, a marked reduction of Chromium concentration was observed after 24 hours, which was 65.18. Hence, 77% reduction of Chromium concentration was seen at 540nm. Moreover, a sharp growth of bacteria was observed in sample S_2 from 1.188 to 2.987 at 600nm. But in the negative control, no significant reduction of Chromium concentration or bacterial growth was obtained. Therefore, it can be said that, sample S_2 is not only resistant towards Chromium, but also proficient of lowering the number of Chromium at 37ºC, pH 8.5.

Table 4.8: Isolate-S_2: Chromium reduction profile Vs. Cell Growth at 42ºC, pH 7

Sample			**Negative Control**	
Time (Hours)	**Chromium concentration on (µM) at 540 nm**	**Bacterial concentration at 600 nm**	**Chromium concentration on (µM) at 540 nm**	**Bacterial concentration at 600 nm**
0	335.6099291	0.413	350.787234	0.084

1.5	301.8510638	0.909	350.1489362	0.098
3.0	158.7304965	1.181	346.3191489	0.091
4.5	138.3758865	2.105	326.3191489	0.098
6.0	118.8723404	2.182	325.893617	0.086
24	31.56737589	2.404	338.0212766	0.079

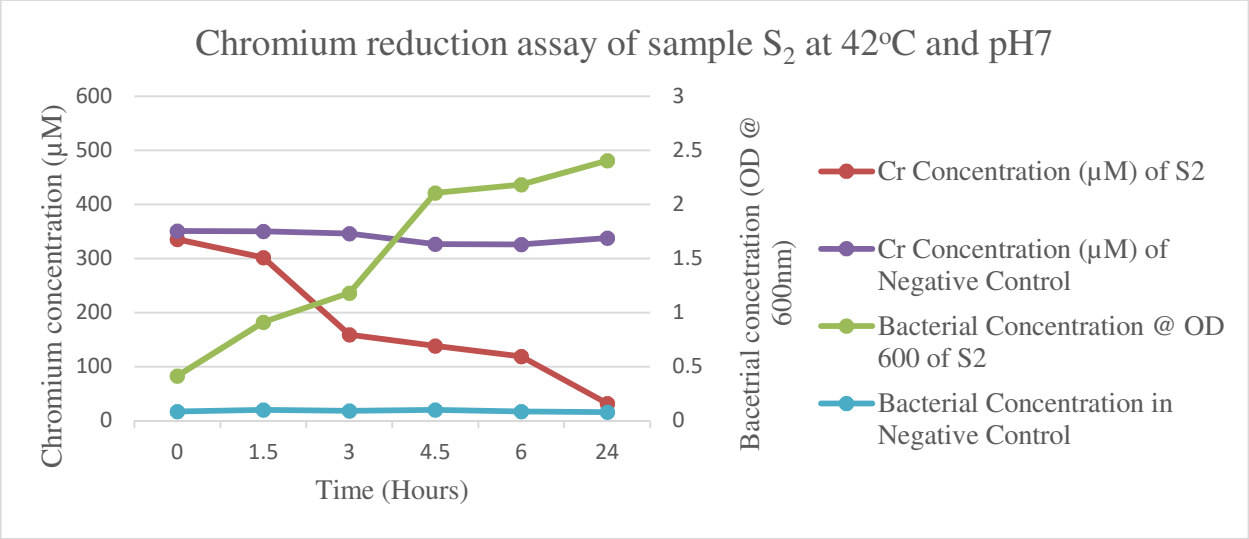

Figure 4.8: Chromium reduction Vs. Cell Growth in S_2 isolate at 42ºC, pH 7

From the above **Figure 4.8** it can be said that, at 0hour in sample S_2, Chromium concentration was around 335.61 at 540nm. After passing 3hour, a dramatic decrease of Chromium concentration was observed which was 158.73 in that sample. Finally, after 24 hours Chromium concentration was rapidly reduced into 31.57. Thus, 90% reduction of the concentration of Chromium was obtained in 24 hours at 540nm. Moreover, in sample S_2, a sharp growth of bacterial concentration was seen at 600nm from 0.413 to 2.404. However, in the negative control, no growth of bacteria or no noticeable reduction of Chromium was observed. Therefore, it has been clear that, sample S_2 is resistant to Chromium and can also decrease the number of Chromium at 42ºC, pH 7.

Table 4.9: Isolate-S_2: Chromium reduction profile Vs. Cell growth at 42ºC, pH 5.5

Sample			Negative Control	
Time (Hours)	**Chromium concentration on (µM) at 540 nm**	**Bacterial concentration at 600 nm**	**Chromium concentration on (µM) at 540 nm**	**Bacterial concentration at 600 nm**
0	335.6099291	0.316	350.787234	0.084
1.5	291.070922	0.732	350.1489362	0.098
3.0	132.9858156	0.885	346.3191489	0.091
4.5	116.106383	1.304	326.3191489	0.098
6.0	95.60992908	1.499	325.893617	0.086
24	15.53900709	1.41	338.0212766	0.079

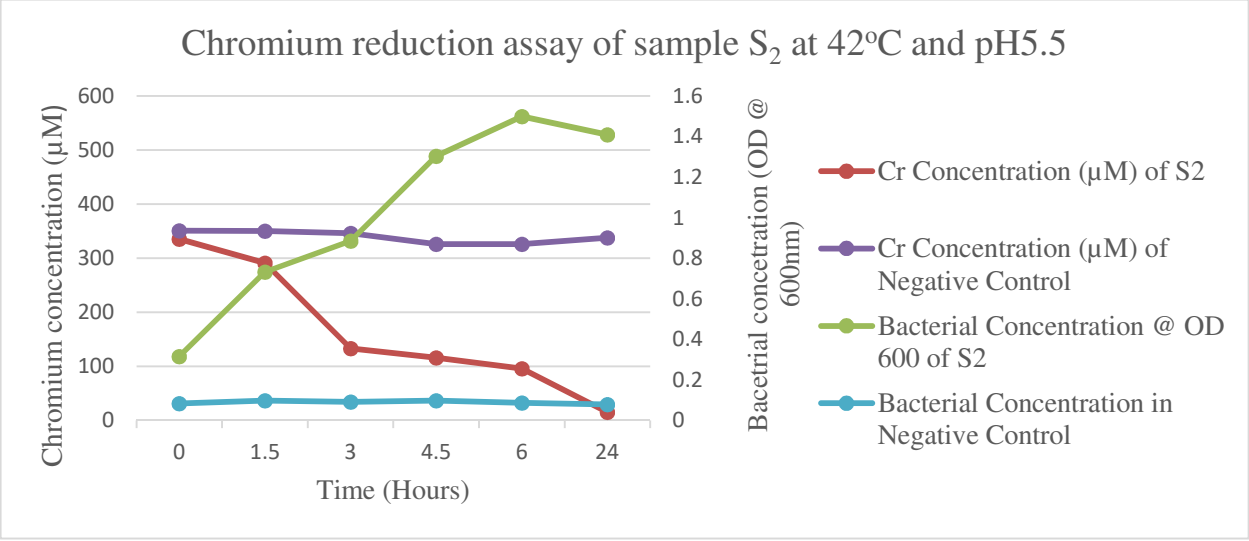

Figure 4.9: Chromium reduction Vs. Cell Growth at 42ºC, pH 5.5

From the above **Figure 4.9** it was clear that, in sample S_2 at 0hour, Chromium concentration was 335.61 at 540nm. Then, a dramatic fall of the concentration of Chromium was seen after 3hour, which was 132.98 at 540nm. Chromium concentration continued to decrease in the following hours and lastly, a marked reduction of Chromium concentration was observed after 24 hours, which was 15.54. Hence, 96% reduction of Chromium concentration was obtained between 24 hours at 540nm. Moreover, in sample S_2, a sharp growth of bacterial concentration was observed from 0.316 to 1.41 at 600nm in 24 hours. But no reduction of Chromium or bacterial growth was seen in the negative control. Therefore, it can be said that, sample S_2 is not only resistant towards Chromium, but also proficient of lessening the Chromium at 42ºC, pH 5.5.

Table 5.0: Isolate-S_2: Chromium reduction profile Vs. Cell growth at 42ºC, pH 8.5

Sample			**Negative Control**	
Time (Hours)	**Chromium concentration on (µM) at 540 nm**	**Bacterial concentration at 600 nm**	**Chromium concentration on (µM) at 540 nm**	**Bacterial concentration at 600 nm**
0	298.4468085	0.457	350.787234	0.084
1.5	223.8368794	0.823	350.1489362	0.098
3.0	146.035461	0.936	346.3191489	0.091
4.5	135.4680851	1.593	326.3191489	0.098
6.0	121.4255319	1.95	325.893617	0.086
24	14.04964539	2.007	338.0212766	0.079

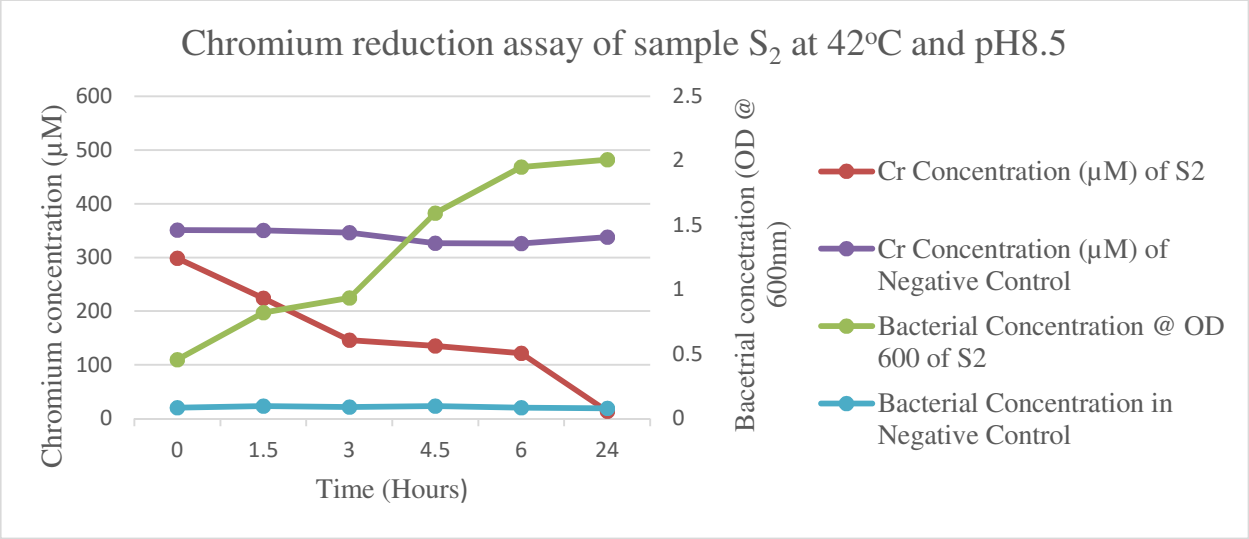

Figure 5.0: Chromium reduction Vs. Cell Growth in S_2 isolate at 42ºC, pH 8.5

From the above **Figure 5.0** it can be observed that, concentration of Chromium was approximately 298.45 at 540nm in sample S_2 at 0hour. After that, Chromium concentration was decreasing rapidly and at 6hour, the concentration was 121.43 at 540nm. Finally, after 24 hours, the concentration of Chromium was dramatically reduced and became 14.05 of sample S_2. Thus, between 24 hours, 95% reduction of the concentration of Chromium occurred at 540nm. Meanwhile, a sharp growth of bacterial concentration was obtained at 600nm from 0.457 to 2.007 in the sample of S_2. However, no bacterial growth or reduction of Chromium was happened in the negative control. Therefore, it can be said that, sample S_2 can decline the Chromium concentration and it is also capable to show resistance to Chromium at 42ºC, pH 8.5.

4.3 Antibiotic resistance among Chromium resistant isolate S_2:

This test was done by distributing and fixing 11 discs of Antibiotic on the surface of MHA plates which have been gone through inoculation and then incubation was done for 24 hours. After incubation, the activities of antibiotic discs against S_2 isolate were determined by measuring the diameter of zone of inhibition in millimeter with the help of a transparent scale. The outcomes which has been attained are given below:

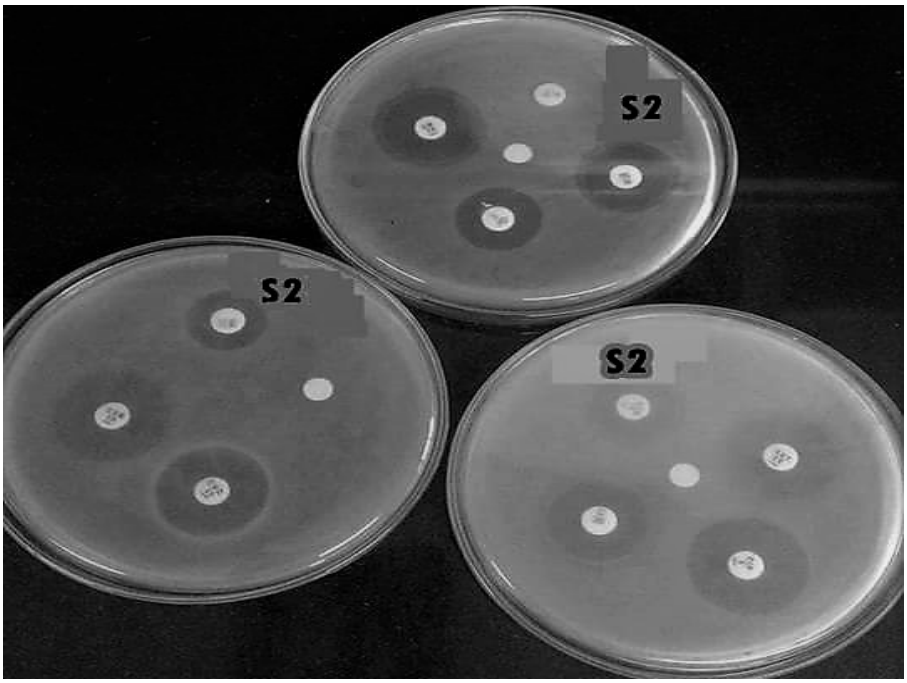

Figure 5.1: Zone of Inhibition of Antibiotic discs in S_2 isolate

Table 5.1: Isolate-S_2: Antibiotic resistance profile

Name of Antibiotic disc	Diameter of Zone of Inhibition (ZI) of S_2 on (mm)
1. Chloramphenicol (C:30mg)	25
2. Ciprofloxacin (Cip:5mg)	26
3. Gentamicin (CN:10mg)	24
4. Ofloxacin (OF:5mg)	20
5. Vancomycin (VA:30mg)	22
6. Sulphametronazol/Trimethoprim (SXT:25mg)	27

7. Azithromycin (AZM:15mg)	17
8. Neomycin (N:30mg)	19
9. Ceftriaxone (CRO:30mg)	18
10. Cefuroxime Sodium (CXM:30mg)	16
11. Penicillin-G (P:10mg)	12

From the **Table 5.1** it can be said that, for bacterial isolate S_2, the highest zone of inhibition was recorded for Sulphametronazol/Trimethoprim which was 27mm. Therefore, the performance of Sulphametronazol/Trimethoprim was more potent than all other antibiotics against the bacterial isolate of S_2 because this antibiotic disc was capable for killing the most bacterial cells of S_2 strain surrounding the disc. S_2 could not show that much resistance against this antibiotic or could not resist the effects of this antibiotic and S_2 was mostly susceptible against Sulphametronazol/Trimethoprim. In addition, the lowest zone of inhibition was recorded for Penicillin-G which was 12mm. Hence, function of Penicillin-G was very poor than all other antibiotics against S_2 isolate because Penicillin-G could not inhibit the bacterial growth of S_2 strain significantly. S_2 isolate was mostly resistant against Penicillin-G and less susceptible against this antibiotic. Overall, isolate S_2 was more or less susceptible against the remaining antibiotic discs. Ciprofloxacin, Chloramphenicol, Gentamicin, Vancomycin exhibited intensive antibiotic activity from 26mm-22mm respectively and Ofloxacin, Neomycin, Ceftriaxone, Azithromycin, Cefuroxime Sodium showed mild to moderate antibiotic activity from 20mm-16mm accordingly.

4.4 Minimum Inhibitory Concentration of Chromium to inhibit the growth of Chromium resistance bacteria:

4.4.1 MIC of isolate: S_2

In different concentrations of Chromium, different number of colonies have been found and they are tabulated below:

Table 5.2: MIC of isolate S_2

Concentration	**Number of Colonies**
2mM	23
4mM	24
6mM	24
8mM	21
10mM	15
12mM	16
14mM	12
16mM	13
18mM	14
20mM	9
22mM	7
23mM	7
24mM	0
25mM	0
27mM	0
28mM	0
29mM	0
30mM	0

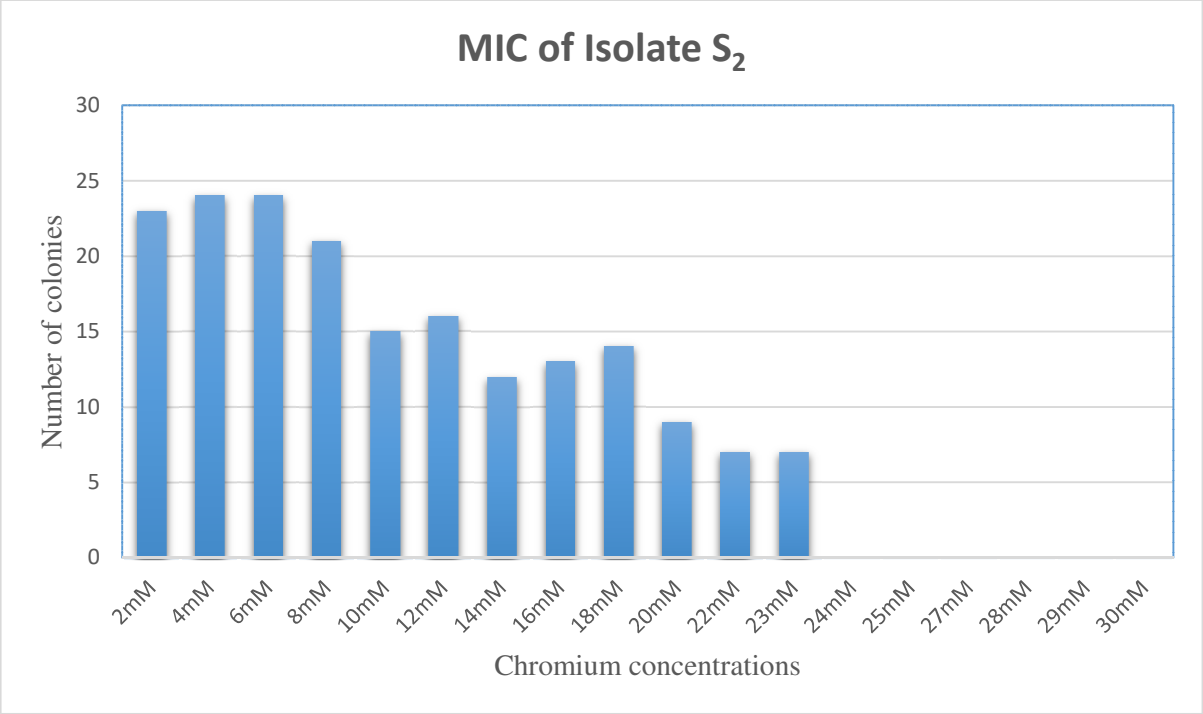

Figure 5.2: MIC of isolate: S_2

From the **Table 5.2** it was seen that, the isolate S_2 tolerates the Chromium concentration up to 23mM, which means, S_2 was able to show resistance till the Chromium concentration of 23mM. But isolate S_2 was totally susceptible from the Chromium concentration of 24mM because no colony was found in 24mM Chromium plate and also in more higher concentration Chromium plate. Thus, 24mM was the Minimum Inhibitory Concentration for S_2 isolate.

4.5 Identification of isolate S_2:

16s rDNA sequencing was used for the identification of the bacterial isolate and then, BLAST was done with the purified sequence data. The result is tabulated below.

Table 5.3: BLAST result of the isolate S_2

Description	Max Score	Total Score	Query cover	E value	Ident	Accession
Bacillus toyonensis **strain BCT-7112 16s ribosomal RNA gene, complete sequence**	1677	1677	100%	0.0	99%	NR 121761.1
Bacillus cereus **ATCC 14579 16s ribosomal RNA (rRNA) gene, complete sequence**	1677	1677	100%	0.0	99%	NR 074540.1
Bacillus thuringiensis **strain NBRC 101235 16s ribosomal RNA gene, partial sequence**	1677	1677	100%	0.0	99%	NR 112780.1
Bacillus pseudomycoides **strain NBRC 101232 16s ribosomal RNA gene, partial sequence**	1672	1672	100%	0.0	99%	NR 113991.1
Bacillus mycoides **strain NBRC 101228 16s ribosomal RNA gene, partial sequence**	1666	1666	100%	0.0	99%	NR 113990.1
Bacillus weihenstephanensis **strain DSM 11821 16s ribosomal RNA gene, partial sequence**	1666	1666	100%	0.0	99%	NR 024697.1

***Bacillus bingmayongensis* strain FJAT-13831 16s ribosomal RNA, partial sequence**	1659	1659	100%	0.0	99%	NR 148248.1
***Bacillus gaemokensis* strain BL3-6 16s ribosomal RNA gene, partial sequence**	1644	1644	100%	0.0	99%	NR 116644.1
***Bacillus manliponensis* strain BL4-6 16s ribosomal RNA gene, partial sequence**	1633	1633	100%	0.0	99%	NR 125530.1
***Bacillus cytotoxicus* strain NVH 391-98 16s ribosomal RNA gene, complete sequence**	1628	1628	100%	0.0	99%	NR 074914.1

From the **Table 5.3** it can be said that, above bacterial strains show maximum similarity score which is 99%. Therefore, strains of *Bacillus toyonensis, Bacillus cereus, Bacillus thuringiensis, Bacillus pseudomycoides, Bacillus mycoides, Bacillus weihenstephanensis, Bacillus bingmayongensis, Bacillus gaemokensis, Bacillus manliponensis or Bacillus cytotoxicus* can be the probable bacterial strain of our sample S_2. To identify the exact bacterial strain, biochemical test can be done in future for conclusive result.

4.5 Discussion

People are discharging huge measures of Chromium into atmosphere because of pervasive utilization of Chromium into industries such as, textile, tannery of leather, generation of pulp & production of dyes and so forth. Cr (VI) is to a great degree toxic and exhibits mutagenic cancer-causing impact on organic framework in light of the solid nature of oxidization of it. Different diminishment and resistance ability have been created by bacteria for adjusting to the poisonousness of chromate.

Into this research, isolation was done for those bacteria which show resistance to Chromium and the capacity for reducing the cancer-causing Chromium effectively was discovered in those bacteria.

This analysis was done with S_2 isolate at various parameters. Plotting of graphs were done through utilizing the outcomes. By investigating all the data, it was obviously observed that, the S_2 isolate showed resistance and meanwhile, was capable for reducing Chromium concentration at different temperature and pH. Isolate S_2 showed significant reduction of Chromium when they were incubated at 37ºC and pH 5.5, then at 42ºC in the pH of 7, 5.5, 8.5. Among all parameters, optimum temperature and pH for the reduction of Chromium concentration was 42ºC, pH 5.5 because 96% reduction was done in this parameter. This examination's result has portrayed and perceived one fresh strain which has resistive and diminishing power against Chromium.

In the test of antibiotic resistance profile, it was clear that, isolate S_2 showed resistance against Ofloxacin, Neomycin, Ceftriaxone, Azithromycin and Cefuroxime Sodium. It showed the greatest resistance against Penicillin-G. Here, the isolate exhibited the greatest susceptibility towards the Sulphametronazol/Trimethoprim. It also exhibited moderate susceptibility towards Ciprofloxacin, Chloramphenicol, Gentamicin and Vancomycin.

In the test of Minimum Inhibitory Concentration (MIC) of the Chromium resistant bacteria, it has been found that, the isolate S_2 tolerated the Chromium concentration up to 23mM. But no colony was found in 24mM Chromium plate. Therefore, the Minimum Inhibitory Concentration for this isolate is about 24mM.

Therefore, it can be said that, microorganisms which show resistance towards Chromium are persistent in the Chromium contaminated regions and they have the ability to reduce chromate into the aerobic situation, a methodology that carries biotechnological and environmental significance.

At last, 16s rDNA sequencing was done to identify the isolated strains of bacteria.

Chapter 5: Conclusion

5.1 Conclusion

The results of this investigation have asserted that, secluded microorganisms are the standout among maximum promising isolate of bacteria that have been able to persist and lessen hexavalent Chromium. The secluded microorganisms carry an amazing potential to biologically reduce Chromium. Rate of decrease of Chromium concentration is relatively associated with the time of incubation. The isolated bacteria is capable to perform as a significant foundation of the Chromium reductase enzyme that can be utilized as one of the source of chemotherapy if future advancement can be done. The information gathered through this examination revealed that, amongst various types of microbial strain that show resistance towards Chromium, the secluded strain is ready to use with different microbes discovered through distinct scientists for relieving toxic effect of the Chromium into normal samples.

5.2 Future Direction

Investigation can be done the correlation between antibiotic resistance profile and Chromium reduction assay. Further studies like cell free extract will help to elucidate the mechanisms responsible for the reduction of Chromium and whether the enzyme is an exo-enzyme or endo-enzyme. Isolate S_2 can be a potential source for Chromium reductase enzyme. Comparative genomic studies might be carried out to find out best candidate for chromium reduction activity.

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APPENDIX

A. 16S rDNA sequence of sample S2:

TTCGGGTGTTACAAACTCTCGTGGTGTGACGGGCGGTGTGTACAAGGCCCGGGA
ACGTATTCACCGCGGCATGCTGATCCGCGATTACTAGCGATTCCAGCTTCATGTA
GGCGAGTTGCAGCCTACAATCCGAACTGAGAACGGTTTTATGAGATTAGCTCCA
CCTCGCGGTCTTGCAGCTCTTTGTACCGTCCATTGTAGCACGTGTGTAGCCCAGG
TCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCG
GCAGTCACCTTAGAGTGCCCAACTTAATGATGGCAACTAAGATCAAGGGTTGCG
CTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATGC
ACCACCTGTCACTCTGCTCCCGAAGGAGAAGCCCTATCTCTAGGGTTGTCAGAG
GATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCC
ACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAGCCTTGCGGCCGTACT
CCCCAGGCGGAGTGCTTAATGCGTTAACTTCAGCACTAAAGGGCGGAAACCCTC
TAACACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTT
TGCTCCCCACGCTTTCGCGCCTCAGTGTCAGTTACAGACCAGAAAGTCGCCTTCG
CCACTGGTGTTCCTCCATATCTCTACGCATTTCACCGCTACACATGGAATTCCAC
TTTCCTCTTCTGCACTCAAGTCTCCCAGTTTCCAATGACCCTCCACGGTTGAGCC
GTGGGCTTTCACATCAGACTTAAGAAACCACCTGCGCGCGCTTTACGCCCAATA
ATTCCGGATAACGCTTGCCACCTACGTATTACCGCGGC

B. BLAST results for similarity scores with sample S2:

i. *Bacillus toyonensis* strain BCT-7112 16s ribosomal RNA gene, complete sequence:

```
Query  1     TTCGGGTGTTACAAACTCTCGTGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTAT  60
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1433  TTCGGGTGTTACAAACTCTCGTGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTAT  1374

Query  61    TCACCGCGGCATGCTGATCCGCGATTACTAGCGATTCCAGCTTCATGTAGGCGAGTTGCA  120
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1373  TCACCGCGGCATGCTGATCCGCGATTACTAGCGATTCCAGCTTCATGTAGGCGAGTTGCA  1314

Query  121   GCCTACAATCCGAACTGAGAACGGTTTTATGAGATTAGCTCCACCTCGCGGTCTTGCAGC  180
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1313  GCCTACAATCCGAACTGAGAACGGTTTTATGAGATTAGCTCCACCTCGCGGTCTTGCAGC  1254

Query  181   TCTTTGTACCGTCCATTGTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTG  240
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1253  TCTTTGTACCGTCCATTGTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTG  1194

Query  241   ACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCACCTTAGAGTGCCCAACTTAA  300
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1193  ACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCACCTTAGAGTGCCCAACTTAA  1134

Query  301   TGATGGCAACTAAGATCAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGAC  360
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1133  TGATGGCAACTAAGATCAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGAC  1074

Query  361   ACGAGCTGACGACAACCATGCACCACCTGTCACTCTGCTCCCGAAGGAGAAGCCCTATCT  420
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1073  ACGAGCTGACGACAACCATGCACCACCTGTCACTCTGCTCCCGAAGGAGAAGCCCTATCT  1014

Query  421   CTAGGGTTGTCAGAGGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAAC  480
             |||||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1013  CTAGGGTTTTCAGAGGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAAC  954

Query  481   CACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAGCCTTGCGGCC  540
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  953   CACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAGCCTTGCGGCC  894

Query  541   GTACTCCCCAGGCGGAGTGCTTAATGCGTTAACTTCAGCACTAAAGGGCGGAAACCCTCT  600
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  893   GTACTCCCCAGGCGGAGTGCTTAATGCGTTAACTTCAGCACTAAAGGGCGGAAACCCTCT  834

Query  601   AACACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCC  660
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  833   AACACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCC  774

Query  661   CCACGCTTTCGCGCCTCAGTGTCAGTTACAGACCAGAAAGTCGCCTTCGCCACTGGTGTT  720
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  773   CCACGCTTTCGCGCCTCAGTGTCAGTTACAGACCAGAAAGTCGCCTTCGCCACTGGTGTT  714

Query  721   CCTCCATATCTCTACGCATTTCACCGCTACACATGGAATTCCACTTTCCTCTTCTGCACT  780
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  713   CCTCCATATCTCTACGCATTTCACCGCTACACATGGAATTCCACTTTCCTCTTCTGCACT  654

Query  781   CAAGTCTCCCAGTTTCCAATGACCCTCCACGGTTGAGCCGTGGGCTTTCACATCAGACTT  840
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  653   CAAGTCTCCCAGTTTCCAATGACCCTCCACGGTTGAGCCGTGGGCTTTCACATCAGACTT  594

Query  841   AAGAAACCACCTGCGCGCGCTTTACGCCCAATAATTCCGGATAACGCTTGCCACCTACGT  900
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  593   AAGAAACCACCTGCGCGCGCTTTACGCCCAATAATTCCGGATAACGCTTGCCACCTACGT  534

Query  901   ATTACCGCGGC  911
             ||||||||||| 
Sbjct  533   ATTACCGCGGC  523
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ii. *Bacillus cereus* ATCC 14579 16s ribosomal RNA (rRNA) gene, complete sequence

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Query  1     TTCGGGTGTTACAAACTCTCGTGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTAT  60
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Sbjct  1443  TTCGGGTGTTACAAACTCTCGTGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTAT  1384

Query  61    TCACCGCGGCATGCTGATCCGCGATTACTAGCGATTCCAGCTTCATGTAGGCGAGTTGCA  120
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1383  TCACCGCGGCATGCTGATCCGCGATTACTAGCGATTCCAGCTTCATGTAGGCGAGTTGCA  1324

Query  121   GCCTACAATCCGAACTGAGAACGGTTTTATGAGATTAGCTCCACCTCGCGGTCTTGCAGC  180
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1323  GCCTACAATCCGAACTGAGAACGGTTTTATGAGATTAGCTCCACCTCGCGGTCTTGCAGC  1264

Query  181   TCTTTGTACCGTCCATTGTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTG  240
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1263  TCTTTGTACCGTCCATTGTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTG  1204

Query  241   ACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCACCTTAGAGTGCCCAACTTAA  300
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1203  ACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCACCTTAGAGTGCCCAACTTAA  1144

Query  301   TGATGGCAACTAAGATCAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGAC  360
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1143  TGATGGCAACTAAGATCAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGAC  1084

Query  361   ACGAGCTGACGACAACCATGCACCACCTGTCACTCTGCTCCCGAAGGAGAAGCCCTATCT  420
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1083  ACGAGCTGACGACAACCATGCACCACCTGTCACTCTGCTCCCGAAGGAGAAGCCCTATCT  1024

Query  421   CTAGGGTTGTCAGAGGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAAC  480
             ||||||||  ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1023  CTAGGGTTTTCAGAGGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAAC  964

Query  481   CACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAGCCTTGCGGCC  540
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  963   CACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAGCCTTGCGGCC  904

Query  541   GTACTCCCCAGGCGGAGTGCTTAATGCGTTAACTTCAGCACTAAAGGGCGGAAACCCTCT  600
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  903   GTACTCCCCAGGCGGAGTGCTTAATGCGTTAACTTCAGCACTAAAGGGCGGAAACCCTCT  844

Query  601   AACACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCC  660
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  843   AACACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCC  784

Query  661   CCACGCTTTCGCGCCTCAGTGTCAGTTACAGACCAGAAAGTCGCCTTCGCCACTGGTGTT  720
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  783   CCACGCTTTCGCGCCTCAGTGTCAGTTACAGACCAGAAAGTCGCCTTCGCCACTGGTGTT  724

Query  721   CCTCCATATCTCTACGCATTTCACCGCTACACATGGAATTCCACTTTCCTCTTCTGCACT  780
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  723   CCTCCATATCTCTACGCATTTCACCGCTACACATGGAATTCCACTTTCCTCTTCTGCACT  664

Query  781   CAAGTCTCCCAGTTTCCAATGACCCTCCACGGTTGAGCCGTGGGCTTTCACATCAGACTT  840
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Sbjct  663   CAAGTCTCCCAGTTTCCAATGACCCTCCACGGTTGAGCCGTGGGCTTTCACATCAGACTT  604

Query  841   AAGAAACCACCTGCGCGCGCTTTACGCCCAATAATTCCGGATAACGCTTGCCACCTACGT  900
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Sbjct  603   AAGAAACCACCTGCGCGCGCTTTACGCCCAATAATTCCGGATAACGCTTGCCACCTACGT  544

Query  901   ATTACCGCGGC  911
             ||||||||||| 
Sbjct  543   ATTACCGCGGC  533
```

iii. *Bacillus thuringiensis* strain NBRC 101235 16s ribosomal RNA gene, partial sequence

```
Query  1     TTCGGGTGTTACAAACTCTCGTGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTAT  60
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Sbjct  1416  TTCGGGTGTTACAAACTCTCGTGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTAT  1357

Query  61    TCACCGCGGCATGCTGATCCGCGATTACTAGCGATTCCAGCTTCATGTAGGCGAGTTGCA  120
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Sbjct  1356  TCACCGCGGCATGCTGATCCGCGATTACTAGCGATTCCAGCTTCATGTAGGCGAGTTGCA  1297

Query  121   GCCTACAATCCGAACTGAGAACGGTTTTATGAGATTAGCTCCACCTCGCGGTCTTGCAGC  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1296  GCCTACAATCCGAACTGAGAACGGTTTTATGAGATTAGCTCCACCTCGCGGTCTTGCAGC  1237

Query  181   TCTTTGTACCGTCCATTGTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTG  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1236  TCTTTGTACCGTCCATTGTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTG  1177

Query  241   ACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCACCTTAGAGTGCCCAACTTAA  300
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Sbjct  1176  ACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCACCTTAGAGTGCCCAACTTAA  1117

Query  301   TGATGGCAACTAAGATCAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGAC  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1116  TGATGGCAACTAAGATCAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGAC  1057

Query  361   ACGAGCTGACGACAACCATGCACCACCTGTCACTCTGCTCCCGAAGGAGAAGCCCTATCT  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1056  ACGAGCTGACGACAACCATGCACCACCTGTCACTCTGCTCCCGAAGGAGAAGCCCTATCT  997

Query  421   CTAGGGTTGTCAGAGGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAAC  480
            |||||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  996   CTAGGGTTTTCAGAGGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAAC  937

Query  481   CACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAGCCTTGCGGCC  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  936   CACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAGCCTTGCGGCC  877

Query  541   GTACTCCCCAGGCGGAGTGCTTAATGCGTTAACTTCAGCACTAAAGGGCGGAAACCCTCT  600
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Sbjct  876   GTACTCCCCAGGCGGAGTGCTTAATGCGTTAACTTCAGCACTAAAGGGCGGAAACCCTCT  817

Query  601   AACACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCC  660
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  816   AACACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCC  757

Query  661   CCACGCTTTCGCGCCTCAGTGTCAGTTACAGACCAGAAAGTCGCCTTCGCCACTGGTGTT  720
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  756   CCACGCTTTCGCGCCTCAGTGTCAGTTACAGACCAGAAAGTCGCCTTCGCCACTGGTGTT  697

Query  721   CCTCCATATCTCTACGCATTTCACCGCTACACATGGAATTCCACTTTCCTCTTCTGCACT  780
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  696   CCTCCATATCTCTACGCATTTCACCGCTACACATGGAATTCCACTTTCCTCTTCTGCACT  637

Query  781   CAAGTCTCCCAGTTTCCAATGACCCTCCACGGTTGAGCCGTGGGCTTTCACATCAGACTT  840
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  636   CAAGTCTCCCAGTTTCCAATGACCCTCCACGGTTGAGCCGTGGGCTTTCACATCAGACTT  577

Query  841   AAGAAACCACCTGCGCGCGCTTTACGCCCAATAATTCCGGATAACGCTTGCCACCTACGT  900
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Sbjct  576   AAGAAACCACCTGCGCGCGCTTTACGCCCAATAATTCCGGATAACGCTTGCCACCTACGT  517

Query  901   ATTACCGCGGC  911
            ||||||||||| 
Sbjct  516   ATTACCGCGGC  506
```

iv. *Bacillus pseudomycoides* strain NBRC 101232 16s ribosomal RNA gene, partial sequence

```
Query  1     TTCGGGTGTTACAAACTCTCGTGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTAT  60
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1416  TTCGGGTGTTACAAACTCTCGTGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTAT  1357

Query  61    TCACCGCGGCATGCTGATCCGCGATTACTAGCGATTCCAGCTTCATGTAGGCGAGTTGCA  120
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Sbjct  1356  TCACCGCGGCATGCTGATCCGCGATTACTAGCGATTCCAGCTTCATGTAGGCGAGTTGCA  1297

Query  121   GCCTACAATCCGAACTGAGAACGGTTTTATGAGATTAGCTCCACCTCGCGGTCTTGCAGC  180
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1296  GCCTACAATCCGAACTGAGAACGGTTTTATGAGATTAGCTCCACCTCGCGGTCTTGCAGC  1237

Query  181   TCTTTGTACCGTCCATTGTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTG  240
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1236  TCTTTGTACCGTCCATTGTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTG  1177

Query  241   ACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCACCTTAGAGTGCCCAACTTAA  300
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1176  ACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCACCTTAGAGTGCCCAACTTAA  1117

Query  301   TGATGGCAACTAAGATCAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGAC  360
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1116  TGATGGCAACTAAGATCAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGAC  1057

Query  361   ACGAGCTGACGACAACCATGCACCACCTGTCACTCTGCTCCCGAAGGAGAAGCCCTATCT  420
             |||||||||||||||||||||||||||||||||||| |||||||| ||||||||||||
Sbjct  1056  ACGAGCTGACGACAACCATGCACCACCTGTCACTCTGCCCCCGAAGGGGAAGCCCTATCT  997

Query  421   CTAGGGTTGTCAGAGGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAAC  480
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  996   CTAGGGTTGTCAGAGGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAAC  937

Query  481   CACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAGCCTTGCGGCC  540
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  936   CACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAGCCTTGCGGCC  877

Query  541   GTACTCCCCAGGCGGAGTGCTTAATGCGTTAACTTCAGCACTAAAGGGCGGAAACCCTCT  600
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  876   GTACTCCCCAGGCGGAGTGCTTAATGCGTTAACTTCAGCACTAAAGGGCGGAAACCCTCT  817

Query  601   AACACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCC  660
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  816   AACACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCC  757

Query  661   CCACGCTTTCGCGCCTCAGTGTCAGTTACAGACCAGAAAGTCGCCTTCGCCACTGGTGTT  720
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  756   CCACGCTTTCGCGCCTCAGTGTCAGTTACAGACCAGAAAGTCGCCTTCGCCACTGGTGTT  697

Query  721   CCTCCATATCTCTACGCATTTCACCGCTACACATGGAATTCCACTTTCCTCTTCTGCACT  780
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  696   CCTCCATATCTCTACGCATTTCACCGCTACACATGGAATTCCACTTTCCTCTTCTGCACT  637

Query  781   CAAGTCTCCCAGTTTCCAATGACCCTCCACGGTTGAGCCGTGGGCTTTCACATCAGACTT  840
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  636   CAAGTCTCCCAGTTTCCAATGACCCTCCACGGTTGAGCCGTGGGCTTTCACATCAGACTT  577

Query  841   AAGAAACCACCTGCGCGCGCTTTACGCCCAATAATTCCGGATAACGCTTGCCACCTACGT  900
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  576   AAGAAACCACCTGCGCGCGCTTTACGCCCAATAATTCCGGATAACGCTTGCCACCTACGT  517

Query  901   ATTACCGCGGC  911
             ||||||||||| 
Sbjct  516   ATTACCGCGGC  506
```

v. *Bacillus mycoides* strain NBRC 101228 16s ribosomal RNA gene, partial sequence

```
Query  1     TTCGGGTGTTACAAACTCTCGTGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTAT  60
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1416  TTCGGGTGTTACAAACTCTCGTGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTAT  1357

Query  61    TCACCGCGGCATGCTGATCCGCGATTACTAGCGATTCCAGCTTCATGTAGGCGAGTTGCA  120
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1356  TCACCGCGGCATGCTGATCCGCGATTACTAGCGATTCCAGCTTCATGTAGGCGAGTTGCA  1297

Query  121   GCCTACAATCCGAACTGAGAACGGTTTTATGAGATTAGCTCCACCTCGCGGTCTTGCAGC  180
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1296  GCCTACAATCCGAACTGAGAACGGTTTTATGAGATTAGCTCCACCTCGCGGTCTTGCAGC  1237

Query  181   TCTTTGTACCGTCCATTGTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTG  240
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1236  TCTTTGTACCGTCCATTGTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTG  1177

Query  241   ACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCACCTTAGAGTGCCCAACTTAA  300
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1176  ACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCACCTTAGAGTGCCCAACTTAA  1117

Query  301   TGATGGCAACTAAGATCAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGAC  360
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1116  TGATGGCAACTAAGATCAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGAC  1057

Query  361   ACGAGCTGACGACAACCATGCACCACCTGTCACTCTGCTCCCGAAGGAGAAGCCCTATCT  420
             |||||||||||||||||||||||||||||||||||||||||||||||||||| ||||||
Sbjct  1056  ACGAGCTGACGACAACCATGCACCACCTGTCACTCTGCTCCCGAAGGAGAAGCTCTATCT  997

Query  421   CTAGGGTTGTCAGAGGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAAC  480
             |||| ||| ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  996   CTAGAGTTTTCAGAGGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAAC  937

Query  481   CACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAGCCTTGCGGCC  540
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  936   CACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAGCCTTGCGGCC  877

Query  541   GTACTCCCCAGGCGGAGTGCTTAATGCGTTAACTTCAGCACTAAAGGGCGGAAACCCTCT  600
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  876   GTACTCCCCAGGCGGAGTGCTTAATGCGTTAACTTCAGCACTAAAGGGCGGAAACCCTCT  817

Query  601   AACACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCC  660
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  816   AACACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCC  757

Query  661   CCACGCTTTCGCGCCTCAGTGTCAGTTACAGACCAGAAAGTCGCCTTCGCCACTGGTGTT  720
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  756   CCACGCTTTCGCGCCTCAGTGTCAGTTACAGACCAGAAAGTCGCCTTCGCCACTGGTGTT  697

Query  721   CCTCCATATCTCTACGCATTTCACCGCTACACATGGAATTCCACTTTCCTCTTCTGCACT  780
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  696   CCTCCATATCTCTACGCATTTCACCGCTACACATGGAATTCCACTTTCCTCTTCTGCACT  637

Query  781   CAAGTCTCCCAGTTTCCAATGACCCTCCACGGTTGAGCCGTGGGCTTTCACATCAGACTT  840
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  636   CAAGTCTCCCAGTTTCCAATGACCCTCCACGGTTGAGCCGTGGGCTTTCACATCAGACTT  577

Query  841   AAGAAACCACCTGCGCGCGCTTTACGCCCAATAATTCCGGATAACGCTTGCCACCTACGT  900
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  576   AAGAAACCACCTGCGCGCGCTTTACGCCCAATAATTCCGGATAACGCTTGCCACCTACGT  517

Query  901   ATTACCGCGGC  911
             ||||||||||| 
Sbjct  516   ATTACCGCGGC  506
```

vi. *Bacillus weihenstephanensis* strain DSM 11821 16s ribosomal RNA gene, partial sequence

```
Query  1     TTCGGGTGTTACAAACTCTCGTGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTAT  60
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1430  TTCGGGTGTTACAAACTCTCGTGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTAT  1371

Query  61    TCACCGCGGCATGCTGATCCGCGATTACTAGCGATTCCAGCTTCATGTAGGCGAGTTGCA  120
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1370  TCACCGCGGCATGCTGATCCGCGATTACTAGCGATTCCAGCTTCATGTAGGCGAGTTGCA  1311

Query  121   GCCTACAATCCGAACTGAGAACGGTTTTATGAGATTAGCTCCACCTCGCGGTCTTGCAGC  180
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1310  GCCTACAATCCGAACTGAGAACGGTTTTATGAGATTAGCTCCACCTCGCGGTCTTGCAGC  1251

Query  181   TCTTTGTACCGTCCATTGTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTG  240
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1250  TCTTTGTACCGTCCATTGTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTG  1191

Query  241   ACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCACCTTAGAGTGCCCAACTTAA  300
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1190  ACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCACCTTAGAGTGCCCAACTTAA  1131

Query  301   TGATGGCAACTAAGATCAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGAC  360
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1130  TGATGGCAACTAAGATCAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGAC  1071

Query  361   ACGAGCTGACGACAACCATGCACCACCTGTCACTCTGCTCCCGAAGGAGAAGCCCTATCT  420
             |||||||||||||||||||||||||||||||||||||||||||||||||||| ||||||
Sbjct  1070  ACGAGCTGACGACAACCATGCACCACCTGTCACTCTGCTCCCGAAGGAGAAGCTCTATCT  1011

Query  421   CTAGGGTTGTCAGAGGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAAC  480
             |||| ||| ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1010  CTAGAGTTTTCAGAGGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAAC  951

Query  481   CACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAGCCTTGCGGCC  540
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  950   CACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAGCCTTGCGGCC  891

Query  541   GTACTCCCCAGGCGGAGTGCTTAATGCGTTAACTTCAGCACTAAAGGGCGGAAACCCTCT  600
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  890   GTACTCCCCAGGCGGAGTGCTTAATGCGTTAACTTCAGCACTAAAGGGCGGAAACCCTCT  831

Query  601   AACACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCC  660
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  830   AACACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCC  771

Query  661   CCACGCTTTCGCGCCTCAGTGTCAGTTACAGACCAGAAAGTCGCCTTCGCCACTGGTGTT  720
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  770   CCACGCTTTCGCGCCTCAGTGTCAGTTACAGACCAGAAAGTCGCCTTCGCCACTGGTGTT  711

Query  721   CCTCCATATCTCTACGCATTTCACCGCTACACATGGAATTCCACTTTCCTCTTCTGCACT  780
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  710   CCTCCATATCTCTACGCATTTCACCGCTACACATGGAATTCCACTTTCCTCTTCTGCACT  651

Query  781   CAAGTCTCCCAGTTTCCAATGACCCTCCACGGTTGAGCCGTGGGCTTTCACATCAGACTT  840
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  650   CAAGTCTCCCAGTTTCCAATGACCCTCCACGGTTGAGCCGTGGGCTTTCACATCAGACTT  591

Query  841   AAGAAACCACCTGCGCGCGCTTTACGCCCAATAATTCCGGATAACGCTTGCCACCTACGT  900
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  590   AAGAAACCACCTGCGCGCGCTTTACGCCCAATAATTCCGGATAACGCTTGCCACCTACGT  531

Query  901   ATTACCGCGGC  911
             ||||||||||| 
Sbjct  530   ATTACCGCGGC  520
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vii. *Bacillus bingmayongensis* strain FJAT-13831 16s ribosomal RNA, partial sequence

```
Query  1     TTCGGGTGTTACAAACTCTCGTGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTAT  60
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1385  TTCGGGTGTTACAAACTCTCGTGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTAT  1326

Query  61    TCACCGCGGCATGCTGATCCGCGATTACTAGCGATTCCAGCTTCATGTAGGCGAGTTGCA  120
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1325  TCACCGCGGCATGCTGATCCGCGATTACTAGCGATTCCAGCTTCATGTAGGCGAGTTGCA  1266

Query  121   GCCTACAATCCGAACTGAGAACGGTTTTATGAGATTAGCTCCACCTCGCGGTCTTGCAGC  180
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1265  GCCTACAATCCGAACTGAGAACGGTTTTATGAGATTAGCTCCACCTCGCGGTCTTGCAGC  1206

Query  181   TCTTTGTACCGTCCATTGTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTG  240
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1205  TCTTTGTACCGTCCATTGTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTG  1146

Query  241   ACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCACCTTAGAGTGCCCAACTTAA  300
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1145  ACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCACCTTAGAGTGCCCAACTTAA  1086

Query  301   TGATGGCAACTAAGATCAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGAC  360
             |||||||||||||||||||||||||||||||||||||||||||||| ||||||||||||||
Sbjct  1085  TGATGGCAACTAAGATCAAGGGTTGCGCTCGTTGCGGGACTTAACCMAACATCTCACGAC  1026

Query  361   ACGAGCTGACGACAACCATGCACCACCTGTCACTCTGCTCCCGAAGGAGAAGCCCTATCT  420
             |||||||||||||||||||||||||||||||||||||||| |||||||| ||||||||||||
Sbjct  1025  ACGAGCTGACGACAACCATGCACCACCTGTCACTCTGCCCCCGAAGGGGAAGCCCTATCT  966

Query  421   CTAGGGTTGTCAGAGGATGTCAAGACCTGGTAAGG-TTCTTCGCGTTGCTTCGAATTAAA  479
             |||||||||||||||||||||||||||||||||||| ||||||||||||||||||||||||
Sbjct  965   CTAGGGTTGTCAGAGGATGTCAAGACCTGGTAAGGGTTCTTCGCGTTGCTTCGAATTAAA  906

Query  480   CCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAGCCTTGCGGC  539
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  905   CCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAGCCTTGCGGC  846

Query  540   CGTACTCCCCAGGCGGAGTGCTTAATGCGTTAACTTCAGCACTAAAGGGCGGAAACCCTC  599
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  845   CGTACTCCCCAGGCGGAGTGCTTAATGCGTTAACTTCAGCACTAAAGGGCGGAAACCCTC  786

Query  600   TAACACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTC  659
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  785   TAACACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTC  726

Query  660   CCCACGCTTTCGCGCCTCAGTGTCAGTTACAGACCAGAAAGTCGCCTTCGCCACTGGTGT  719
             |||||||||||||||||||||||||||||||||||| |||||||||||||||||||||||
Sbjct  725   CCCACGCTTTCGCGCCTCAGTGTCAGTTACAGACCARAAAGTCGCCTTCGCCACTGGTGT  666

Query  720   TCCTCCATATCTCTACGCATTTCACCGCTACACATGGAATTCCACTTTCCTCTTCTGCAC  779
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  665   TCCTCCATATCTCTACGCATTTCACCGCTACACATGGAATTCCACTTTCCTCTTCTGCAC  606

Query  780   TCAAGTCTCCCAGTTTCCAATGACCCTCCACGGTTGAGCCGTGGGCTTTCACATCAGACT  839
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  605   TCAAGTCTCCCAGTTTCCAATGACCCTCCACGGTTGAGCCGTGGGCTTTCACATCAGACT  546

Query  840   TAAGAAACCACCTGCGCGCGCTTTACGCCCAATAATTCCGGATAACGCTTGCCACCTACG  899
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  545   TAAGAAACCACCTGCGCGCGCTTTACGCCCAATAATTCCGGATAACGCTTGCCACCTACG  486

Query  900   TATTACCGCGGC  911
             ||||||||||||
Sbjct  485   TATTACCGCGGC  474
```

viii. *Bacillus gaemokensis* strain BL3-6 16s ribosomal RNA gene, partial sequence

```
Query  1     TTCGGGTGTTACAAACTCTCGTGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTAT  60
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1337  TTCGGGTGTTACAAACTCTCGTGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTAT  1278

Query  61    TCACCGCGGCATGCTGATCCGCGATTACTAGCGATTCCAGCTTCATGTAGGCGAGTTGCA  120
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1277  TCACCGCGGCATGCTGATCCGCGATTACTAGCGATTCCAGCTTCATGTAGGCGAGTTGCA  1218

Query  121   GCCTACAATCCGAACTGAGAACGGTTTTATGAGATTAGCTCCACCTCGCGGTCTTGCAGC  180
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1217  GCCTACAATCCGAACTGAGAACGGTTTTATGAGATTAGCTCCACCTCGCGGTCTTGCAGC  1158

Query  181   TCTTTGTACCGTCCATTGTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTG  240
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1157  TCTTTGTACCGTCCATTGTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTG  1098

Query  241   ACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCACCTTAGAGTGCCCAACTTAA  300
             |||||||||||||||||||||||||||||||||||||||||||||||||||||||||| ||
Sbjct  1097  ACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCACCTTAGAGTGCCCAACTAAA  1038

Query  301   TGATGGCAACTAAGATCAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGAC  360
             || ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1037  TGCTGGCAACTAAGATCAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGAC  978

Query  361   ACGAGCTGACGACAACCATGCACCACCTGTCACTCTGCTCCCGAAGGAGAAGCCCTATCT  420
             |||||||||||||||||||||||||||||||||| ||| |||||||| ||||||||||||
Sbjct  977   ACGAGCTGACGACAACCATGCACCACCTGTCACTTTGCCCCCGAAGGGGAAGCCCTATCT  918

Query  421   CTAGGGTTGTCAGAGGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAAC  480
             |||||||| ||| ||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  917   CTAGGGTTTTCAAAGGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAAC  858

Query  481   CACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAGCCTTGCGGCC  540
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  857   CACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAGCCTTGCGGCC  798

Query  541   GTACTCCCCAGGCGGAGTGCTTAATGCGTTAACTTCAGCACTAAAGGGCGGAAACCCTCT  600
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  797   GTACTCCCCAGGCGGAGTGCTTAATGCGTTAACTTCAGCACTAAAGGGCGGAAACCCTCT  738

Query  601   AACACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCC  660
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  737   AACACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCC  678

Query  661   CCACGCTTTCGCGCCTCAGTGTCAGTTACAGACCAGAAAGTCGCCTTCGCCACTGGTGTT  720
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  677   CCACGCTTTCGCGCCTCAGTGTCAGTTACAGACCAGAAAGTCGCCTTCGCCACTGGTGTT  618

Query  721   CCTCCATATCTCTACGCATTTCACCGCTACACATGGAATTCCACTTTCCTCTTCTGCACT  780
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  617   CCTCCATATCTCTACGCATTTCACCGCTACACATGGAATTCCACTTTCCTCTTCTGCACT  558

Query  781   CAAGTCTCCCAGTTTCCAATGACCCTCCACGGTTGAGCCGTGGGCTTTCACATCAGACTT  840
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  557   CAAGTCTCCCAGTTTCCAATGACCCTCCACGGTTGAGCCGTGGGCTTTCACATCAGACTT  498

Query  841   AAGAAACCACCTGCGCGCGCTTTACGCCCAATAATTCCGGATAACGCTTGCCACCTACGT  900
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  497   AAGAAACCACCTGCGCGCGCTTTACGCCCAATAATTCCGGATAACGCTTGCCACCTACGT  438

Query  901   ATTACCGCGGC  911
             ||||||||||| 
Sbjct  437   ATTACCGCGGC  427
```

ix. *Bacillus manliponensis* strain BL4-6 16s ribosomal RNA gene, partial sequence

```
Query  1     TTCGGGTGTTACAAACTCTCGTGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTAT  60
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1372  TTCGGGTGTTACAAACTCTCGTGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTAT  1313

Query  61    TCACCGCGGCATGCTGATCCGCGATTACTAGCGATTCCAGCTTCATGTAGGCGAGTTGCA  120
             ||||||| |||||||||||| ||||||||||||||||||||||||||||||||||||||||
Sbjct  1312  TCACCGCAGCATGCTGATCTGCGATTACTAGCGATTCCAGCTTCATGTAGGCGAGTTGCA  1253

Query  121   GCCTACAATCCGAACTGAGAACGGTTTTATGAGATTAGCTCCACCTCGCGGTCTTGCAGC  180
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1252  GCCTACAATCCGAACTGAGAACGGTTTTATGAGATTAGCTCCACCTCGCGGTCTTGCAGC  1193

Query  181   TCTTTGTACCGTCCATTGTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTG  240
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1192  TCTTTGTACCGTCCATTGTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTG  1133

Query  241   ACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCACCTTAGAGTGCCCAACTTAA  300
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1132  ACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCACCTTAGAGTGCCCAACTTAA  1073

Query  301   TGATGGCAACTAAGATCAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGAC  360
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1072  TGATGGCAACTAAGATCAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGAC  1013

Query  361   ACGAGCTGACGACAACCATGCACCACCTGTCACTCTGCTCCCGAAGGAGAAGCCCTATCT  420
             |||||||||||||||||||||||||||||||||||  ||||||||  ||||||||||||
Sbjct  1012  ACGAGCTGACGACAACCATGCACCACCTGTCACTCTGTCCCCGAAGGGAAAGCCCTATCT  953

Query  421   CTAGGGTTGTCAGAGGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAAC  480
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  952   CTAGGGTTGTCAGAGGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAAC  893

Query  481   CACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAGCCTTGCGGCC  540
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  892   CACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAGCCTTGCGGCC  833

Query  541   GTACTCCCCAGGCGGAGTGCTTAATGCGTTAACTTCAGCACTAAAGGGCGGAAACCCTCT  600
             |||||||||||||||||||||||||||||||||||||||||||| |||||||||||| ||
Sbjct  832   GTACTCCCCAGGCGGAGTGCTTAATGCGTTAACTTCAGCACTAAGGGGCGGAAACCCCCT  773

Query  601   AACACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCC  660
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  772   AACACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCC  713

Query  661   CCACGCTTTCGCGCCTCAGTGTCAGTTACAGACCAGAAAGTCGCCTTCGCCACTGGTGTT  720
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  712   CCACGCTTTCGCGCCTCAGTGTCAGTTACAGACCAGAAAGTCGCCTTCGCCACTGGTGTT  653

Query  721   CCTCCATATCTCTACGCATTTCACCGCTACACATGGAATTCCACTTTCCTCTTCTGCACT  780
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  652   CCTCCATATCTCTACGCATTTCACCGCTACACATGGAATTCCACTTTCCTCTTCTGCACT  593

Query  781   CAAGTCTCCCAGTTTCCAATGACCCTCCACGGTTGAGCCGTGGGCTTTCACATCAGACTT  840
             ||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  592   CAAGTTTCCCAGTTTCCAATGACCCTCCACGGTTGAGCCGTGGGCTTTCACATCAGACTT  533

Query  841   AAGAAACCACCTGCGCGCGCTTTACGCCCAATAATTCCGGATAACGCTTGCCACCTACGT  900
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  532   AAGAAACCACCTGCGCGCGCTTTACGCCCAATAATTCCGGATAACGCTTGCCACCTACGT  473

Query  901   ATTACCGCGGC  911
             ||||||||||| 
Sbjct  472   ATTACCGCGGC  462
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x. *Bacillus cytotoxicus* strain NVH 391-98 16s ribosomal RNA gene, complete sequence

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Query  1     TTCGGGTGTTACAAACTCTCGTGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTAT  60
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Sbjct  1436  TTCGGGTGTTACAAACTCTCGTGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTAT  1377

Query  61    TCACCGCGGCATGCTGATCCGCGATTACTAGCGATTCCAGCTTCATGTAGGCGAGTTGCA  120
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1376  TCACCGCGGCATGCTGATCCGCGATTACTAGCGATTCCAGCTTCATGTAGGCGAGTTGCA  1317

Query  121   GCCTACAATCCGAACTGAGAACGGTTTTATGAGATTAGCTCCACCTCGCGGTCTTGCAGC  180
             |||||||||||||||||||||||||||||||||||||||||||||||||||| ||   |
Sbjct  1316  GCCTACAATCCGAACTGAGAACGGTTTTATGAGATTAGCTCCACCTCGCGGTCTCGCGAC  1257

Query  181   TCTTTGTACCGTCCATTGTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTG  240
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Sbjct  1256  TCTTTGTACCGTCCATTGTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTG  1197

Query  241   ACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCACCTTAGAGTGCCCAACTTAA  300
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Sbjct  1196  ACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCACCTTAGAGTGCCCAACTGAA  1137

Query  301   TGATGGCAACTAAGATCAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGAC  360
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Sbjct  1136  TGATGGCAACTAAGATCAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGAC  1077

Query  361   ACGAGCTGACGACAACCATGCACCACCTGTCACTCTGCTCCCGAAGGAGAAGCCCTATCT  420
             |||||||||||||||||||||||||||||||||||||| |||||||| ||||||||||||
Sbjct  1076  ACGAGCTGACGACAACCATGCACCACCTGTCACTCTGCCCCCGAAGGGGAAGCCCTATCT  1017

Query  421   CTAGGGTTGTCAGAGGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAAC  480
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Sbjct  1016  CTAGGGTTGTCAGAGGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAAC  957

Query  481   CACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAGCCTTGCGGCC  540
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Sbjct  956   CACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAGCCTTGCGGCC  897

Query  541   GTACTCCCCAGGCGGAGTGCTTAATGCGTTAACTTCAGCACTAAAGGGCGGAAACCCTCT  600
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  896   GTACTCCCCAGGCGGAGTGCTTAATGCGTTAACTTCAGCACTAAAGGGCGGAAACCCTCT  837

Query  601   AACACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCC  660
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Sbjct  836   AACACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCC  777

Query  661   CCACGCTTTCGCGCCTCAGTGTCAGTTACAGACCAGAAAGTCGCCTTCGCCACTGGTGTT  720
             |||||||||||||||||| |||||||||||||||| |||||||||||||||||||||||
Sbjct  776   CCACGCTTTCGCGCCTCAGCGTCAGTTACAGACCAGAGAGTCGCCTTCGCCACTGGTGTT  717

Query  721   CCTCCATATCTCTACGCATTTCACCGCTACACATGGAATTCCACTTTCCTCTTCTGCACT  780
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Sbjct  716   CCTCCATATCTCTACGCATTTCACCGCTACACATGGAATTCCACTCTCCTCTTCTGCACT  657

Query  781   CAAGTCTCCCAGTTTCCAATGACCCTCCACGGTTGAGCCGTGGGCTTTCACATCAGACTT  840
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Sbjct  656   CAAGTCTCCCAGTTTCCAATGACCCTCCACGGTTGAGCCGTGGGCTTTCACATCAGACTT  597

Query  841   AAGAAACCACCTGCGCGCGCTTTACGCCCAATAATTCCGGATAACGCTTGCCACCTACGT  900
             |||||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  596   AAGAAACCGCCTGCGCGCGCTTTACGCCCAATAATTCCGGATAACGCTTGCCACCTACGT  537

Query  901   ATTACCGCGGC  911
             ||||||||||| 
Sbjct  536   ATTACCGCGGC  526
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