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STUDY ON CONSORTIUM OF BACTERIA IN CHROMIUM LADEN AQUATIC ECOSYSTEM OF INDIA

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ABSTRACT

The Chromium is most dangerous heavy metal persistent in the environment. It exists in hexavalent Cr (VI) ion which is mutagenic, carcinogenic and toxic to all life forms. Few microorganisms have developed various mechanisms of adsorption, methylation, oxidation and reduction of chromium and converting it into less toxic trivalent Cr (III) form. These organisms are bacteria, fungi, phytoplankton and zooplankton which form food web based on the physicochemical parameters of that aquatic system. The present study tries to identify all the bacteria occupying aquatic and soil ecological niche laden with chromium. Total of 28 bacteria were isolated and the 16S rRNA were used to study the phylogenetic relationship in these organisms. Bacteria are ranked as most adaptable organism because of its fast transferable plasmid helping in enzymatic transformation of Cr and precipitating metal from aqueous phase. These organisms can be exploited for bioremediation and biosorbent in bioreactors.

INTRODUCTION

Chromium is one of the most widely used heavy metals in industrial processes (Wang 2000). Chromate is a serious environmental pollutants due to its wide use in industries like corrosion control, pigment manufacturing electroplating, metal finishing, chromate preparation, leather tanning (Ackerley et al., 2004; Thakur et al., 2001; Shaili and Thakur, 2006). It is also used in cooling towers of heavy metal industry, atomic power plants and nuclear weapons production (Barceloux,, D. G. 1999).

Chromium exists in nine valence states ranging from -2 to + 6 (Liliana and Eliseo 2008) but in aquatic environment it exists in two stable oxidation states, trivalent chromium and hexavalent chromium. Trivalent chromium Cr (III) is less toxic and mobile.

Hexavalent chromium Cr (VI) is recognized as one of the most dangerous environmental pollutants due to its easy solubility, toxicity and carcinogenic ability to cause mutations and cancer in humans. Cr (VI) typically exists in one of these two forms; chromate (CrO_4^{2-}) or dichromate ($Cr_2O_7^{2-}$) (Shen and Wang 1994) and is 100 fold more toxic than trivalent chromium (Meghraj et al., 2003).

The presence of high levels of chromate in the environment also has an inhibitory effect on most microorganisms (Ajmal et al., 1984). However, microorganisms have evolved resistance mechanisms that lead to the selection of resistant variants that can tolerate metal toxicity (Losi and Frankenberger, 1994; Wong and Trevors 1998). There are reports of live microbial systems for the purpose of remediation of contaminated soils and water (Kratochvil et al., 1998). Bioremediation or removal of toxic heavy metal from effluent is generally mediated by diverse group of organisms namely bacteria fungi and planktons (Pandi et al 2007). These microorganisms have the exceptional ability to adapt to and colonize noxious metal polluted environments, which are inhabitable by higher organisms. These microorganisms have developed the capabilities to protect themselves from heavy metal toxicity by various mechanisms such as adsorption, uptake methylation, oxidation and reduction (Megharaj et al 2003). Many questions about the response of complex systems to toxicity are difficult to address by describing different natural environments (Hart 1945). Changes in taxonomic composition and abundance of communities change with response to concentrations of effluents (Koteswari and Ramnibai 2004). The response of taxonomic composition as the end point of microcosm toxicity is reasonably sensitive to many types of toxicants (Plafkin et al., 1989).

The 16s ribosomal RNA gene is a commonly used tool for identifying bacteria for taxonomical studies. Traditional characterization depended upon phenotypic traits like gram positive or gram negative, bacillus or coccus, etc. Taxonomists today consider analysis of an organism’s DNA more reliable than classification based solely on phenotypes. We can also identify or classify only the bacteria within a given environmental samples using 16S rRNA. While there is a homologous gene in eukaryotes, the 18S Rrna gene is relatively short at 1.5 kb, making it faster and cheaper to sequence than many other unique bacterial genes.

In the present study we have tried to pool in the entire bacteria consortium present in the various water bodies in and around the leather and electroplating industries of Tamil Nadu India.

MATERIAL AND METHODS

Sample collection:

In this two year (December 2007 to January 2010) extensive study, water and soil samples were collected and analyzed from chromium laden water bodies, like outlets of leather industry in Ambur District, Common Effluent Treatment Plant (CETP) at Ranipetai and various electroplating industries of Tamil Nadu. The samples were transported to the Environmental Biotechnology Laboratory in black bottles at 4ºC.

Sample culturing:

The water samples were cultured on the differential gradient agar of chromium after serial dilution with a non-inoculated differential gradient agar as a control. The agar plates with pH 1 to 12 were also inoculated in order to isolate acidophiles. The bacterial cells were.

Sample identification:

The standard microbial procedures like gram’s stain, spore stain were employed to visualize the shape and size of the microorganism. The biochemical tests were performed to identify the bacterial group. The cellular fatty acid chromatography fitted with flame ionization detector, automatic sampler, integrator and computer was used to confirm the results.

Bioinformatics Evaluation:

The partial fragments of 16S RNA were collected from NCBI (online) and were subjected to multiple sequence alignment in BIOEDIT (v_7.0.5) and parallel verification was performed in CLUSTALW. The tree file generated by the CLUSTALW was visualized using software DENDROSCOPE (v_2.3) to derive the different patterns of phylograms.

RESULTS:

The extensive study showed 28 organisms overlapping at various sites of chromium rich environments as given in Table 1. Out of these 28 organisms only 10 were found to be gram positive bacteria which were all motile except *Micrococcus*. Except 5 bacteria namely *Agrobacterium, Geobacter metallireducens, Micrococcus roseu,* *Micrococcus* and *Streptomyces* all were highly motile. The colony morphology showed that only 5 bacteria colony namely Bacillus *cereus, Bacillus subtilis, Micrococcus roseus, Micrococcus sp.* and *Pseudomonas fluorescens* were flat whereas others had convex elevation. Intriguing colony colors were captivating in these consortia. Among these greenish colonies dominated.

The UTR of these bacteria in Fig 1 and Fig 2 showed many similarities. The specific region of DNA that has proved to be most informative for evolutionary relatedness is 16S Rrna, the gene that encodes the RNA component of the smaller subunit of the bacterial ribosome.

DISCUSSION

The ecological perturbation in an aquatic environment generally produces certain predictable changes in the community structure (Patrick 1971). Species with low tolerance are eliminated, while those species best suited for survival enriched habitats become extensively dominant. Relatively few species were identified which suggests that the early community development was dominated by pioneer species capable of surviving in chromium accumulated environments (Pratt et al., 1993).

Among the heterotrophic population, bacteria show more tolerance to heavy metals in both aerobic and anaerobic bacteria. The gene encoding resistances to metals tolerance are located at transmissible plasmids. Under environmental conditions of metal stress resistant populations will adapt faster by the spread of R- factors than by mutation and natural selection, thus leading to a very rapid increase in their numbers (Bhattacherjee et al 1988). Bacteria and Pseudomonas use Chromium (VI) as terminal electron acceptor during their respiration (Viktoriya et al., 2003). Chromium (VI) is also taken up via sulphate or thiosulphate transporter and oxidixed biological molecules resulting in toxicity (Krishna et al., 2004). These microorganisms have developed the capabilities to protect themselves from heavy metal toxicity by various mechanisms such as adsorption, uptake methylation, oxidation and reduction (Megharaj et al 2003). Many questions about the response of complex systems to toxicity are difficult to address by describing different natural environments (Hart 1945). Metal ions are converted into insoluble form by specific enzyme reactions and are removed from the aqueous phase (Brierly et al., 1986). The cell surfaces of microorganisms are negatively charged owing to the presence of various anionic structures. This gives microorganisms an ability to bind metal cation. (Chen and Hao, 1998). Intact microbial cell, live or dead and their products can be highly efficient bioaccumulator (Kovacevic et al., 2000).

These organisms discussed in this paper can be used for bioremediation and biosorbent in bioreactors as they continue to do charitable service by adsorbing and transforming chromium from chromium rich ecosystems it into less toxic forms.

Table 1: Bacteria consortium in chromium rich aquatic environment

Name of the organism	Colony color	Colony-form/ Margin/ elevation	Motility Test	Grams Test	Catalase Test
Aeromonasdechromatica	White		Motile		Positive
Aeromonas sp.	White	Circular, Entire,Convex	Motile		Positive
Alcaligenes eutrophus	Green	Circular,Entire,Convex	Motile	-	Positive
Agrobacterium	Pink	Circular,Entire,Convex	Non motile	+	Positive
Arthrobacter sp.	White(glossy)	Round,Entire,Convex	Motile	-	Positive
Bacillus cereus	Peacock blue	Irregular,Undulate,Flat	Motile	-	Positive
Bacillus circulans	Pale yellow	Irregular,Wavy,Convex	Motile	+	Positive
Bacillus subtilis	Brown	Circular,Undulate,Flat	Motile	+	Positive
Bacillus thuringiensis	Turquoise	Circular,Entire,Convex	Motile	+	Positive
Desulfovibrio vulgaris	Red	Circular,Entire,Convex	Motile	+	Negative
Desulfovibrio	Red	Circular,Entire,Convex	Motile	+	Negative
desulfuricans	Pale yellow	Silky,Entire,Convex	Motile	-	Positive
Enterobacter cloacae	Pink	Silky,Entire,Convex	Motile	-	Positive
Enterobacter aerogenes	Yellow	Irregular,Entire,Convex	Motile	-	Positive
Escherchia coli	Green		Non motile	-	Negative
Geobacter	Pink	Circular, Undulate, Flat	Non motile	-	Positive
metallireducens	Yellow	Circular,Undulate, Flat	Non motile	-	Positive
Micrococcus roseus	Purple		Motile	+	Positive
Micrococcus sp.	Green	Circular, Entire, Convex	Motile	+	Positive
Microbacterium spp.	Green	Circular, Undulate, Flat	Motile	+	Positive
Pseudomonas sp.	Brown		Motile	-	Positive
Pseudomonas fluorescens	Blue-Green	Irregular,Undulate,	Motile	-	Positive
Pseudomonas ambigua	Blue	Convex	Motile	-	Positive
Pseudomonas aeruginosa	Green	Circular,Entire,Raised	Motile	-	Positive
Pseudomonas putida			Motile		Positive
Pseudomonas	Grey		Non motile	-	Positive
chromatophila	Green	Filamentous,Undulate,Co	Motile	-	Positive
Pseudomonas	Blue	nvex	Motile	-	Positive
dechromaticans				+	
Streptomyces				-	
Thiobacillus thiooxidans					
Thiobacillus ferrooxidans					

Figure 1. Phylogenetic comparison of UTR of these 28 bacteria

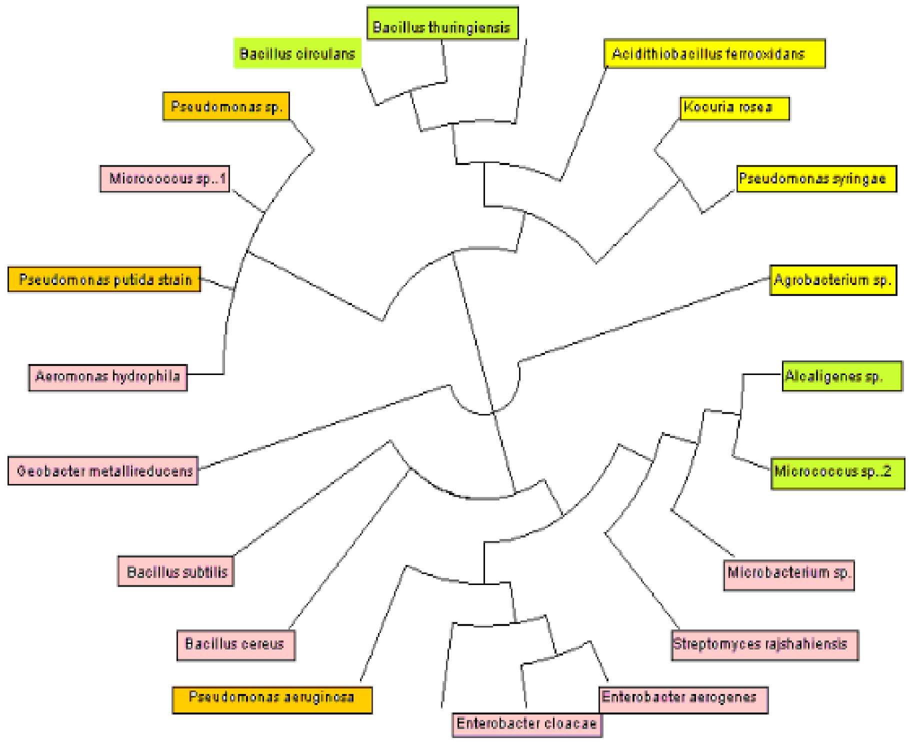
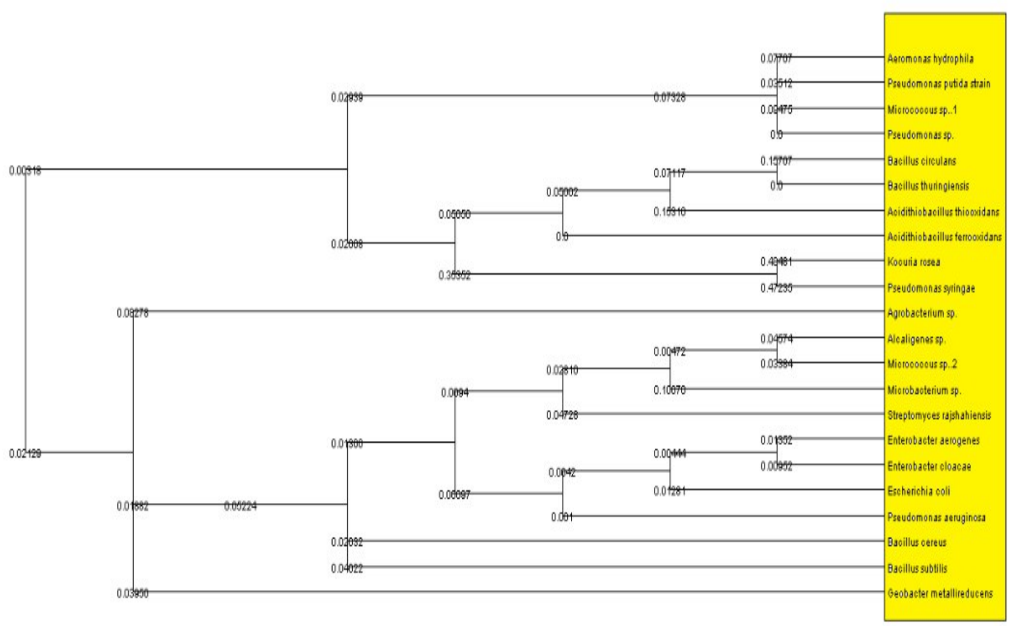


Figure 2. Phylogenetic comparison of UTR of these 28 bacteria



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