The study on microbial chromium reduction by pseudomonas bacteria

Science, Biology

World remains affected by the metal pollution and especially heavy metals represents the major danger which can lead to number of diseases (Marilena and Castanas, 2008). Similar to other metals, Chromium (Cr) is naturally occurring element present in earth’s crust and mined as chromite. In nature, it represents different oxidation states and the most stable are hexavalent and trivalent states. Trivalent chromium is less toxic, remains in trace amount and required by plants and animals (Chowdhury et al., 2003). Where Cr (VI) and its compounds are mutagenic as well as carcinogenic in nature (Langard, 1982; Costa and Catherine, 2006). Due to its soluble nature, hexavalent chromium generally persists in water for a longer period of time and that is the reason, why it gets easily spread in the environment and has adverse effects on plants, animals, and microbial flora. Since chromium is an industrially important metal and majorly used in industries for manufacturing of stainless steel, wood preservation, dyes etc. It can easily reach to the environment (Lunk, 2015).

Environment has a great impact on the quality of every creature. Here problems arise due to polluted environment which is not only experienced by plants and animals, but it also affects the microbial habitat. As microorganisms are ubiquitous and abundant in biosphere, they are always getting exposed to different kinds of environments. In one of the aspects, interaction of metals with microbes certainly affects their growth and development (Bharagava and Mishra, 2015; Shi et al., 2002; Fernando et al., 1977). Soil is the main reservoir for pollutants and trace elements discharged through various anthropogenic activities. Prolong exposure of heavy metals to the microbes is the main cause for the development of resistant strains by

bringing number of genetic changes (Clausen, 2000). Microbes, especially bacteria developed various mechanisms like oxidation, adsorption, accumulation, uptake, and reduction to adjust themselves in these unfavorable conditions (Carlo et al., 2014; Nezha et al., 2015; Das et al., 2015).

Among number of metals, ability to tolerate the chromium presence and its further reduction was recorded to be prominent in different bacterial genus reported till date viz., Pseudomonas spp (Konovalova et al., 2003; Garbisu et al., 1998; Ishibashi et al., 1990), Enterobacter spp, (Wang et al., 1990), Escherichia coli (Shen and Wang 1993), Bacillus spp (Chaturvedi, 2012) Ochrobactrum intermedium (Batool et al., 2012), Lysinibacillus spp, (Montenegro et al., 2015; Kipkurui et al., 2016, Acinetobacter spp., (Méndez et al., 2017), Microbacterium spp (Panneerselvam et al., 2013; Sarkar et al., 2016)and Rhodococcus spp (Banerjee et al., 2017). It is also understood that chromium reduction and resistance are independent processes among different species (Verma et al., 2009). Here microbial chromium reduction was first reported in Pseudomonas species by Romanenko and Koren’Kov (1977).

MATERIALS AND METHODS

Soil Sampling

As per the report of Directorate of Geology and Mining, Government of Maharashtra Nagpur, chromite reserves from 20°47’00″ N, 79°39’00″ E at Pauni, Bhandara district was selected for sampling. Soil Samples were subjected to X- ray fluorescence analysis at Mineral testing Lab, Indian

Bureau of Mines, Nagpur for determination of chromium concentration in the given soil. In addition, electrical conductivity, organic carbon content, total nitrogen (Kjeldhal method), phosphorus and potassium were also subjected to investigation.

Isolation of Chromium Tolerant Strains

1g chromium rich soil samples from the given site was serially diluted and processed further by enrichment culture technique followed by plating of every dilution. Luria Bertani medium before adding the diluent of soil inoculum was supplemented with 50 ppm of potassium dichromate ($K_2Cr_2O_7$) and all plates were incubated at 37°C ± 2 for 24-48 hrs. The plates were then examined for morphologically distinct colonies and sub cultured to maintain the isolates and identification carried out using conventional biochemical tests (Cappuccino and Sherman, 1987) and by16S rRNA sequencing.

Evaluation of Chromium Tolerance:

Ability of chromium tolerant bacteria was evaluated when 1 O. D. fresh culture of the isolate was inoculated in 30 ml of LB Broth containing different concentration of potassium dichromate ($K_2Cr_2O_7$) ranging from 100-1300 ppm and allowed to incubate at 37ºC ± 2 for 24-48 hrs. The growth of bacteria was determined by measuring the optical density on spectrophotometer at 600 nm.

Molecular Characterization

Total DNA isolation and extraction from bacterial cells for PCR analysis was done by Genomic CTAB protocol. PCR amplification of the 16S rRNA gene fragment was done by using 27forward (AGAGTTTGATCMTGGCTCAG) and1492reverse (ACGGYTACCTTGTTACGACTT) primers. The amplification mixture contains of 32. 0 µl nuclease free water, 5. 0 µl PCR buffer 10x, 2. 0 µl dNTP (10 mM), 4. 0 µl forward primer (10 µM), 4. 0 µl reverse primer (10 µM), 1. 0µl Taq DNA polymerase enzyme (1U/ µl) and 200ng DNA template. PCR reaction was programmed as: Initial denaturation of 3 min. at 94 ºC, denaturation of 1 min. at 94 ºC, primer annealing for 1 min. at 54 ºC, extension of 2 min. at 72 °C, final extension for 5 min. at 72 °C; total 30 cycles and stored at 4 °C. Amplicon was sequenced and analyzed by BLAST to find the best scored close homolog and further accession number of isolate was obtained through NCBI genbank database. In a phylogenetic analysis, top five best homologs were aligned in CLUSTALW and later on designed for phylogram in MEGA5 software.