Studies on chromium transforming bacteria isolated from Tannery waste disposal site of Kanpur

Abstract submitted to

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Abstract

The purpose of this thesis is to isolate and characterize a few hexavalent chromium reducing bacteria. The tannery effluent sample was collected from the outside of a tannery located at Jajmau area of Kanpur. The collected tannery effluent sample was brownish in color and odorous. In the physicochemical analysis of tannery wastewater sample, most of the parameters including pH, BOD, COD, TS, TDS, and TSS, were found to be higher than recommended permissible standard limits. The concentration of Cr in the tannery effluent sample was also much higher than the permissible limit.

Total eighteen chromium-resistant bacteria were isolated from the tannery effluent sample. Based on the 16S rRNA gene sequencing and phylogenetic analysis, these eighteen bacteria belong to seven different genera including *Bacillus*, *Pseudomonas*, *Kocuria, Cellulosimicrbium*, *Klebsiella*, *Microbacterium, Brucella.* The members of genus *Bacillus* constitute 28% of total chromium transforming isolates; followed by members of genus *Microbacterium* (17%) *Brucella* (17%), *Pseudomonas* (11%), *Cellulosimicrobium* (11%), *Kocuria* (11%) and *Kleibsiella* (5%). Furthermore, all eighteen bacteria were screened for their ability to reduce hexavalent chromium with increasing concentrations (600–1200 ppm) of potassium dichromate. Results showed that one bacterium, *Bacillus licheniformis* strain KNP exhibited hexavalent chromium reduction up to concentrations of 1000 ppm. *Bacillus licheniformis* strain KNP was selected for further study.

Cells of *Bacillus licheniformis* strain KNP was motile, Gram-positive, endo-spore forming and rod-shaped. Colonies formed on nutrient agar were creamy white,

mucoid, translucent and raised, 3–4 mm in diameter after 24 h of incubation at 30 °C. Cells were positive for catalase test but negative for oxidase. Strain KNP exhibited positive results for gelatin hydrolysis, casein hydrolysis, starch hydrolysis, and citrate utilization. Strain KNP showed negative results for urease production, indole test.

Bacillus licheniformis KNP grew well in presence of the hexavalent chromium and completely reduced chromium (500 ppm) within 48 hours. *Bacillus licheniformis* KNP grew well in presence of the hexavalent chromium and completely reduced Cr (VI) (500 ppm) within 48 hours. In comparison with control, Cr (VI)-treated KNP cells showed a decrease in cell size, aggregation of cells, and slight deformity in cell shape. Furtheremore, the Cr-treated cells of strain KNP accumulated a small amount of Cr. A Cr (VI) molecule interacts with functional groups and chemical bonds in KNP cells.

To identify the genes involved in the chromium resistant and reduction, whole genome of *Bacillus licheniformis* strain KNP was sequenced. The total size of the draft assembly was 4,280,093 bp, distributed into 21 contigs with an N50 value of 4,186,229. The genome has 45.9% G + C content, 4255 coding sequences and 86 putative RNA genes.

Functional annotation of genome of strain KPN discovered presence of three chromate transporters that involve in chromium resistance via chromate efflux mechanism. Furthermore, putative genes related to the chromium reduction for instance nitroreductase, quinone reductase and azoreducatase were also detected in the genome of strain KNP. In addition, genes coding the proteins involuted

resistance and reduction of arsenic for instance arsenate reductase (thioredoxin), arsenical pump-driving ATPase, arsenical efflux pump membrane protein ArsB, arsenate reductase family protein, arsenite efflux transporter metallochaperone ArsD, arsenic transporter were also acknowledged in the KNP genome. Numerous other genes involved in heavy metal resistance including heavy metal translocating P-type ATPase, Nramp family divalent metal transporter, nickel ABC transporter, divalent metal cation transporter, nickel/metallophore periplasmic binding protein, metal ABC transporter ATP-binding protein, metal ABC transporter permease, MerR family transcriptional regulator, copper-sensing transcriptional repressor CsoR, BlaI/MecI/CopY family transcriptional regulator, metal ABC transporter substrate-binding protein, TetR/AcrR family transcriptional regulator, MarR family transcriptional regulator, LysR family transcriptional regulator, Transcriptional regulator MntR, GbsR/MarR family transcriptional regulator, Zn(II)-responsive metalloregulatory transcriptional repressor CzrA, MgtC/SapB family protein, Spx/MgsR family RNA polymerase-binding regulatory protein were also identified in the KNP genome. Moreover, genes related to bacterial chemotaxis were also identified in the KNP genome.