ISOLATION AND CHARACTERIZATION OF A Cr(VI)-REDUCING *Klebsiella pneumoniae* WITH POTENTIAL APPLICATION IN BIOREMEDIATION AND WASTE WATER TREATMENT.

Paloma Lara, Enrique Morett, Katy Juárez; Instituto de Biotecnología, UNAM, Av. Universidad 2001, Chamilpa, Cuernavaca, Morelos. CP 62210. larafp@ibt.unam.mx

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Introduction. Hexavalent chromium is a dangerous mutagen and oxidizing agent, highly soluble and able to permeate biological membranes (1). The uncontrolled Cr(VI) industrial wastes and their improper disposal has resulted in an anthropogenic pollution of various environments including soils and aquifers. Diverse bacteria have developed several strategies to resist high concentrations of Cr(VI), mainly through chromate efflux and Cr(VI) reduction to Cr(III), which is highly insoluble, and thousand-fold less toxic and unable to permeate biological membranes (1). Chromium-reducing microorganisms with high levels of resistance have taken great interest in recent decades due to their potential application in bioremediation processes of Cr-contaminated environments as a safe and cost-effective technology alternative to the expensive traditional physicochemical methods. The present study was aimed at the characterization of a Cr(VI)-resistant and Cr(VI)-reducing *Klebsiella pneumoniae* isolated from long-term Cr-contaminated aquifer located in León Guanajuato, México.

Methods. The identification of the isolated strain ChroAq1 was initially performed by sequencing the *rDNA 16S*, *gyrA*, *rpoB* and *parC* genes and recently by the complete genome sequence. The isolated strain was examined for its tolerance to hexavalent chromium [Cr(VI)] both in aerobic and in anaerobic conditions. It was determined the ability to reduce Cr(VI) to Cr(III) by growing cells and cell-free extracts, soluble fraction and membrane fraction. The cellular fractionation was performed by a protocol modified from a method described by Myers in 1998 (2). In addition, the ability of the isolated strain to reduce Fe(III) both in soluble and insoluble oxides was also studied.

Results. The isolated strain ChroAq1 was identified as *Klebsiella pneumoniae.* This microorganism tolerated concentrations about 1.8mM of Cr(VI) in anaerobic conditions and 22mM in aerobic conditions. This level of resistance is remarkable in comparison with other microorganisms reported like *Pantoea agglomerans SP1* wich resist 100µM in aerobic conditions (3). *Klebsiella pneumoniae* ChroAq1 showed the ability to reduce Cr(VI) in anaerobic growth conditions but not aerobically. Complete reduction of Cr(VI) was achieved within 336h and white-grayish Cr(III) precipitate was visible at the bottom of the tube. The cell-free extract of *K. pneumoniae* ChroAq1 showed a chromate-reductase activity with NADH as electron donor. Cell fractionation allowed us to conclude that the NADH-dependent chromate-reductase activity is in the soluble cell fraction. *K. pneumoniae* ChroAq1 it is also capable to reduce iron oxides both soluble and insoluble.

Conclusions. *K. pneumoniae ChroAq1 has* a remarkable resistance and ability to reduce Cr(VI) to Cr(III) great potential application in bioremediation processes and waste water treatment

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