Environmental Engineering and Management Journal *March 2012, Vol.11, No. 3, Supplement, S156*
http://omicron.ch.tuiasi.ro/EEMJ/

"Gheorghe Asachi" Technical University of Iasi, Romania

P90

CHARACTERIZATION OF Cr(VI)-HYPER-RESISTANT *Pseudomonas alcaliphila* 34 BIOFILM

L. Santopolo[1], F. Decorosi[1], E. Marchi[1], L. Frediani[1], L. Giovannetti[1], S. Zecchi[2], D. Nosi[2], A. Tani[2], C. Viti[*1]

[1]Department of Agricultural Biotechnology; [2]Department of Anatomy, Histology and Forensic Medicine, University of Florence, Italy

Abstract

Biofilm, the form commonly taken by microorganism in environment, consist of surface-attached cells embedded in a self-produced extracellular polymeric matrix. *Pseudomonas alcaliphila* 34, a Cr(VI) hyper-resistant, Cr(VI) reducing, and biofilm producing bacterium, previously characterized from a phenotypical point of view by the standard Penotype Microarray (PM) technology for biotechnological proposes, was used as a model. The bacterium was grown into 96 wells polistirene microplates in mineral medium (TMM) supplemented with gluconate and Cr(VI) at different concentrations for 24 h. Then cultures were removed by the wells and biofilm was quantified by cristal violet assay. Metabolic activity of biofilm was also quantified in a separate experiment, using a tetrazolium dye.

The results showed that bacterial biofilm production got enhanced by Cr(VI) concentrations ranging from 6.25 to 50 mM, compared to the control condition [0 mM Cr(VI)]. On the contrary biofilm metabolic activity decreased with increasing Cr(VI) concentrations. This suggested that Cr(VI) enhances formation of biofilm, mainly composed by metabolic inactive cells. In order to confirm this result, biofilms of *P. alcaliphila* 34 grown in TMM added with gluconate or TMM added with gluconate and 6.25 mM of Cr(VI) were stained with Syto19 (a freely diffusible, nucleic acid intercalator that labels all cells in the microbial population regardless of viability) and propidium iodide (a membrane impermeant DNA intercalator that only stains cell with compromised membrane integrity) and analysed by confocal laser scanning microscopy. Since extracellular DNA (eDNA) is one of the main polymers contributing to the biofilm structure, we assessed the role of eDNA in biofilm structure of *P. alcaliphila* 34: cultures untreated and treated with Cr(VI) at different concentrations were grown in presence or absence of DNase I and subsequently biofilm production was measured by cristal violet assay while biofilm metabolic activity was analyzed by a tetrazolium dye.Our results showed that, for all samples tested, biofilm formation and biofilm metabolic activity were significantly reduced in cultures treated with DNase, suggesting that eDNA plays a key role in the formation of *P. alcaliphila* biofilm both in presence and absence of Cr(VI).

Acknowledgements

The research was supported by the MIUR (PRIN2008)