Taller: Estrategias de enseñanza en Microbiología

The target of this study was to obtain the best culture conditions for the production together with a chemical characterization of *Pseudomonas veronii 2E* EPS. EPS can be used for the removal of environmentally relevant metals in wastewater treatments thanks to its complexing capacity. In order to study the effect of different carbon and nitrogen sources on the production of EPS, two types of culture media were used: PY (per liter: peptone 5g, yeast extract 2.5g, glucose 0.5-20g or citrate 5g or glycerol 20g or succinate 5g) and a minimal medium M9 (per liter: K_2HPO_4 7,3g, KH_2PO_4 3g, NH_4Cl 1.0g, NaCl 0.5g, glucose 20g or glycerol 20g or succinate 5g or glutamate 5g or citrate 5g, supplemented with yeast extract 0.1g). The carbon source concentration was changed on PY, while different carbon sources and ammonium chloride as nitrogen source were used on M9. A third experiment to study the effect of temperature on EPS production was performed: *P. veronii 2E* was cultivated in a minimal medium M9 at two different temperatures (25°C and 30 °C). In all cases cells were separated by centrifugation (7000g) and the soluble EPS in supernatants was precipitated adding ethanol. After centrifugation, EPS content was determined by dry weight. Optimal results were obtained using M9-glicerol 2% at 25°C, indicating a temperature and carbon source dependence.

The biochemical composition of purified EPS (dialyzed,MW>12,400Da) was assessed using colorimetric methods : Lowry et. al (1951) for protein content; Anthrone assay for neutral sugar; Blumenkrantz (1973) for uronic acid and ammonium molybdate assay for phosphorus. IR spectroscopy and potentiometric titrations were done to explore functional groups present in the EPS.

Chemical characterization, combined with future analysis of the monosaccharides constituting the polymer and electrochemically monitored titrations will help to understand the EPS structure and complexing capacity responsible for the interaction with metals.

Código de Resumen: BB-003

Sección: Bioremediación y Biocontrol

Modalidad: Poster

SIDEROPHORES PRODUCTION AND *IN VITRO* ANTIFUNGAL ACTIVITY OF TWO PLANT GROWTH PROMOTING BACTERIA: *Gluconacetobacter diazotrophicus* AND *Burkholderia tropica*

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In the last decades there has been an increased interest in the use of Plant Growth Promoting Bacteria (PGPB) for biological control as an alternative to reduce the population of phytopathogenic organisms and to decrease the use of chemicals in agriculture. It has been demonstrated that PGPB can exert this function by diverse mechanisms such as competition for an ecological niche or a substrate, production of inhibitory allelochemicals and induction of systemic resistance in host plants to a broad spectrum of pathogens. Two N_2-fixing endophytic bacteria, *Gluconacetobacter diazotrophicus* Pal 5 and *Burkholderia tropica* MTo293, were described as potential PGPB, but their capabilities as biocontrol agents have been poorly characterized. In this way, batch cultures with these bacteria growing independently under different nutritional conditions were carried out to evaluate siderophores production and, on the other hand, plate assays were made using different phytopathogenic fungi in order to determinate their abilities to inhibit fungal growth. Both microorganisms produced siderophores under iron depletion, independently of other nutritional conditions. Six fungi strains were tested and both microorganisms showed growth inhibition against *Fusarium gramineae* (*Fusarium* head blight) and *Alternaria alternata* (diseases on tomato and others). These results show that the PGPB tested in this work are promising for their use as biocontrol agents of plant diseases. Future studies will be necessary to find the nature of the biological products (siderophores or others) that produce this inhibition, and antifungal activity should be tested *in vivo* to support these results.

Código de Resumen: BB-004

Sección: Bioremediación y Biocontrol

Modalidad: Poster

SOIL WASHING CONTAMINATED WITH HEAVY METALS BY USING BACTERIAL BIOEMUSIFIER AT LABORATORY SCALE.

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Heavy metal cannot be degradable into innocuous products and they tend to be strongly absorbed on the matrix of soils and sediments. These characteristics limit their solubilization and subsequent removal. An effective method to increase the metal-desorption of soil and sediments involves washing technologies assisted with surface active compounds as such bioemulsifiers. However, there is little information found in the literature regarding bacterial bioemulsfiers used for this purpose.

In previous studies, it have being demonstrated the ability to produce bioemulsifier by an actinobacterium, *Amycolatopsis tucumanensis* DSM 45259, using different carbon and nitrogen sources. Also it was showed that both production and hence functional properties of bioemulsifier is associated mainly to carbon sources used for biosynthesis.

Following these studies, the objective of the present work was to study the applicability of bioemulsifiers produced by *A. tucumanensis* DSM 45259 from different carbon a nitrogen sources, as washing agents in environmental remediation technologies, as well as to determine whether Cu(II) or Cr(VI) presence affecting the bioemuslfier production.

To achieve this, soil samples were artificially contaminated with Cu(II) or Cr(VI) added as $CuSO_4.5H_2O$ and $K_2Cr_2O_7$, respectively, at final concentration of 200 mg kg^{-1} of soil. Washing experiments were performed using 2.0 g of contaminated soil in flasks. Soils were washed with 10 ml of aqueous solutions of the partially purified bioemulsifiers, using deionized water as control. Emulsification index of each bioemulsifier solution was previously adjusted to 60%. The washing procedures were performed by shaking at 30 ºC between 12 to 24 h. Soil samples were centrifuged at 10,000g and the concentration of Cu(II) and Cr(VI) in supernatants were analyzed by atomic absorption spectrometry and Cr(VI) concentration was measured using a colorimetric method.

Under these assayed conditions, no significant Cu(II) removal could be detected after 12 h of washed either with H_2Od or bioemulsifier solutions. However, *A. tucumanensis* bioemulsifiers seemed to be effective for Cr(VI) recovery, whose removal from soil increased 2 fold while compared to H_2Od. Cr removed in the washing experiments remains in its hexavalent state. The increase of the in the washing time, did not improve the Cu(II) and Cr(VI) removal. Analysing the different effects of carbon and nitrogen sources and metal type, the last one was the most relevant variable that influence on the washing efficiency.

In relation to the production of bioemulsifier by *A. tucumanensis* DSM 45259 in the presence of metals, the results showed that the assayed concentrations of Cu(II) and Cr(VI) (10, 20 and 30 ppm) in the culture media did not affect the bioemulsifier production.

These are the first advances conducted in our research group focused on the direct application of microbial products in heavy metal remediation strategies.

Código de Resumen: BB-005

Sección: Bioremediación y Biocontrol

Modalidad: Poster

TOLERANCE OF *Lactobacillus kefir* TO LEAD, CADMIUM, NICKEL AND ZINC IONS

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Heavy metals such as lead, cadmium, nickel and zinc are natural elements with a high toxicity (depending on the concentration, persistence and speciation). To overcome this problem, microorganisms have evolved coping strategies to either transform the element to a less-harmful form or bind the metal intra- or extracellularly, thereby preventing any harmful interaction in the host cell.

The interactions between Lactic Acid Bacteria (LAB) and metal ions are very poorly investigated. Therefore, the objective of this work was to investigate the influence of heavy metal ions (Pb^{2+}, Cd^{2+}, Ni^{2+} and Zn^{2+}) on the growth of two strains of *Lactobacillus kefir.*

L. kefir strains CIDCA 8348 (aggregating) and JCM 5818 (non-aggregating) were used. Bacteria were grown in de MRS broth containing different concentrations of metal ions ranging from 0 to 10 mM and incubated at 30ºC for 76 h. The bacterial growth was determined by measuring the absorbance at 600 nm. The *lag time* and the EC50 (concentration of metal ion that produces 50% inhibition of bacterial growth) were determined from growth kinetics. Optical and transmission microscopy observations for each strain were carried out using the EC50. The metal ions uptake was determined using atomic absorption spectrometry.