DECHLORINASE ACTIVITY AND CHLORDANE REMOVAL BY *Streptomyces* STRAINS AS PURE AND MIXED DEFINED CULTURES

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Chlordane (CLD) is a toxic fumigating agent widely used in the past, which is now found in air, soil and water resources. Technical chlordane consists in 147 components, and it has been included in the list of the 12 persistent organic pollutants of Stockholm Convention (2001) because of its persistence, toxicity and tendency to biomagnification. Bioremediation is an attractive cleaning technique of polluted environments. The use of actinobacteria for this purpose, results an effective biotechnological approach due to their metabolic versatility and furthermore their use in mixed cultures can increase the catabolic pathways available for biodegrading these contaminants.

The aim of this work was to evaluate the chlordane removal capacity and dechlorinase activity by pure and mixed actinobacteria cultures, under controlled laboratory conditions, and to select one mixed culture for further morphological studies.

Streptomyces spp. M7, A2, A5, A6, A13 previously isolated in the laboratory and *Streptomyces coelicolor* A3 (2) were cultivated individually in minimal medium (MM) with CLD for acclimation. These strains, as pure cultures and consortia from two to six microorganisms, were cultivated in MM with CLD (1.66 mg L^{-1}). Microbial cells were used to obtain cell-free extracts for dechlorinase activity assays and the supernatants of these cultures were used to determine residual CLD by gas chromatography. The selected mixed culture according to their dechlorinase activity and capacity to remove CLD was grown in MM either with glucose or chlordane as carbon source and analyzed at 72 h in an optical microscope the probability of morphological changes.

Dechlorinase activity ranged between 0.00 to 1291.28 µmolCl^-/h/mg protein and CLD removal percentages was between 82.6 to 95.5%. The mixed culture consisting of *Streptomyces* sp. A2-A13-*Streptomyces coelicolor* A3(2) showed the best enzyme activity but not the minimal residual CLD concentration. Because no linear relationship between residual CLD and enzyme activity was obtained, the ratio between these two parameters was evaluated, and the mixed culture *Streptomyces* sp. A2-A5-A13 with the minimal obtained relationship was selected. In CLD presence, the microscopic analysis of this culture showed scarce vegetative cells and numerous spores, which results of the hyphal fragmentation.

These *Streptomyces* strains were able to grow as mixed cultures, in CLD presence, and showed ability to dechlorinate and remove this toxic compound from the culture medium. Therefore the mixed culture of *Streptomyces* sp. A2-A5-A13 could be a promising tool for CLD biodegradation.

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Sección: Bioremediación y Biocontrol

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BIOMINERALIZATION IN *Candida fukuyamaensis* RCL-3 UNDER COPPER OVERLOAD

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Copper (Cu) plays an essential role in cellular metabolism due to its versatility as a biological catalyst. It is required as a catalytic cofactor in many enzymes involved in diverse cellular processes. While trace amounts of copper are essential for life, copper can easily react with oxygen or hydrogen peroxide (H_2O_2) generating reactive oxygen species (ROS) that may damage cell constituents through the oxidation of proteins, cleavage of DNA and RNA, and lipid peroxidation. *Candida fukuyamaensis* RCL-3 (NCBI number AY743221), yeast strain isolated from a copper filter plant at the province of Tucumán, Argentina, has the ability of supporting high amounts of copper metal by a slowdown in its growth rate. Bioremediation mechanisms as bioaccumulation, biospeciation, biomineralization has been descripting in yeast. In order to understand the mechanism involved in *C. fukuyamaensis* RCL-3 resistance to copper it was conducted an approach. Atomic absorption spectroscopy results showed decrease copper concentration (from 0.5 to 0.14 ± 0.05 mM) in the culture medium after 16 h inoculation. At the same time, change in cells coloration to brownish color was observed. Is known that cooper sulfide (CuS) mineralization on the surface of cells causes the cells turns brown. Upon addition of KCN to Cu-grown *C. fukuyamaensis* RCL-3 cells, the brownish coloration was bleached instantly, and cooper ions were solubilized. Sulfate reduction as the brown coloration of Cu-treated cells was attenuated when ammonium chloride was substituted for ammonium sulfate in the growth media.

The results obtained in the present work show that when exposing *C. fukuyamaensis* RCL-3 to 0.5mM copper concentration, is produced a biomineralization process probably involved as cell bioremediation mechanisms.

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BIOCONTROL OF BACTERIAL SPECK *Pseudomonas syringae* pv. *tomato* DC3000 OF TOMATO SEEDLINGS BY *Pseudomonas* spp. STRAINS SVBP6 AND RBAN4

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Pseudomonas syringae pv. *tomato* DC3000 (*Pto* DC3000) is the cause of bacterial speck in tomato (*Solanum lycopersicum*). This bacterium represents an important model in molecular plant pathology. Tomato is one of the most important vegetable crops worldwide. The effective controls of bacterial speck are currently explored in crop plants. In response to the high environmental pollution caused by toxic agrochemicals, new ecological alternatives such as biological control are being developed and implemented for the control of plant diseases.

Pseudomonas spp. strains are widely recognized for their ability to antagonize the growth of pathogens and to induce systemic disease resistance in plants. The *Pseudomonas putida*-related strain SVBP6 was isolated from an agricultural bulk soil and the *Pseudomonas koreensis*-related RBAN4 strain is a rhizospheric isolate from native pasture of a non agricultural soil. In this work, the ability of SVBP6 and RBAN4 strains to reduce the incidence of bacterial speck in tomato seedlings was tested. Four-day-old seedlings of the susceptible genotype cv. Platense, were placed on Murashige and Skoog's medium (MS) containing 0.5 % agar and the corresponding bacterial suspension. The bioassays were set up independently with the strains SVBP6 or RBAN4 (OD_{600} = 0.1 on the plate). After 4 days of treatment, seedlings were placed on MS-agar plates (0.8% agar) and infected with 1×10^8 cells ml^{-1} *Pto* DC3000 by flooding method. Symptoms of bacterial damage in tomato seedlings were evaluated at 7 days post-infection. Pretreatment of tomato seedlings with either *Pseudomonas* strain before inoculation with *Pto* DC3000 significantly decreased the infected area of cotyledons compared with controls (no treatment or *Escherichia coli* treatment). To evaluate the *Pto* DC3000 load, disks were cut from SVBP6- or RBAN4-pretreated cotyledons, homogenized in sterile distilled water, and serial dilutions were plated onto KB agar medium to measure the number of Colony Forming Units (CFU). In SVBP6- or RBAN4-pretreated seedlings the number of CFU were $2.14 \pm 1.65 \times 10^7$ and $3.15 \pm 1.89 \times 10^6$ CFU ml^{-1}, respectively compared with non-pretreated seedlings ($8.93 \pm 0.995 \times 10^9$ CFU ml^{-1}). Accumulation of the antioxidant enzyme, ascorbate peroxidase (APX) protein, and of chitinase as a pathogenesis-related protein, was detected in SVBP6- and RBAN4-pretreated seedlings compared with controls, suggesting that both *Pseudomonas* strains could trigger inducible defense mechanisms *in planta*. Currently, our goal is to elucidate the mechanism underlying the biological control of *Pto* DC3000 by SVBP6 or RBAN4 strains in tomato.

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MECHANISMS INVOLVED IN CHROMIUM (VI) REMOVAL BY *Serratia* sp. C8

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Different processes by which microorganisms remove chromium Cr(VI) have been described, including bioadsorption, bioaccumulation and enzymatic reduction to the less toxic form Cr(III). The present work deals with the study of the mechanisms involved in Cr(VI) removal in *Serratia* sp. C8, a strain previously isolated from tannery sediments with ability to tolerate and reduce this heavy metal.

Two different methodologies (cell inactivation by heat and acid pH) were used to demonstrate that the strain was capable to bioadsorb around 7.5-8.5% of 10 mg/l Cr on their surface. Extracellular chromate reductase activity was not detected in *Serratia* sp. C8, however the results suggested that the enzyme would be present in cytoplasm, since the soluble fraction was responsible of 34% Cr(VI) reduction. To determine the final localization of initially incorporated Cr(VI) to the culture media, total