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development was analyzed. The average of fresh and dry weight of the vegetative tissue was measured in these inoculated and control plants. As a result, both parameters showed a significant increase when OI43 was present. In parallel, isolate OI43 was analyzed as a biocontroller. Mushrooms used include *Rhizoctonia solani, Fusarium verticillioides, Fusarium sp., Fusarium graminearum* and *Macrophomina phaseolina*. Isolate OI43 showed significant biocontrol capacity for *Macrophomina phaseolina.* Finally, in order to identify the isolate OI43, 16sRNA and GyrA genes were amplified using specific oligonucleotides. Since, these sequences were not enough to identify the isolate, the OI43 genome was sequenced. The taxonomic classification was obtained by MiGA, a data management and processing system for microbial genomes and metagenomes. According to this tool, the dataset most likely belongs to the order *Bacillales* (p-value: 0.002) and probably belongs to the family *Bacillaceae* (p-value: 0.019).

BB18-CLONING AND EXPRESSION IN *E. coli* OF THE *LINA* GENE FROM *Streptomyces* sp. M7, ENCODING A DECHLORINASE ENZYME

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Streptomyces sp. M7 is an actinobacterium isolated from contaminated sediments with heavy metals and pesticides in Northern Argentina and is able to grow in the presence of hexachlorocyclohexane isomers (γ-HCH, α-HCH, β-HCH) as carbon sources. This property is due to the presence of a high and low metabolic pathway for xenobiotics degradation, where the first enzyme corresponds to LinA, dehydrochlorinase/dehydrohalogenase. This is able to catalyze the breaking of the highly resistant C-Cl bonds of hexachlorocyclohexane, an essential step for its subsequent biodegradation into less toxic metabolic intermediates and/or final products such as carbon dioxide and water. In this work, the *linA* gene belonging to *Streptomyces* sp. M7 that codes for dehydrochlorinase (DHC) was synthesized (GenBank: MH703800). The sequence was adapted according to high-frequency-usage codons in *E. coli*. In the sequence redesign process, two restriction sites, *NcoI* and *XhoI*, were added, which are in the bacterial vector pET28a+ used for the expression of 6xHis-tagged N-terminal proteins with a thrombin site. Previously, the synthetic DNA sequence was constructed and cloned in a pTOP Blunt V2 plasmid that was later treated with both restriction enzymes, giving rise to an expected fragment of 475 bp. The released fragment was cloned into the pET28a+ vector and was transformed in competent *E. coli* BL21 (DE3) host cells with T7 phage promoter. In the same way, transformants were obtained with the expression vector without the cloning fragment and used as control. It should be noted that the plasmid sequence construction and analysis were performed using the Vector NTI software. Subsequently, for the differential expression assay optimization, different conditions were analyzed: 0.4 mM, 0.7 mM, and 1 mM of IPTG, temperatures of 30 and 37 °C and variable times. The most favorable setups were 1 mM of IPTG, 37 °C, and 2 hours of incubation. Subsequently, the cell-free extract was obtained by sonication and analyzed by SDS-PAGE. The stained gels showed a differential band of approximately 39,000 Daltons, confirming the expression of the LinA-6xHis fusion protein.

This approach will allow the analysis of the LinA enzyme, as well as its subsequent application in bioremediation technologies.

BB19- MYCOREMEDIATION OF CITRUS WASTEWATER BY WHITE-ROT FUNGI IMMOBILIZED IN *Luffa cylindrica.*

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Citrus-processing industries produce large volumes of wastewater (WW) characterized by a high content of organic matter, presence of pesticides, and terpenes. Although several strategies were developed for their treatment, white-rot fungi (WRF) have emerged as a promising alternative owing to their high tolerance and ability to degrade xenobiotics. WW can inhibit fungal growth due to the presence of toxic compounds and/or bacterial proliferation, therefore fungal immobilization on low-cost lignocellulosic materials is proposed as an effective approach to increase their stability. The aim of this work was to evaluate two WRF immobilized on lignocellulosic material for the mycoremediation of real citrus WW. The strains *Phlebia brevispora* BAFC 633 and *Pleurotus pulmonarius* LBM 105 were immobilized on *Luffa cylindrica*. Citrus WW and *L. cylindrica* were kindly provided by Cooperativa Citrícola Agroindustrial de Misiones Ltda. (Leandro N. Alem, Misiones) and Espudela (Jardín América, Misiones), respectively. The *L. cylindrica* was washed with tap water, rinsed with distilled water, oven-dried at 40 ± 2 °C, and cut into 1 cm^3 pieces. All the experiments were carried out in triplicate in 250 mL Erlenmeyer flasks containing 1 g of *L. cylindrica*. Initial moisture was adjusted to 75 % w/w with Czapek medium (sucrose 30 g/L; K_2HPO_4 1 g/L; KCl 0.5 g/L; $MgSO_4$ $7H_2O$ 0.5 g/L; $NaNO_3$ 20 g/L). Autoclave-sterilized flasks were inoculated with three agar plugs (~7 mm ⌀) of each strain and were incubated for different time periods (0, 3, 6, 9, and 12 days) at 28 ± 1 °C under static conditions. After incubation, 50 mL of filtered citrus WW was added to the cultures, and flasks were incubated for 10 extra days (treatment), destructive samples were taken every 48 h. The control consisted in WW without immobilized fungi. Supernatants were obtained by centrifugation at 4600 *xg* for 10 min. Chemical oxygen demand (COD) and toxicity were determined following standard protocols (open reflux method and *Lactuca sativa* seed germination/root elongation test, respectively). COD variation was estimated as:

$$\% \text{ } = \frac{(\text{ } - \text{ })}{\text{ }} * 100$$

where A and B are COD before and after treatment respectively. A significant COD reduction was observed for both strains. *P. brevispora* BAFC 633 reached 83.01 ± 1.81 COD reduction after 10 days of treatment without prior incubation. For *P. pulmonarius* LBM 105 a 92.82 ± 5.18 % COD reduction was determined after 10 days of treatment without prior incubation, and 94.26 ± 5.18 % with 9 days of incubation. However, toxicity test showed that the treatment with *P. pulmonarius* LBM 105 without