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Molecular Detection and Characterization of Novel Lipase Genes of the Lipolytic Yeast *Candida palmioleophila*

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In this study we analyzed the genetic variability of lipase gene sequences from eight oil and grease-degrading strains of *Candida palmioleophila* and to relate it to their degrading ability. The genetic variability of lipase genes was analyzed by PCR-restriction fragment length polymorphism (PCR-RFLP) and low-stringency single specific primer- PCR (LSSP-PCR), in order to obtain specific DNA fingerprints from each strain, which were subsequently compared by bioinformatic programs. DNA fingerprints were contrasted to the ability of each strain to remove palm oil in liquid culture. The results showed that at least three genes encoding lipases are present in *C. palmioleophila*, two of them resembling the *LIP*2 and *LIP*6 genes of *C. albicans.* DNA fingerprints obtained by LSSP-PCR revealed differences in the sequences of *C. palmioleophila* lipase genes, which allowed to group the strains according to their degrading activity. C. palmioleophila strains SACL05, SACL08 and SACL11, which showed the highest removal of palm oil after 72 h (77 to 79 % removal), were grouped in a single clade in dendrograms. Similarly, strains SACL01, SACL03, SACL06 and SACL09, which showed intermediate removal activity of palm oil (54 to 76%) grouped in a different clade. This suggests the genetic variability in lipase genes is directly related to the differences found in the efficiency of degradation of oils. On the other hand, DNA fingerprints obtained by PCR-RFLP did not allow to differentiate the strains and did not generate changes in the bands patterns between the analyzed strains. In conclusion, this study reported for the first time the detection and characterization of lipase genes from the lipolytic yeast *Candida palmioleophila*, and their association to the degradation of oils.

1. Introduction

Lipases are an important group of enzymes produced by a wide range of microorganisms, useful in different fields such as the pharmaceutical, industrial, chemical and environmental due to their regioselectivity, enantioselectivity and selectivity in the chain length of the substrate in which they act. For these reasons, there is a growing demand for identifying new lipolytic enzymes for the development and optimization of bioprocesses (Sharma et al. 2011). Lipases can be used in environmental applications, specifically in wastewater treatment processes from oil refineries, as toxic wastes are generated during all processing processes causing serious contamination in soils and water. These enzymes are produced by animals, plants, and microorganisms in general, which catalyze the ester-chain hydrolysis of triacylglycerols and release fatty acids and glycerol (Guncheva and Zhiryakova 2011). The most representative genera of microorganisms producing lipolytic enzymes are *Candida*, *Pseudomonas*, *Burkholderia*, *Thermomyces*, *Rhizopus*, *Bacillus*, *Staphylococcus*, *Geobacillus*, *Acinetobacter*, *Rasltonia* and *Yarrowia* (Bell et al. 2002).
The lack of knowledge about new microorganisms exhibiting lipase activity has led to new investigations about the prospection of new lipolytic strains and to identify new lipases with biotechnological potential, especially for the mitigation of the environmental impact. Previous studies of our group have shown that different strains of the lipolytic yeast *C. palmioleophila* present different removal efficiencies of fats and oils under the same conditions, even though they show 100% similarity in their ribosomal sequences (Rodriguez-Mateus et al. 2016). These microorganisms could have a high potential for their use as bioremediation agents of effluents

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contaminated with fats and oils. The microbial degradation of fats and oils in these microorganisms is mainly modulated by extracellular lipases; however, to date, there are no studies about *C. palmioleophila lipases*, or their coding genes. The variability in these genes could significantly influence the activity and efficiency of these enzymes. Thus, the objective of this study was to detect and characterize genes encoding lipases in *C. palmioleophila* in order to correlate their genetic variations to their reported ability to metabolize grease and oils.