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(22nd SPPS Meeting)

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two main approaches: (i) using sequence data from the ribosomal internal transcribed spacer regions (ITS) and elongation factor 1-alpha (EF); and (ii) using specific PCR-based marker (IGS1). Eighty nine isolates assumed to be *A. pisi, D. pinodes* and *D. pinodella* of diverse geographical origins were used. Following DNA extraction, ITS and EF were amplified in all tested isolates. The partial sequences were used for identification and clarification of intra- and inter-species relationships. The phylogenetic analysis using ITS sequences revealed that the most *A. pisi* isolates formed clusters with high bootstrap values, but differentiation between *D. pinodes* and *D. pinodella* isolates was not possible. Phylogeny based on EF sequences enabled differentiation of *D. pinodella* isolates, but *A. pisi* and *D. pinodes* could not be separated. Amplification with primers specific for IGS1 marker resulted in different amplification profiles in all three fungi, enabling their identification and differentiation.

Keywords: ascochyta, differentiation, sequence, IGS1

Optimization of reaction conditions for phenol removal in batch reactor with horseradish peroxidase immobilized within tyramine-alginate micro-beads

PP5-28

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Removal of phenolic compounds from wastewaters was previously studied using different enzymatic approaches. In the presence of hydrogen peroxide, peroxidases are able to oxidize phenol-like compounds and form non-soluble polymers that could be easily removed from aqueous phase. Horseradish peroxidase (HRP) is the most investigated peroxidase used for phenol removal from waste effluents, but it can be easily inactivated during this process by excess of hydrogen peroxide. In order to increase operational stability of the enzyme, immobilization on different materials and various peroxide delivery systems were tested. In our previous work, we studied bioinspired hydrogels based on natural cell wall polymers and enzymes, for efficient removal of phenols from water. In this work, tyramine-alginate hydrogels that we have previously developed were used for horseradish peroxidase encapsulation within micro-beads obtained in a coupled emulsion polymerization reaction. The aim of this research was to study the influence of tyramine- alginate concentration and hydrogen peroxide delivery system on operational stability and efficiency of phenol removal by immobilized peroxidase. The best result of 96% phenol removal from water solution was achieved by peroxidase immobilized within 20% (w/v) tyramine-alginate micro-beads using delivery system for hydrogen peroxide composed of 0.187 U mL^{-1} of glucose oxidase and 4 mmol L^{-1} of glucose. The reusability studies showed that these biocatalysts can be used up to five cycles with slight decrease in their catalytic performance.

Keywords: immobilization, horseradish peroxidase, phenol removal, tyramine, alginate

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