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ANALYZING THE VIABILITY OF BIOACTIVE COMPOUNDS RECOVERY BY SOLID-STATE FERMENTATION USING *Trametes versicolor* AND *Phanerochaete chrysosporium*

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Introduction. Solid-state fermentation (SSF) processes can be an interesting technology for the extraction and/or production of plant metabolites, able to provide high quality and high activity extracts while precluding any toxicity associated to the use of organic solvents. *Larrea tridentata,* also known as creosote bush or chaparral, is a lignin-rich plant native from the semi-arid regions of Northern Mexico and South-western of United States.
The purpose of the present study was to evaluate the potential two basidiomycetes (*Trametes versicolor* and *Phanerochaete chrysosporium*) known by their ability to degrade lignin, to recover or enhance the extraction of bioactive compounds (nordihydroguaiaretic acid (NDGA), kaempferol and quercetin) from *Larrea tridentata* leaves.

Methodology. SSF cultivations were performed in 100 mL Erlenmeyer flasks containing approximately 2.0 g of sterilized powdered plant. The plant material was moistened with the following culture medium to attain 70% moisture content (g/L): K_2HPO_4 (1.0), $CaCl_2$ (0.07), $MgSO_4$ (0.35), $FeSO_4.7H_2O$ (0.035), $ZnSO_4$ (0.023) and $CuSO_4$ (0.0035), adjusted to pH 5 and sterilized at 121 °C for 15 min. The moistened material was inoculated with 2×10^7 spores/g plant, and statically incubated at 25 and 37 °C (*T. versicolor* and *P. chrysosporium*, respectively). All the experiments were conducted in tetraplicate. Samples for analysis were collected after 6 days for *P. chrysosporium*, and after 6 and 12 days for *T. versicolor*. The total content of each Erlenmeyer was collected as a sample and the fermented broth was extracted by filtration (extract A). The dried fermented plant was then subjected to extraction by mixing 1 g of material with 20 mL of methanol 90% (extract B). The mixtures were heated during 30 min in a water-bath at 65 ºC and the produced extracts were filtered. All samples were stored at -20 ºC until further analysis. Bioactive compounds were quantified by HPLC.

Results and Discussion. *T. versicolor* and *P. chrysosporium* are white-rot fungi known by their ability to produce extracellular oxidative enzymes, in particular lignin and manganese peroxidases and laccase, which degrade plant wood compounds such as lignin (1, 2). The bioactive compounds concentration recovered during SSF with these strains are summarized in Table 1.

Table 1. Bioactive compounds (mg/g plant DW) recovery during SSF of *Larrea tridentata* leaves by *Trametes versicolor* and *Phanerochaete chrysosporium*.

Trametes versicolor			NDGA	Kaempferol	Quercetin
Extract A	Day 6	IC	0.17	0.15	0.52
		Sample	0.19	0.16	0.52
		FC	0.15	0.14	0.48
	Day 12	Sample	0.28	0.27	0.67
		FC	0.18	0.20	0.51
Extract B	Day 6	IC	24.69	18.35	18.62
		Sample	22.76	18.00	13.45
		FC	21.00	16.20	11.94
	Day 12	Sample	20.57	16.52	11.92
		FC	21.55	17.45	12.58

Phanerochaete chrysosporium			NDGA	Kaempferol	Quercetin
Extract A	Day 6	IC	0.17	0.15	0.52
		Sample	0.30	0.34	0.26
		FC	0.30	0.26	0.64
Extract B	Day 6	IC	24.09	18.35	18.62
		Sample	24.72	20.53	14.71
		FC	17.12	13.55	9.20

IC and FC: Control assays (plant without spores' inoculation, at the beginning and at the end of the process, respectively); Sample (plant inoculated with spores).

Due to the high content of lignin (36% w/w) in *Larrea tridentata* leaves, a hypothesis had been formulated considering that these fungal strains could liberate bioactive compounds from cellular degradation of this plant material during SSF. Nevertheless, the present results showed that, although the fungal strains were able to growth when cultivated in the plant material, neither a significant liberation nor an improvement of chemical extraction of bioactive compounds with methanol occurred by submitting the plant to SSF.

Conclusion. Solid-state fermentation using *Trametes versicolor* and *Phanerochaete chrysosporium* was not an effective technique for the liberation of bioactive compounds from *Larrea tridentata* leaves.

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