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Identification of Leachables from Trametes versicolor in Biodegradation Experiments

Åke Stenholm*
Department of Medicinal Chemistry,Uppsala University, BMC Box 574, SE-751
E-mail: ake.stenholm@ilk.uu.se

ABSTRACT

The transport of fungal-derived compounds from Trametes versicolor to the environment was investigated. Fatty acids and sphingoids were identified at the outlet of a bioreactor containing an acidic nutrient solution and immobilized fungal mycelia. The analyses were conducted using UHPLC-Q-TOF-MS (/MS). Eleven fatty acids, including C20:0, C18:1-OH and C20:0-OH that have not been previously described for this species, were detected. The identities of myristic acid (C14:0), palmitic acid (C16:0) and stearic acid (C18:0) were confirmed using reference standards. Six sphingoids, including Sph (t18:0), Sph (t18:1), Sph (d18:0), Sph (d18:1), Sph (d16:0) and Sph (d16:1), were tentatively identified, and the identities of Sph (d18:0) and Sph (d18:1) were confirmed by reference standards. The findings show that an array of compounds, with concentrations at the µgL-1 level, was easily transported from the fungal mycelia. This is of concern when the investigated species is used in biodegradation experiments of xenobiotics and conclusions are to be drawn on the quality of the treated water. The study thus shows that the chemical composition of water treated with Trametes versicolor is also influenced by the immobilized fungus itself. The lipids that were detected, including fatty acids and sphingoids do not present any threat to the environment since they are not toxic. At µgL-1 concentration levels, they are soluble in water.

The enzyme-catalysed biodegradation of environmentally hazardous compounds in lab-scale experiments is well documented, including research that has studied the biodegradation potential of extracellular enzymes secreted by white rot fungi (WRF) mycelia [1].The particular interest in these fungal species is related to their effective biodegradation of recalcitrant pharmacologically active compounds including endocrine disrupting compounds (EDCs) [2,3]. These studies typically emphasize three objectives: (A) removal of target compounds; (B) correlation of this removal to measured enzymatic activities; and (C) identification of degradation products, including toxicity assessments [4-6]. However, there is limited knowledge regarding which constituents are leached from the mycelial cells into the medium.

Secretion and excretion are two concepts that are commonly used to describe the processes through which compounds penetrate cell walls. Secretion is the active process of releasing and transporting chemical substances, like extracellular enzymes (exoenzymes), out of a cell, while excretion is the passive transport of waste products that have no further utility. Active processes do not only comprise the secretion of exoenzymes (exocytosis) from fungal cells, but can include endocytosis, in which extracellular macromolecules are engulfed by the cell membrane and imported into the cell [7]. Exocytosis describes a process in which membrane-bound vesicles containing enzymes, toxins and lipids -fuse with the plasma membrane in hyphae [8]. Like the plasma membrane, the vesicle membranes also contain lipid bilayers. Upon fusing, the vesicle membrane proteins and lipids move to the outer region of the fungal plasma membrane via the Spitzenkörper, i.e. a vesicle supply centre present in hyphae.

Results

In all experiments, diclofenac d-4 was present at a concentration of 100 µgL-1.The detected compounds were present at approximate µgL-1 levels. All substances were detected using positive mode ESI MS. For fatty acids (FAs), negative mode ESI (MS/MS) was used to confirm the chemical structures of the most abundant FAs.

Keywords: Xenobiotics; Immobilized fungus; Sphingoids; lipids