



Extended Abstract

## Extraction of Proteins from Microalgae Using Lytic Enzymes Produced by *Trichoderma* Strains †

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Filamentous fungi are well known as sources of industrial enzymes with high capacity for extracellular protein production [1]. The aim of this study was to use lytic enzymes produced by Trichoderma strains grown on whey for the extraction of proteins from microalgae. By this approach, the use of lateral flows to produce high value bioactive compounds is achieved, bioactive compounds which can be further formulated and used as nutritional supplements or plant biostimulants. Trichoderma isolates were grown on whey, a by-product of the food industry. T. asperellum and T. atroviride were incubated for 2 weeks in minimal medium (MM) supplemented with 20% whey, using potato dextrose broth (PDB) at the same dry matter concentration as the control. Protease and cellulase activities were assayed using casein and Folin-Ciocalteu reagent, and, respectively, carboxymethylcellulose (CMC), microcrystalline cellulose (MCC), and 3,5-dinitrosalicylic acid (DNS) reagent [2]. Sterile filtrates of Trichoderma isolates grown on whey and PDB were used for the enzymatic extraction of proteins from microalgae at different temperatures. The molecular weights of the proteins were determined by SDS-PAGE. The protease (caseinase) activity of the *T. asperellum* strain (T36) was significantly increased due to the incubation in whey medium. Sterilization of whey by filtration induced the highest activity, while autoclaving appeared to decrease the induction of the caseinase activity. For the cellulase activity, the whey induced an increase in the enzymatic activity of T. asperellum (T36) compared to control. High cellulase and protease activity was observed only for autoclaved whey in the case of T. atroviride. The extracellular enzymes secreted by *Trichoderma* strains amplified the cell lysis of microalgae. The SDS-PAGE profile of extracted proteins at 25 °C showed a wide distribution of molecular weights, with several intense bands between 5 and 30 kDa. In this study, we developed a biotechnological method for using co-products resulting from the diary industry for inducing lytic enzymes in Trichoderma cultures capable of amplifying the protein extraction from microalgae cells. The biomass resulting from *Trichoderma* growing on whey can be used as plant biostimulant.

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## References

1. Jun, H.; Kieselbach, T.; Jönsson, L.J. Enzyme production by filamentous fungi: analysis of the secretome of Trichoderma reesei grown on unconventional carbon source. *Microb. Cell Fact.* **2011**, *10*, 68.

2. Gajera, H.P.; Vakharia, D.N. Production of lytic enzymes by *Trichoderma* isolates during in vitro antagonism with *Aspergillus niger*, the causal agent of collar rot of peanut. *Braz. J. Microbiol.* **2012**, 43, 43–52.

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