Taller: Estrategias de enseñanza en Microbiología

FMN-dependent oxidoreductases that catalyze the insertion of an oxygen atom from molecular oxygen next to a carbonyl group at the expense of NAD(P)H and the other atom is reduced to water. Lactones are very versatile precursors for the synthesis of natural products, analogs and bioactive compounds. Moreover, some BVMOs can catalyze the oxidization of linear ketones as well as selenium- and boron-containing compounds, sulfoxidations, epoxidations and N-oxidations.

The aim of our study is to expand the number of biocatalysts available for chemical applications. In this work we explored the predicted proteome of *Leptospira biflexa* (Paris) using protein sequences of known BVMOs as queries for protein blast searches and, as a result, one putative BVMO sequence was retrieved. To examine the relationships between the identified sequence and previously characterized BVMOs, they were aligned and phylogenetic trees were inferred. The putative BVMO from *L. biflexa* is related to BVMOs with variable substrate preferences. This novel protein exhibits the characteristic consensus sequence and dinucleotide-binding motifs of BVMOs. In order to evaluate its substrate preference, the identified BVMO-encoding gene was cloned and functionally expressed in *Escherichia coli* BL21(DE3). Whole-cell biotransformations were carried out and linear aliphatic, monocyclic, bicyclic and aromatic ketones were tested as substrates. We observed that the BVMO from *L. biflexa* was able to oxidize some cyclic compounds and linear short-chain ketones.

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ADAPTED AND OPTIMIZED COLORIMETRIC METHOD FOR THE RAPID ON-LINE QUANTIFICATION OF SCLEROGLUCAN DURING A SUBMERGED FERMENTATION PROCESS

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Scleroglucan is an extracellular neutral β-1,3-β-1,6-glucan frequently produced by *Sclerotium* fungal species during submerged fermentation processes. Due to its physicochemical, rheological and biological properties, scleroglucan became particularly attractive for diverse food, agro industrial, biomedical and oil recovery applications. Currently, the most widely used technique for polymer quantification consists in its purification from culture broths and dry weight determination. This method has the inconvenience of being poorly sensitive at low concentrations and time-consuming, therefore, being not suitable for real-time monitoring. Recently, *Jörg Nitschke et al.* (*Food Chemistry,* 2011. 127: 791–796) developed a colorimetric Congo red-based method to quantify β-1,3-glucans in mycelia and fruiting bodies from edible mushrooms. Congo red would incorporate into the β-1,3-β-1,6-glucans triple helix thus leading to a bathochromic shift that can be used for colorimetric quantification. Based on this previous report, this work is aimed at adapting and optimizing this novel technique in order to on-line quantify scleroglucan production during submerged fermentation. For this purpose, several dye (0.6-1 g/L Congo red) and NaOH (80-200 µL of NaOH 1 or 1.2 N) concentrations were tested to achieve the greater bathochromic shift when using commercial scleroglucan (LSCL) as standard. Reproducibility of bathochromic shift was also evaluated with lab-scale produced scleroglucans. A scleroglucan calibration curve (0.1-0.9 g/L) could be satisfactorily constructed. Linearity, sensitivity and specificity within this working range were assessed at different wavelengths and time points (0, 30 min, 1, 3, 5, 8 and 24 h post reaction). Finally, to validate the methodology, a fermentation process with *Sclerotium rolfsii* ATCC 201126 was performed, and scleroglucan quantification was simultaneously accomplished by conventional (dry weight) and Congo red methods. Selected conditions allowed the reliable and sensitive scleroglucan Congo red quantification during fermentation. Both commercial and lab-scale produced scleroglucans could be successfully used for the standard curve preparation. This novel methodology proved to be highly effective and sensitive for the on-line quantification throughout scleroglucan production, and the obtained results were comparable to those from the conventional technique (dry weight). The method optimized for scleroglucan measurement showed to be inexpensive, practical, reliable, specific and time-effective, being also potentially useful for other triple-helical β-glucans. Additionally, on-line monitoring of scleroglucan production represents a critical tool for taking real-time appropriate decisions during fermentation process, particularly when working at large scale.

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WHEY FERMENTED WITH KEFIR MICROORGANISMS: PROTECTION AGAINST *Salmonella* ENTERITIDIS INFECTION IN BROILER CHICKENS