SCREENING AND ASSESSMENT OF LACCASE PRODUCTION BY DIFFERENT FUNGAL STRAINS

Elena Mariana Mundo[1,2], Ma. de Lourdes Acosta[2],Daniel Morales[1] y María del Refugio Trejo[1];
[1]Centro de Investigacion en Biotecnología, [2]Centro de Investigacion en Ciencias Biologicas, Universidad Autonoma del Estado de Morelos Av. Universidad 1001, Col. Chamilpa, Cuernavaca, Morelos. sapito_m87@hotmail.com

Key words: screening, laccase, basidiomycetes

Introduction. Laccases (benzenediol:oxygen oxidoreductases; EC1.10.3.2) are multicopper enzymes belonging to the group of blue oxidases. Laccases exist widely in nature and are defined as oxidoreductases which oxidize diphenols and similar substances and use molecular oxygen as an electron acceptor (Baldrian 2004). Their substrate hability makes them highly interesting for diverse applications, including textile dye bleaching, pulp bleaching and bioremediation. Enzymatic catalysis is an environmentally friendly alternative. The present work reports the production of laccases by a local isolates of *Trametes versicolor, Phanerochaete chrysospoirum and Pleurotus djamour roseus* by submerged fermentation.

Methodology. The inocula were prepared by suspending the mycelia from one plate in sterile 0.9% (w/v) Sodium chloride. One plate was used to inoculate three parallel fungi cultivations. The production of extracellular laccase in each organism was followed in three cultivations in 250 mL shake flasks containing 100 mL of liquid medium with 120 rpm agitation during 20 days. For enzyme production and growth of the different fungi strains 6 cultures media were used: Brand Flakes (BF), integral wheat flour (IWF), integral wheat flour (IWF+saccharose), Wheat Bran (WB) natural), Potato dextrose (PE) and Malt extract (ME). Standard laccase activity was determined by oxidation of ABTS at room temperature. The reaction solution was composed of 1mM ABTS in a 900 µL-acetate buffer, pH 3.6. A suitable amount of enzyme was added, and the oxidation of ABTS was followed by measuring the absorbance increase at 436 nm. One unit of laccase activity was defined by the amount of enzyme able to oxidize 1 µmol ABTS/min under these conditions.

Results. The results obtained show that the fungal strains studied are capable of producing laccase in the different media evaluated with varying levels of volumetric activities.

In the production curves, two periods of higher volumetric activity levels were observed: the first, day 2 to 9, and the second, day 14 to 20. These observations could indicate the presence of isoforms that appear at different stages of mycelial growth. Likewise, the influence of the culture media on the production of volumetric activity has been demonstrated. The highest laccase volumetric activity for *T. versicolor* was obtained in the ME medium (364U/L). Meanwhile, for the *P. chrysosporium* strain, laccase production was 169 U/L. The lowest laccase activity was obtained with the *P. djamour roseus* strain with a value of 77 U/L. With respect to the media evaluated, we observed that wheat bran and potato extract gave best the results. Meanwhile, *P. chrysosporium* was able to produce laccase in culture media with WB, BF, IWF+S and IWF. The highest laccase production from the *P. djamour* roseus strain was achieved with in the malt extract medium.

Table 1. Effect of culture medium on laccase production by basidiomycetes

Medium	*T. Versicolor*	*P. Chrysospoirum*	*P. Djamour r.*
WB	309	169	38.6
BF	92	143	25.9
IWF+S	77.4	150	14.4
IWF	47.2	143	45.4
PE	364	10.8	42.7
ME	273	6.7	77.8

Conclusions. Laccase production for the different fungi studied was influenced by the culture medium. The production of laccase in the absence of inducers (lignocellulosic subsrates) was observed.

Acknowledgements. Leobardo Serrano (IBT-UNAM).

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