

XIX CONGRESO DE LA SOCIEDAD ARGENTINA DE MICROBIOLOGÍA GENERAL

22 al 25 de octubre del 2024

Centro cultural y Pabellón Argentina de la Universidad Nacional de Córdoba, Córdoba, ARGENTINA.



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OPTIMIZATION AND CHARACTERIZATION OF ENDO-1,4-?-XYLANASE ACTIVITY IN *Lentinus sajor-caj* LBM266

Barua, Celeste^{1,2,3} - Coniglio, Romina^{1,2} - Turike, Ileana^{1,2} - Zapata, Pedro^{1,2} - Fonseca, María^{1,2}

1) Universidad Nacional de Misiones - Facultad de Ciencias Exactas, Químicas y Naturales - Instituto de Biotecnología Misiones Dra. María Ebe Reca (InBioMis) - Laboratorio de Biotecnología Misiones (Biotecmol) - Posadas - Misiones - Argentina.

2) CONICET, Buenos Aires, Argentina.

Contacto: baruaceleste@gmail.com

Endo-1,4-?-xylanase (EX) cleaves the xylan backbone at 1,4-?-linkages in hemicellulosic biomass. EX holds significant potential in juice clarification due to its effectiveness in degrading hemicellulosic biomass in fruit juices. The cost of the available enzymes has driven the search for more cost-effective production sources. This study focused on optimizing a culture medium of *Lentinus sajor-caj* LBM 266 to enhance the EX activity. Also, we aimed to determine the optimal pH and temperature for EX activity and assess its stability across different temperatures and pH levels. A screening assay with 36 runs and 4 central points evaluated the effects of 9 factors: 6 lignocellulosic substrates (10 g/L) and 3 nitrogen sources (5 g/L). These factors were assessed in various combinations using a 1/16 2⁹⁻⁴ factorial design created with STATGRAPHICS CENTURION software. Factors significantly affecting EX production were selected and a composite central design was done. Then, 6 replicates were performed to validate these results. For both, the screening and central composite experiments, *L. sajor-caj* LBM 266, from the InBioMis culture collection, was reactivated on Malt Extract (12.7 g/L)-Agar (15 g/L) and incubated for 10 days at 28 °C. Then, one mycelial disc was inoculated into 20 mL of Malt Extract medium (12.7 g/L) with the combinations according to the models and incubated at 28 °C for 12 days. EX activity was quantified using the DNS method. The effects of pH (2.6, 3, 4, 4.8, 5, 6, 7, 8, 9, 10) and temperature (4, 10, 20, 30, 40, 50, 55, 60, 65, 70, 80) on EX activity were evaluated, including thermo-stability (4, 30, 50, 55 °C) and pH stability (3, 4.8, 7, 10). The screening assay identified wheat bran, citrus, and banana peels significantly influencing EX activity ($p < 0.05$). In the validation assay, an EX activity of 5242.45 U/L was obtained, increasing the activity obtained in the screening. A relative activity of $95.04 \pm 7.01\%$ was obtained at pH 4.8 ($p < 0.05$). Stability across pH levels decreased to 80-90% after 1 h but remained relatively stable above 60% for the remaining 72 h at all pH values tested. A relative activity between $77.16 \pm 0.51\%$ and $97.11 \pm 4.08\%$ ranged from 40-55 °C ($p > 0.05$). Thermo-stability decreased after 1 h to 98% at 3 °C, 60-75% at 30, 50, and 55 °C, but remained above 50% at all temperatures. These findings highlight *L. sajor-caj* LBM266 as a promising

candidate for enzyme production with potential applications in biotechnological processes. Future research should explore the effectiveness of this strain's supernatants in juice clarification.

Palabras clave: Endo-1,4-xylanase – *Lentinus sajor-cajú* – optimization – pH – temperature