World Academy of Science, Engineering and Technology
International Journal of Biological and Ecological Engineering
Vol:14, No:09, 2020

Purification, Biochemical Characterization and Application of an Extracellular Alkaline Keratinase Produced by Aspergillus sp. DHE7

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Abstract : The aim of this study was to purify and characterize a keratinolytic enzyme produced by Aspergillus sp. DHE7 cultured in basal medium containing chicken feather as substrate. The enzyme was purified through ammonium sulfate saturation of 60%, followed by gel filtration chromatography in Sephadex G-100, with a 16.4-purification fold and recovery yield of 52.2%. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis revealed that the purified enzyme is a monomeric enzyme with an apparent molecular mass of 30 kDa — the purified keratinase of Aspergillus sp. DHE7 exhibited activity in a broad range of pH (7- 9) and temperature (40℃-60℃) profiles with an optimal activity at pH eight and 50℃. The keratinolytic activity was inhibited by protease inhibitors such as phenylmethylsulfonyl fluoride and ethylenediaminetetraacetate, while no reduction of activity was detected by the addition of dimethyl sulfoxide (DMSO). Bivalent cations, Ca^{2+} and Mn^{2+}, were able to greatly enhance the activity of keratinase by 125.7% and 194.8%, respectively, when used at one mM final concentration. On the other hand, Cu^{2+} and Hg^{2+} inhibited the enzyme activity, which might be indicative of essential vicinal sulfhydryl groups of the enzyme for productive catalysis. Furthermore, the purified keratinase showed significant stability and compatibility against the tested commercial detergents at 37ºC. Therefore, these results suggested that the purified keratinase from Aspergillus sp. DHE7 may have potential use in the detergent industry and should be of interest in the processing of poultry feather waste.

Keywords : Aspergillus sp. DHE7, biochemical characterization, keratinase, purification, waste management
Conference Title : ICBEB 2020 : International Conference on Biological Engineering and Biotechnology
Conference Location : Tokyo, Japan
Conference Dates : September 10-11, 2020