ABSTRACT

Fungal α-*N*-acetylgalactosaminidase –
Enzyme screening, production and characterization

Extracellular α-*N*-acetylgalactosaminidase was so far isolated mostly from animal and microbial sources (*Clostridium perfrongens*, *Aspergillus niger*, *Acremonium* sp. No. 413). Cultivation of pathogenic bacteria *Clostridium perfringens* was complicated by production of toxins and by the need of anaerobic cultivation. Two published fungal production strains (*Aspergillus niger*, *Acremonium* sp. No. 413) are not available to public and unfortunately they are not taxonomically properly determined.

Therefore, screening for α-*N*-acetylgalactosaminidase in the fungal strains from public collection (CCF, CCIM) was performed. Fungal strains were cultivated in a complex liquid media containing soya flour as inductor under aerobic condition. Good strains from *Nigri* section were found to be producers of α-*N*-acetylgalactosaminidase; the best producer beiing ***Aspergillus niger* CCIM K2**

pH Optimum of the enzyme reaction was 1.5 at 35 °C. The enzyme was active over the range pH 2 – 6 and quite unstable at neutral or alkaline pH levels. Temperature optimum of enzymatic reaction was found to be 55 °C at pH 2.5 and 45 °C at pH 1.5. The enzyme was stabile in solutions in the presence of mercaptoethanol and dithiothreitol and it was stable over 14 days' storage at 4 °C in concentrated solutions without preservatives. α-*N*-Acetylgalactosaminidase accepted both *p*NP-α-Gal*p*NAc and *o*NP-α-Gal*p*NAc as a substrate.

The purified α-*N*-acetylgalactosaminidase from *Aspergillus niger* CCIM K2 was obtained from cultivation broth by ion-change chromatography (Fractogel EMD SO_3^- (S)) and gel chromatography (Sephacryl S-200 and Superdex 200), however α-galactosidase activity was still detectible. This activity could be result of the contamination of α-galactosidase or an intrinsic broader specificity of α-*N*-acetylgalactosaminidase. Molecular mass estimated was approximately 116 kDa by SDS polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions.