African Journal of Microbiology Research Vol. 5(9) pp. 1018-1028, 4 May, 2011
Available online http://www.academicjournals.org/ajmr
ISSN 1996-0808 ©2011 Academic Journals

Full Length Research Paper

Production, purification and characterization of α-amylase from *Trichoderma harzianum* grown on mandarin peel

Saleh A. Mohamed[1,2]*, Esam I. Azhar[3,4], Morooj M. Ba-Akdah[1], Nisreen R. Tashkandy[4] and Taha A. Kumosani[1,5]

[1]Biochemistry Department, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia.
[2]Molecular Biology Department, National Research Center, Dokki, Cairo, Egypt.
[3]Medical Laboratory Technology Department, Faculty of Applied Medical Sciences, King Abdulaziz University, Jeddah, Saudi Arabia.
[4]Special Infectious Agents Unit, Biosafety Level 3, King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia.
[5]Expermintal Biochemistry Unit, King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia.

Accepted 28 March, 2011

The production, purification and characterization of α-amylase from *Trichoderma harzianum* grown on mandarin peel were investigated. The effect of incubation time and mandarin peel concentration on the production of α-amylase by *T. harzianum* was studied. α-Amylase A3 was purified from *T. harzianum* to electrophoretic homogeneity by using DEAE-Sepharose and Sephacryl S-200 columns. The enzyme had molecular weight of 70 kDa using gel filtration and SDS-PAGE. The affinity of the substrates toward A3 was in the order of amylopectin > glycogen > starch > β-cyclodextrin > dextrin > α-cyclodextrin. These findings tend to suggest that the enzyme has high affinity toward high-molecular mass substrates. The K_m and V_{max} values of the enzyme for hydrolyzing potato soluble starch and glycogen were 6.53, 4.5 mg/ml and 2 and 2.2 µmol reducing sugar/ml, respectively. The maximum activity of enzyme against soluble starch was determined at pH 4.5 and 40°C. α-Amylase A3 was stable up to 40°C for 30 min of incubation and retained 70 and 50% of its activity at 50 and 60°C, respectively. While all the examined metal cations were effective in inhibiting the enzyme, Ca^{2+} considerably enhanced the activity. The metal chelators, EDTA, sodium citrate and sodium oxalate had inhibitory effects on A3. The rate of breakdown of starch was higher than the rate of formation of reduced sugar indicating A3 is endo-acting enzyme. These properties of A3 with its remarkable activity meet the prerequisites needed for liquefaction and saccharification of starch industry.

Key words: *Trichoderma harzianum*, mandarin, α-amylase, purification, characterization.

INTRODUCTION

Amylases are widespread in animals, fungi, plants, and are also found in the unicellular eukaryotes, bacteria and archaea (da Lagea et al., 2007). Though plants and animals produce amylases, enzymes from microbial sources are generally used in industrial processes. This is due to a number of factors including productivity, thermostability of the enzyme as well as ease of cultivating microorganisms (Reddy et al., 1999). The major advantages of the enzymatic route are the selectivity with its associated high yield and exclusivity toward the desired product (Kim and Dale, 2004). Bacteria used in commercial production are the *Bacillus* spp. (Olafimihan and Akinyanju, 1999; Pandey et al., 2000; Gupta et al., 2003). Others, such as *Escherichia* spp, *Pseudomonas*, *Proteus*, *Serratia* and *Rhizobium* also yield appreciable quantity of the enzyme (Oliviera et

*Corresponding author. E-mail: saleh38@hotmail.com.