ABSTRACT

L-glutaminases (L-glutamine amidohydrolase EC.3.5.1.2) is proposed as a prospective candidate for enzyme therapy of cancer and also as an important additive during enzymatic digestion of shoyu koji since it could enhance glutamate content of soysauce. Commercial production of glutaminase could make possible its wide application in these areas, which would demand availability of potential sources and suitable fermentation techniques.

The present investigation highlighted marine environment as a potential source of efficient glutaminase producing bacteria mainly species of Pseudomonas, Aeromonas, Vibrio, Alcaligenes, Acinetobacter, Bacillus, and Planococci. Among them Pseudomonas fluorescens ACMR 171, P.fluorescens ACMR 43, Vibrio costicola ACMR 267 and V. cholerae ACMR 347 were chosen as the ideal strains for glutaminase production. They could grow and produce maximal glutaminase as extracellular enzymes under submerged condition at a wide range of parameters ranging from pH 5 to 8, 25-40°C, NaCl concentration of 0-5%, substrate concentration 0.5-3% within 18-24 hours of incubation. Glucose, at 0.5% level, enhanced significant level of enzyme production in all strains

except V.costicola ACMR 267 which preferred 1% glucose concentration for the same. Beef extract, followed by lysine, peptone and glutamic acid were observed to enhance the level of enzyme production. The strains were found to possess longer generation time in mineral media than in nutrient broth except V.cholerae ACMR 347 exhibited the opposite trend. Extracellular glutaminase fraction from all strains were in higher titres than intracellular enzymes during growth in mineral media, nutrient broth and nutrient broth added with glutamine.

Glutaminase from all strains were purified employing $(NH_4)_2SO_4$ fractionation followed by dialysis and ion exchange chromatography. The purified glutaminase from all strains were observed to be active and stable over a wide range of pH and temperature. Glutaminases purified from the strains possessed a Km of 1.0×10^{-4} M for P.fluorescens ACMR 171, 4.6×10^{-5} M for P.fluorescens ACMR 43, 9.54×10^{-5} M for V.costicola ACMR 267 and 1.5×10^{-5} M for V.cholerae ACMR 347. Enzymes from all strains were observed to be highly salt tolerant upto 25% NaCl. Heavy metals viz., Hg, Fe, Ca, Mn, Pb, Co, Cu and Zn affected the enzyme activity adversely. Glutamic acid, aspartic acid and EDTA had not influenced the activity of glutaminase

while phosphate ion and tris ion enhanced the activity of glutaminase. Glutamine was identified as the preferential substrate for glutaminase from all strains and a combination of L-asparagine and L-glutamine resulted in a reduced enzyme activity.

Production pattern of glutaminase by these strains in SSF was also examined. There too a wide range of operational parameters such as moisture content of 40-60%, pH 4-10, temperatures 15-45°C, 0-5% NaCl concentrations could effect significant levels of enzyme production. Interestingly V.cholerae ACMR 347 produced maximal enzyme production in the absence of NaCl in both SmF and SSF. Extraction parameters for maximal recovery of glutaminase from SSF was also standardised. A comparative analysis for enzyme production in both SmF and SSF showed that SSF process can yield many fold enzymes. Glucose enhanced the level of enzyme production by all the strains in SmF while reducing the enzyme production in SSF except in P.fluorescens ACMR 171. Optimization studies of environmental variables that normally influence the yield of glutaminase indicated that the optimal requirements of these bacteria for maximal glutaminase production remained stable irrespective of the medium, they are provided with for enzyme production. However, solid state fermentation technique was observed to be the most suitable process for the production of glutaminase.