STUDIES ON BACTERIAL LYSINE DECARBOXYLASE FROM MYANMAR SCOMBROID FISH

PhD (DISSERTATION)

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ABSTRACT

Enzyme lysine decarboxylase converts lysine to cadaverine. Cadaverine forming bacteria were isolated from scombroid fish, viz., *Auxis thazard* (nga-mae-lone), *Scomberomorus guttatus* (nga-kun-shut), and *Rastrelliger kanagurta* (pa-la-tu) by using TCBS (Thiosulphate citrate bile salts sucrose) agar medium and MAC (Mac Conkey) agar medium followed by lysine, ornithine, arginine and base quick method. Identification of cadaverine forming bacteria were made by biochemical tests and API (Analytical Profile Index) 20E strips after isolation of bacteria. *Vibrio parahaemolyticus* was isolated and identified on TCBS agar and *Klebsiella pneumoniae* and *Pseudomonas cepacia* on MAC agar. Enumeration of total bacteria was made by using standard plate count method. Cadaverine forming bacteria were in low proportion and accounted for less than 7% of the total bacterial load in the fish samples studied. Studies on lysine decarboxylase (LDC) (EC 4.1.1.18) from the isolated bacteria, which catalyze the decarboxylation of lysine to cadaverine were carried out. Moller decarboxylase broth was used for growing these bacteria at 37°C. The lysine decarboxylase were extracted from these bacteria using acetone powder method. In the enzyme catalyzed reaction, hydrochloric acid was used as an terminator. The product cadaverine was converted into the corresponding dinitrophenol derivative by reaction with fluorodinitrobenzene, with subsequent visible spectrophotometric determination of this derivative at 400 nm. The parameters such as pH, reaction time, temperature, substrate concentration, and enzyme concentration for bacterial lysine decarboxylase catalyzed decarboxylation of lysine were necessarily studied. Evaluation of the maximum velocity (V_{max}), Michaelis-Menten constant (K_m), were carried out by using various graphical methods such as Michaelis-Menten, Lineweaver-Burk, Eadie-Hofstee,

Eisenthal-Cornish Bowden, etc., linear regression method and KINSIM software (Computer simulation of enzyme kinetic data). Kinetic studies showed that K_m for *Klebsiella pneumoniae* was about 2 fold greater than those for *Pseudomonas cepacia* and *Vibrio parahaemolyticus* whereas V_{max} values of all three species were comparable. The enzyme unit was 17.05 EU for lysine decarboxylase of *Klebsiella pneumoniae* and was found to be greater than those of the other species. The reaction order (n) concerning the decarboxylation of lysine catalyzed by lysine decarboxylase was also determined and found to be first order. The enzyme was competitively inhibited by semicarbazide with a K_i of 1.394 mM and by urea with a K_i of 6.592 mM.

Finally, lysine decarboxylase enzyme from *K.pneumoniae* was purified on DEAE- Sephadex A - 50 column by using potassium phosphate as eluent. In the UV absorption spectrum of the purified enzyme protein, no absorption was found above 350 nm and it can be observed that the purified enzyme proteins were free from colour materials. The molar extinction coefficients at 205 and 280 nm were determined to be 68.9 and 24.0, respectively.

Keywords : Scombroid fish, *Vibrio parahaemolyticus, Klebsiella pneumoniae*, *Pseudomonas cepacia*, biochemical tests, lysine decarboxylase, cadaverine, Moller decarboxylase broth, KINSIM