

**STUDIES ON BACTERIAL ORNITHINE  
DECARBOXYLASE FROM MYANMAR  
SCOMBROID FISH**

**PhD (DISSERTATION)**

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## ABSTRACT

Biogenic amines are indicators of fish spoilage with economic and human health effects. Putrescine, one of the biogenic amines, is a potentiator of scombroid poisoning which results from the consumption of scombroid fish. Putrescine is the bacterial decarboxylation product of ornithine by the enzyme ornithine decarboxylase (ODC). Putrescine forming bacteria (PFB) were isolated from the TCBS (thiosulphate-citrate-bile salts-sucrose) agar and MacConkey agar media and these putrescine forming bacteria (PFB) were found to be 10% of the total bacterial load. Identification of the ODC - producing microorganisms by LAOB (Lysine, Arginine, Ornithine, and Base), Gram's staining, biochemical tests, and Analytical Profile Index (API) 20 E strips showed that ornithine decarboxylase producing microorganisms were *Morganella morganii*, *Enterobacter cloacae*, and *Vibrio parahaemolyticus*. Moller decarboxylase broth was used for the growth of these bacteria. Ornithine decarboxylase (ODC) was extracted by using acetone powder method from the bacterial suspension. ODC - catalyzed reaction of the decarboxylation of ornithine was studied using L - ornithine as substrate and hydrochloric acid as a terminator in this investigation. The product putrescine was converted into dinitro benzene derivative by reaction with 1- fluoro 2,4-dinitro - benzene (FDNB), with subsequent spectrophotometric determination of this derivative at 400 nm. The effects of pH of propionate buffer, time, temperature, enzyme concentration, and substrate concentration on the ODC-catalyzed reaction were studied. The enzyme ornithine decarboxylase (E.C 4.1.1.17) has a pH optimum of 5.5 and a temperature optimum of 37°C. From enzyme kinetic data., maximum velocity ( $V_{max}$ ) and Michaelis-Menten constant ( $K_m$ ) were determined by using Michaelis-Menten, Lineweaver-Burk, Eadie-Hofstee, Hanes-Wilkinsons, and Eisenthal-Cornish Bowden plots and KINSIM programme. From the kinetic studies *Morganella morganii* ODC was found to have

the highest enzymic activity due to its low  $K_m$  (0.331 mM) and high  $V_{max}$  (0.0406 mM min<sup>-1</sup>) values. The reaction order (n) for the ODC-catalyzed reaction was also calculated and found to obey first order kinetics. The enzyme unit (EU) value of *Morganella morganii* ODC was found to be 2 fold higher than the other two. Inhibitory effects of amines (*viz.*, putrescine, histamine, and cadaverine) and mercuric ion were also studied and mercuric ion showed strong inhibitory effect (70.19%). Among amines, inhibition of putrescine was 22.03% while other amines, histamine and cadaverine, were found to be less inhibited, *i.e.*, 14.02 and 3.01% respectively. In this study mercuric ion reversibly and non-competitively inhibited the enzyme reaction with  $K_i$  value of 4.5693 mM. The prepared *Morganella morganii* ODC was used for the determination of ornithine in the human urine and the concentrations of ornithine were found to be 16.67 and 18.09 nmol/ml urine which fall in the range of a normal human urinary level of 7 to 21 nmol/ml.

**Keywords** : Sombroid fish, ODC-producing microorganisms, ornithine decarboxylase, putrescine, Lineweaver-Burk, Eadie-Hofstee, Hanes-Wilkinsons, and Eisenthal-Cornish Bowden, KINSIM, Michaelis-Menten constant.