## BACTERIOLYTIC ACTIVITY OF CROTOXIN-B AND LYSOZYME ON GRAM-POSITIVE AND NEGATIVE BACTERIA.

<u>Baesse, L.G.</u>, Santos, H.L., Homsi-Brandeburgo, M.I., Rodrigues, V.M., Hamaguchi, A.

Instituto de Genética e Bioquímica, Universidade Federal de Uberlândia, Uberlândia-MG, Brasil

Lysozyme is an enzyme found in several biological fluids, catalyzing the hydrolysis of bacterial peptidoglicans. This work's purpose was the partial purification of Lysozyme-c and to verify its lytic activity on bacteria, associated or not with Crotoxin-B (PLA<sub>2</sub>). Lysozyme-c was purified from hen egg white on Q-Sepharose, in NH<sub>4</sub>HCO<sub>3</sub> buffer 0,05mol/L; pH 9.0, 25°C. When submitted to SDS-PAGE 15%, the void fractions showed only one band. These fractions were pooled and bacteriolytic activity of Lysozyme was verified by turbidimetric method on Micrococcus lysodeikticus. Crotoxin is the major component of Crotalus durissus terrificus snake venom, responsible for myotoxic and neurotoxic effects. Crotoxin was extracted from the crude venom by centrifugation and chromatography on SephadexG-75, in NH<sub>4</sub>HCO<sub>2</sub> 0.1mol/L with NaCl 0.1mol/L, pH 3.0, 25°C. Crotoxin-B subunit (CB) was isolated from Crotoxin on DEAE-SephadexA25 in TRIS-HCI 0.05mol/L with Urea 7.0mol/L, pH 7.2, 25°C, and its phospholipasic activity was determined. Bacteriolytic activity of CB (150 µg/mL) associated with lysozyme (150 µg/mL) on *E. coli* was significant, inhibiting 31% the bacterial growing, compared with control, whereas pure CB inhibited only 6.11% and crude venom alone (CV)(150 µg/mL) inhibited 34.72%. CB, CV, Crotapotin and Crotoxin (5 µg/mL) each one) only showed muramidase activity on *M. lysodeikticus* when they were associated with Lysozyme.

KEY WORDS: Lysozyme-c, Crotoxin, bacteriolytic activity, snake venom. FINANCIAL SUPPORT: FAPEMIG

CORRESPONDENCE TO: leobaesse@yahoo.com.br.