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

Baesse, L.G., Santos, H.L., Homsi-Brandeburgo, M.I., Rodrigues, V.M., Hamaguchi, A.<br>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 ( $\mathrm{PLA}_{2}$ ). Lysozyme-c was purified from hen egg white on Q-Sepharose, in $\mathrm{NH}_{4} \mathrm{HCO}_{3}$ buffer $0,05 \mathrm{~mol} / \mathrm{L}$; $\mathrm{pH} 9.0,25^{\circ} \mathrm{C}$. 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 $\mathrm{NH}_{4} \mathrm{HCO}_{2} 0.1 \mathrm{~mol} / \mathrm{L}$ with $\mathrm{NaCl} 0.1 \mathrm{~mol} / \mathrm{L}$, pH 3.0, $25^{\circ} \mathrm{C}$. CrotoxinB subunit (CB) was isolated from Crotoxin on DEAE-SephadexA25 in TRIS-HCI $0.05 \mathrm{~mol} / \mathrm{L}$ with Urea $7.0 \mathrm{~mol} / \mathrm{L}, \mathrm{pH} 7.2,25^{\circ} \mathrm{C}$, and its phospholipasic activity was determined. Bacteriolytic activity of CB (150 $\mu \mathrm{g} / \mathrm{mL}$ ) associated with lysozyme (150 $\mu \mathrm{g} / \mathrm{mL}$ ) on $E$. coli was significant, inhibiting $31 \%$ the bacterial growing, compared with control, whereas pure CB inhibited only $6.11 \%$ and crude venom alone $(C V)(150 \mu \mathrm{~g} / \mathrm{mL})$ inhibited $34.72 \%$. CB, CV, Crotapotin and Orotoxin ( $5 \mu \mathrm{~g} / \mathrm{mL}$ each one) only showed muramidase activity on $M$. Iysodeikticus when they were associated with Lysozyme.
KEY WORDS: Lysozyme-c, Crotoxin, bacteriolytic activity, snake venom.
FINANCIAL SUPPORT: FAPEMIG
CORRESPONDENCE TO: leobaesse@yahoo.com.br.

