

## 9 Studies on other bacterial pathogens

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#### 9.1 Mode of action of *Yersinia enterocolitica* heat stable enterotoxin (YSTa) in rat intestinal epithelial cell

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The principal objective of this study was to evaluate the mechanism of action of heat stable enterotoxin secreted by *Yersinia enterocolitica*. *Yersinia enterocolitica* heat stable enterotoxin (YSTa) was purified from the culture filtrate using ammonium sulfate precipitation, DEAE Sephacel and Sephacryl S-100 HR column chromatography. Fractions were tested for enterotoxicity by suckling mice assay. It was found that purified YSTa raised  $[Ca^{2+}]_i$  in a dose dependent manner and the optimal level of  $[Ca^{2+}]_i$  was achieved by incubating cells with 10ng YSTa. We reported earlier that Y-STa stimulated phospholipase C activity. It was also found that Y-STa induced rise in intracellular calcium level by calcium influx from extracellular environment as well as  $IP_3$  mediated calcium mobilization from intracellular calcium store. In further support of the involvement of  $IP_3$  mediated calcium mobilization in the mechanism of action of Y-STa evidence of the intracellular  $IP_3$  level was directly measured and found that Y-STa increased the intracellular  $IP_3$  level.

During the reported period an attempt has been made to evaluate the involvement of phospholipase C in the Y-STa induced calcium influx and to identify the specific isoform of phospholipase C involved in the mechanism of action of Y-

STa. Incubation of rat enterocytes with  $10\mu M$  U73122 for 5 min at  $37^\circ C$  completely inhibited the Y-STa induced calcium rise. This suggested that phospholipase C might have a role in Y-STa induced calcium influx in rat intestinal epithelial cells. To identify the specific isoform of phospholipase C involved in the mechanism of action of Y-STa isolated rat enterocytes were treated with Y-STa for a given time period at  $37^\circ C$ . The reaction of Y-STa was stopped by the addition of 2ml of ice cold BSS (pH 7.4). Cells were washed twice and then homogenized with 10mM Tris-HCl buffer pH 7.5 containing 0.1% bovine albumin and centrifuged at  $600 \times g$  for 10 min. Supernatant was collected and incubated with  $12\mu l$  of anti phospholipase  $C\gamma$  for 4h at  $4^\circ C$ . At the end of the 4h,  $20\mu l$  of protein A-sepharose were added and incubated for another 1.5h with very gentle tapping. The precipitate was collected by centrifugation and washed with immunoprecipitation buffer. The immunoprecipitate obtained with anti phospholipase  $C\gamma$  was then separated by 10% SDS-PAGE and transferred to nitrocellulose membrane (pore size  $0.45\mu m$ , BIO-RAD, USA) using a transblot apparatus (BIO-RAD, USA). The blot was incubated at  $37^\circ C$  for 2h in 3% gelatine in Tris-buffered saline containing 0.1% Tween twenty (TTBS) under constant shaking. After washing with TTBS it was incubated with primary antibody (anti phosphotyrosine diluted 1:5000), washed with TTBS and incubated with the secondary antibody conjugated with alkaline phosphatase. It was found that tyrosine phosphorylation of phospholipase  $C\gamma$  (which denotes phospholipase C activity) was maximum after 40 sec of toxin treatment. Further studies are in progress.

