A-06 **EVALUATION OF METHODS FOR THE PREPARATION OF BACTERIAL EXTRACTS FROM SUSPENSIONS OF *Staphylococcus epidermidis* FOR ENZYMOLOGICAL STUDIES**

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A very important prelude to enzyme assay is the preparation of any active cell-free extract and the method employed to achieve this should ideally be the one which does not result in enzyme inactivation during the preparative procedures. In establishing the glucose and serine metabolic pathways in *Staphylococcus epidermidis*, the existence of the various key enzymes in cell-free extracts was procured. The apparent resistance of the cell wall of *S. epidermidis* to the traditional methods available led to a systematic evaluation of a number of methods.

Effectiveness of cell breakage was estimated using the amount of cellular protein released as a marker. Of the different procedures adopted the most successful methods were : (i) homogenizing with gas chromatography beads ; (ii) subjecting cells to osmotic shock after lysostaphin digestion followed by a 6 min. sonication ; both of which released 60% of the total cellular protein.

The specific and total activities of glucose 6-phosphate dehydrogenase and lactate dehydrogenase were higher in extracts prepared by method (ii) than by method (i). Conversely, the specific and total activities of fructose-1,6-bisphosphate aldolase and 6-phosphagluconate dehydrogenase were higher in extracts prepared by method (i) than by method (ii).

Even though the specific activities of lactate dehydrogenase and glucose 6-P dehydrogenase were lower in extracts prepared by method (i), incorporation of 10 mM—$MgCl_2$ and 100 mM—KCl in the extraction buffer increased the specific activities of them suggesting a possible protective role. Phosphate buffers in the alkaline range were not suitable for extraction ; addition of 30 mM—NaF to such buffers had a beneficial effect.

Conclusion : The cell wall of *Staphylococcus epidermidis* as with other Staphylococci in resistant to mechanical stress exerted by the French press and ultrasonicator and prior sensitization of the cell wall with lysosyme or lysostaphin digestion helped the disruption of the organism.