

Cloning and expression of phospholipase D gene from three different isolates of *C. pseudotuberculosis**

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ABSTRACT

Caseous lymphadenitis is a sheep disease caused by Corynebacterium pseudotuberculosis. This organism is also causing oedematous skin disease of buffalo and tuberculous-like lesions of camel in Egypt. Phospholipase D (PLD) is found to be an exotoxin playing a great role in propagation of the disease. Three isolates of C. pseudotuberculosis were isolated from sheep, buffalo and camel. These isolates were found to be different based on their biochemical reactions. The PLD genes of these isolates were amplified by PCR then cloned and expressed in E. coli system. The length of amplified products by PCR was found to be the same in the three isolates although only one set of primers was used. The purified PLD proteins from the three clones that expressed in E. coli have the same molecular weight on SDS-PAGE. Moreover, when the biological activity of recombinant PLD was estimated, the same zone of haemolysis was obtained on blood agar using synergistic haemolysis assay. A preliminary conclusion was made that the PLD genes are the same in the three isolates. However, further study is needed for restriction mapping of these isolates and sequencing of their PLD genes.

Key words: *Corynebacterium*, PLD, cloning, expression, *E. coli*.

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INTRODUCTION

Corynebacterium pseudotuberculosis is a gram-positive pleomorphic bacterium that causes caseous lymphadenitis (CLA) in sheep, goats and deer (Anderson and Nairn, 1985, Brown and Olander, 1987 and Scott *et al.*, 1997), ulcerative lymphangitis in horses (Miers and Ley, 1980) and various suppurative conditions in cattle including skin lesions resembling

tuberculosis (Barakat *et al.*, 1984). In addition, the bacteria have been highly suggested as the cause of a condition called oedematous skin disease (OSD) in water buffaloes in Egypt (Barakat *et al.*, 1985). Recently, *C. pseudotuberculosis* has been recovered from tuberculous-nodes (Selim *et al.*, 1997). Nevertheless, sheep CLA is the major worldwide problem caused by the organism. Not only *C. pseudotuberculosis* causes animal