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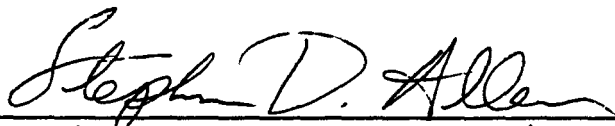
**CLONING AND NUCLEIC ACID SEQUENCING OF A SERINE PROTEASE
GENE FROM *CLOSTRIDIUM SEPTICUM***

Scott William Riddell

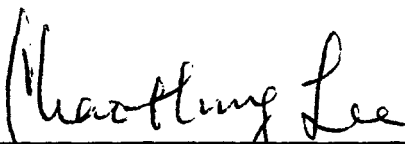
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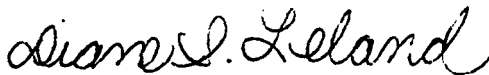


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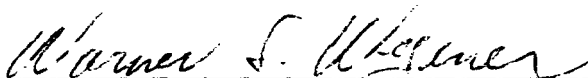


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ABSTRACT

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Cloning and Nucleic Acid Sequencing of a Serine Protease Gene from *Clostridium septicum*

Clostridium septicum is a pathogenic anaerobe that causes serious, potentially fatal infections, especially in compromised hosts. This organism is most commonly isolated from the blood of patients who have an underlying malignancy with or without neutropenia, and the most frequently implicated portal of entry has been the intestinal tract. Although this organism produces several potential virulence factors including two hemolysins, hyaluronidase, gelatinase, and fibrinolysin, data are lacking about their roles in diseases. Early studies in our laboratory demonstrated that cell-free culture filtrates of *C. septicum* were cytotoxic for several different cell lines. The majority of these same filtrates also produced significant fluid accumulation and tissue damage within a rabbit intestinal loop model. The degree of histologic damage induced by culture filtrates was found to correlate with the volume of fluid accumulated. Since tissue damaging factors can also be hemolytic, we created and screened a *C. septicum* genomic library for hemolytic colonies. A clone containing a 4.8 kb insert was identified that conferred hemolytic activity, and the

nucleic acid sequence of the insert DNA was determined. The deduced amino acid sequence from an open reading frame within the insert was found to contain a portion of a subtilisin family serine protease gene. The remainder of the gene was subsequently obtained by polymerase chain reaction (PCR) amplification. Translation of the complete 2.9 kb protease gene revealed separate aspartate, histidine, and serine active site motifs characteristic of the subtilisin family of serine proteases, as well as a conserved binding site pocket. A 36-residue sequence resembling a signal peptide was identified at the amino terminus of the translated protein. Analysis of the nucleotide sequence of the gene showed a potential Shine-Dalgarno sequence 7 base-pairs upstream of the putative initiation codon. Primer extension experiments revealed a putative transcription start site 145 base-pairs upstream of the putative translation start site. The serine protease gene identified in these studies is only the third gene sequenced from *C. septicum* and may contribute to the virulence of the organism. Delineation of the protease gene sequence will allow expression of the gene and analysis of the gene product.

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