

Sequence of the *lktD* gene from *Actinobacillus actinomycetemcomitans*

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Members of the RTX family of protein toxins are secreted without the concomitant cleavage of a signal peptide^{1,2}. In *Pasteurella haemolytica* and *E. coli*, the structural gene for the RTX toxin is upstream of two genes, *lktB/hlyB* and *lktD/hlyD*, required for toxin secretion^{1,3}. In the preceding paper, we reported the sequence of the *lktB* gene from the leukotoxin operon of *Actinobacillus actinomycetemcomitans*; it shared a high degree of sequence similarity with the equivalent proteins from *P. haemolytica* and *E. coli*. This was unexpected since the RTX toxins from these bacteria are secreted while the leukotoxin from *A. actinomycetemcomitans* is reportedly retained in the periplasm⁴. Thus, to determine whether a sequence analogous to *lktD/hlyD* was present in *A. actinomycetemcomitans*, a 6 kb Bam HI fragment containing 2.5 kb of DNA 3' to *lktB* was cloned from strain JP-2. The region immediately downstream of the *lktB* gene was sequenced and an open reading frame was identified. The deduced protein, LktD, demonstrates significant homology to the equivalent polypeptides from related bacteria: it shares 61% identity with the LktD protein from *P. haemolytica*¹ and 68% identity with the HlyD protein from *E. coli*³. Identities are found in all three proteins at 52% of the residues. Moreover, the *lktD*

gene is expressed in *A. actinomycetemcomitans* as shown by Northern blot analysis using an *lktD*-specific probe (Spitznagel, Kraig, and Kolodrubetz, in preparation). The fact that the LktB and LktD proteins of *A. actinomycetemcomitans* are so similar to the leukotoxin/hemolysin secretion proteins from other organisms suggests that further investigations into the possible secretion of LktA from *A. actinomycetemcomitans* are necessary.

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REFERENCES

1. Strathdee, C.A. and Lo, R.Y.C. (1989) *J. of Bacteriol.* **171**, 916–928.
2. Mackman, N., Nicaud, J.-M., Gray, L. and Holland, I.B. (1986) *Curr. Topics Micro. and Imm.* **125**, 159–181.
3. Felmlee, T., Pellett, S. and Welch, R.A. (1985) *J. of Bacteriol.* **163**, 94–105.
4. DiRienzo, J.M. (1986) In A.J.L. Macario and E.C. de Macario (eds). *Monoclonal Antibodies against Bacteria* **3**, 250–293.

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-12 GAACGAACGATAATAAAAACCTGGCTTTGGCATTGTATGATGTGCTTTCCGTTATAAAAAACGTGGAATGAAACTGGAAAATTCGTAACAGCTTGATTCTCCGGTTAGA
    M K T W L L A L Y D V L S R Y K N V W N E T W K I R K Q L D S P V R
103 GAAAAAGATGAAATGAATTTCTGCCCGCGCATTTGGAATTAATGAAACACCTGTATCAAAATGCCACGATTCGTGTCTTATCCATTATGCTCTTTAACTTTAGCGATT
    E K D E N E F L P A H L E L I E T P V S M A P R F V S Y S I M L F L T L A I
217 ATGTTTCTATCTTTAGCAATGTGAAAATATCGCAACGGCTTCAGGAAAATTTGCACTAAGCGGGAGAAGCAAGAAAATTAACCCGATTGAAAATTCATTGGTTAAACATATT
    I V S I F S N V E I I A T A S G K F A L S G R S K E I K P I E N S L V K H I
331 TTTGTGAAGAGGGGCAATACGTGAAAAAGGGTGAACATTGTTAAAACTGACCCACTTGGTCCCAAGCGGATACGTAAAAACGAAGACATCGCTTTCTCAAGCTAAACTG
    F V K E G E Y V K K G E L L L K L T A L G A E A D T L K T K T S L S Q A K L
445 GAGGAATTCGTTATAAATCTTTATGGAAGCTGTGAAAAAGATCAATTACCAATATTAGATTTTTCTAAAAATGATTTACCTTTTATGACGGAACGATCAAAAAAGAGTG
    E E F R Y K S L L E A V E K D Q L P I L D F S K I D L P F M T E N D Q K R V
559 ACTTACTGATTGAAGAACAATCTCTACTTGGCAAAAACAACGCCATCAGAAAACCTTGAATCTTAATAAAAAAGAAGCGGAAAAACTCAGTATTGGCCAGCAATAAAAAA
    T L L I E E Q F S T W Q K Q R H Q K T L N L N K K E A E K L S Y L A R I K K
673 TATGAAGGTCTGATTAATACAGAGCAAGTCCGATTGGATGATTTAGAGCCTTATATAAGGAACATGCTATTGCAAAAACATACAGTTTTAGAGAGAAATAAATACAGGAT
    Y E G L I N T E Q V R L D D F R A L Y K E H A I A K H T V L D E E N K Y Q D
787 GCAATCAATGAGCTTGAGGTATATAAGGCGAGTTAATGCAAGTTGAAAACGAAGTTTTATTGGCAAAGAAGAGCAGGAATTAGTCACACAATGTTCAAAAATGATATTTTA
    A I N E L E V Y K A S L M Q V E N E V L L A K E E Q E L V T Q L F K N D I L
901 GACAAGCTAAAAAGCGGACGGATAATGTGAATTTATTGACATTCGAACTGGCAAAAAACAATCAACGCCAGCAAGTCTCTGAAATTCGAGCTCTGTATCAGGTACTGTACAA
    D K L K Q A T D N V N L L T F E L D K N N Q R Q Q V S E I R A P V S G T V Q
1015 CAATTAAGTTTCACACAATAGATGGCGTTGTTACTACGGCTGAAACATTAATGGTAGTTGTGCGGAAAGAAGATCCCTTGAAGTCACTGCATTTATCAAAAATAAGATATT
    Q L K V H T I D G V V T T A E T L M V V V P E E D S L E V T A I Q N K H I
1129 GGTTTTGTGAAGAAGGGCAAGAGTTGTAATTAAGGTGCAAGCCTTCCCTATACCCGTTATGGTTATTTAACGGGAAAAGTGAAAAATTACTTTAGATGCGATTGAACAT
    G F V K E G Q E V V I K V E A F P Y T R Y G Y L T G K V K N I T L D A I E H
1243 CCAAAGCTTGGACTGTATTTAATACGATTATTGAATGGATAAAAAACACTTTCTACCGAAGAAAAGAAATCCCACTTTCCGCAGGATGGAATTAAGTACGCAAAAATTAAG
    P K L G L V F N T I I E L D K K T L S T E E K E I P L S A G M E I T A E I K
1357 ACAGGCATGAGAAGCGTTATAAGCTATCTACTAGCCCATTAGAAGAATCTATTGATAAAAGTTAAGAGAACGTTAATTAAGATAGATAATAACATCC
    T G M R S V I S Y L L S P L E E S I D K S L R E R term

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