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## Legiolysin, a New Hemolysin from *L. pneumophila*

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### Summary

*Legionella pneumophila* generates exotoxins, cytolysins, proteases or hemolysins that damage host cells like erythrocytes or tissue culture cells. The gene for a new *L. pneumophila* hemolysin without a proteolytic activity was identified, cloned in *E. coli* and sequenced. The gene product was analysed by SDS-Polyacrylamide-gel-electrophoresis.

### Zusammenfassung

*Legionella pneumophila* bildet Exotoxine, Zytolysine, Proteasen oder Hämolsine, die Wirtszellen wie Erythrozyten oder Animalzellen schädigen. Das Gen für ein neues *L. pneumophila* Hämolysin ohne proteolytische Aktivität wurde identifiziert, in *E. coli* kloniert und sequenziert. Das Genprodukt wurde durch SDS-Gelelektrophorese analysiert.

*Legionella pneumophila* is a Gram-negative, aerobic inhabitant of freshwater and soil environments. It is also a facultative intracellular pathogen that invades and grows in human alveolar macrophages leading to acute bronchopneumonia referred to as legionellosis. The cytopathology of infected lung tissue suggests involvement of extracellular proteolytic enzymes or toxins in the pathogenesis of the disease.

A *L. pneumophila* zinc metallo-protease has been characterized as a protein of 38 kDa (5), the sequence of the structural gene was found to possess a single large open reading frame (ORF) of 1,629 nucleotides (1). The protease exhibits a hemolytic effect on canine but not on human erythrocytes and a cytotoxic effect on chinese hamster ovary cells (CHO).

We describe here a new hemolysin called legiolysin (Lly) which lyses erythrocytes from man, guinea-pig and dog and exhibits no proteolytic activity. In order to screen for the gene involved in the production of legiolysin, we constructed a gene library of the *L. pneumophila* genome. Total DNA of *L. pneumophila* was cleaved partially with *Sau3A* and cloned into the *BamHI*-cut cosmid vector pLAFR2 (2) and transduced into *E. coli* HB 101. 2885 clones were tested for hemolytic activity on blood agar plates

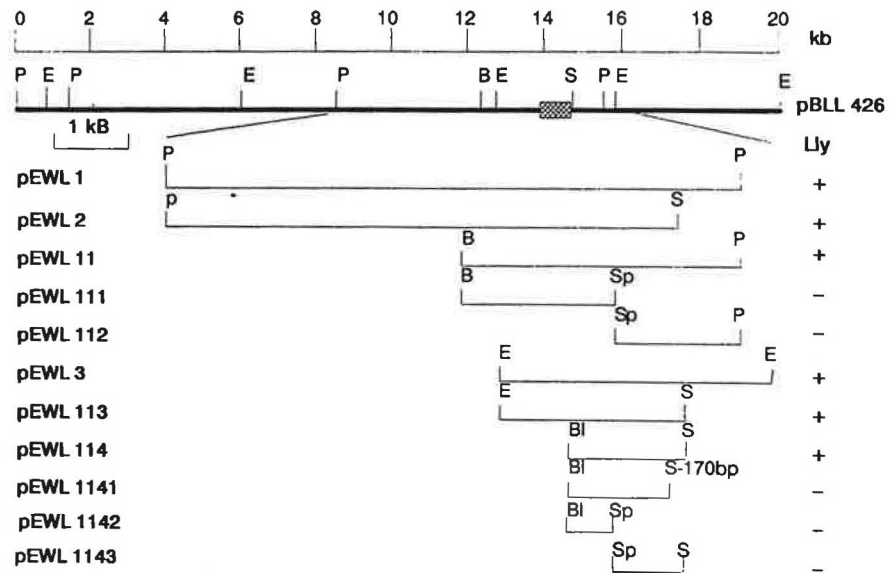


Fig. 1. Restriction map of the chromosome region containing the legiolysin gene from *L. pneumophila*. The restriction fragments contained within the various subclones are indicated. The subclones exhibiting hemolytic activity are marked Lly+, non-hemolytic clones are marked Lly-. The restriction sites are: B = BamHI; Bl = Ball; E = EcoRI; P = PstI; S = SmaI; Sp = SphI.

containing human erythrocytes and proteolytic activity on skim milk plates. Eight clones produced a hemolytic lysis zone but had no proteolytic activity. Eleven clones caused proteolysis but no hemolytic effect on human erythrocytes.

The hemolytic and non-proteolytic clone pBLL426 was used for further analysis of the legiolysin determinant. The *Legionella* DNA insert of pBLL426 was used as a gene probe in Southern hybridization (6) of the eight hemolytic and the eleven proteolytic clones and of total DNA of *L. pneumophila*. The DNA from the proteolytic clones did not hybridize, the DNA from the hemolytic clones and total *Legionella* DNA showed one band of hybridization indicating that the *lly* gene is present only once on the *Legionella* genome.

Clone pBLL426 was used to establish a restriction map (Fig. 1). Different DNA fragments were ligated into the vector puC19 (7) and the subclones were analysed for hemolysin production. A 6.7 kb *PstI* fragment located in pEWL1 conferred the hemolytic phenotype. The hemolysin specific gene seems to be transcribed from its own promoter since hemolysin production occurred after subcloning in both orientations. To identify the hemolysin specific gene from a 4.2 kb *BamHI-PstI* insert in plasmid pEWL11 a set of *in vitro* deletion mutants was constructed. These data show that the hemolysin specific gene must be located between coordinates 13 and 15 in Fig. 1. The smallest insert still coding for hemolysis is a *BamHI-SmaI* fragment in plasmid pEWL114. A comparison to the restriction map of the protease gene established by Quinn et al. (5) revealed a completely different restriction pattern.

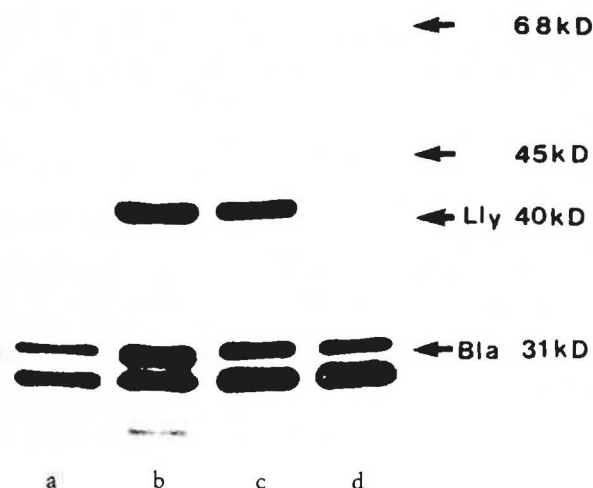


Fig. 2. Gene products identified in minicells of *E. coli* which are determined by recombinant plasmids carrying *Bam*HI-*Pst*I, *Bal*I-*Sma*I or *Bal*I-*Exo*III-fragments from the *Lly*-region of the *L. pneumophila* chromosome. a) <sup>35</sup>S-labeled proteins determined by vector plasmid pUC19 as a control, Bla is  $\beta$ -Lactamase, b) pEWL11, c) pEWL114, d) pEWL1141.

In order to analyze the protein encoded by the legiolysin specific gene, minicells (4) were isolated from strain P678-54 pEWL11, P678-54 pEWL114 and P678-54 pEWL1141 and as a vector control from P678-54 pUC19. The purified minicells were radiolabeled with <sup>35</sup>S-Methionine, lysed in loading buffer according to *Laemmli* (3) and the proteins of the lysate were separated on a 12% SDS-PAGE (Fig. 2). The hemolytic clones pEWL11 and pEWL114 expressed a protein of a molecular weight of 40 kDa, the non-hemolytic clone pEWL1141, where the gene is truncated from the 3' end (Fig. 1), did not show an additional band besides the vector bands.

To characterise the *lly* gene more precisely we have sequenced the corresponding DNA region of about 1000 basepairs (data not shown). There is no homology to the sequence of the protease gene determined by *Quinn* et al. (5). An open reading frame of 813 bp was found for *lly* which could code for a protein of a total of 270 amino acids, corresponding to a molecular mass of 29 kD. The molecular weight of the legiolysin protein determined from the SDS-PAGE differs from the size predicted for legiolysin from the ORF of the DNA sequence. This discrepancy can be due to unusual mobility of the protein in the gel system or to post-translational modification of the protein.

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