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Interfaces properties of Legionella pneumophila : an issue to explain host-cell association?

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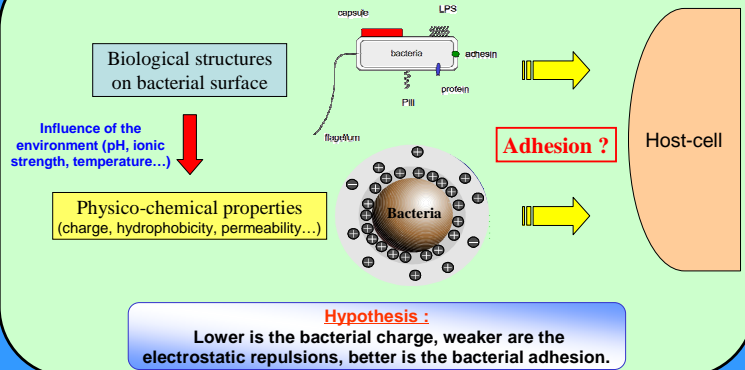
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Introduction :

Legionella pneumophila is naturally found in fresh water as well under planctonic bacteria as associated form within protozoa (infection of amoeba) or biofilms. Upon aerosol formation via man-made water systems, *L. pneumophila* can enter the human lung and cause a severe form of pneumonia, called Legionnaires' disease. The pathogenesis of Legionnaires' disease is largely due to the ability of *L. pneumophila* to invade and grow within macrophages. Unlike its intracellular cycle, first step of *L. pneumophila* adhesion to the host-cell is unexplored. The physicochemical composition of the bacterial cell surface is however an important factor in bacterial pathogenicity, since it is this surface that first interacts with soluble factors (eg. the humoral immune system) and the extracellular matrices of the infected host tissues and cells. Many bacteria, in particular gram negative, display a polysaccharide coat, either as a discrete layer of exopolysaccharide (lipopolysaccharide, capsule), or as a loosely attached slime layer. Interfaces properties of bacteria are known to govern the adhesion mechanisms and consequently they may potentially influence the attachment to the host cell during infection, more particularly early step of adhesion¹.

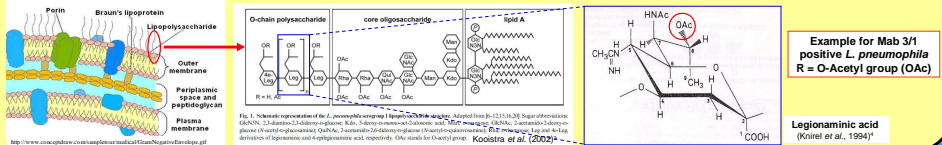
Phenomena governing bacterial adhesion to the host-cell



Objectives :

Characterisation of the bacterial charge of different *Legionella pneumophila* sg1 strains according to the presence or absence of 8 O-Acetyl groups in the side chain of the LPS (respectively Mab 3/1 positive versus Mab 3/1 negative² strains) among clinical genotypes.

Among the exopolysaccharides produced by gram-negative bacteria is lipopolysaccharide (LPS) comprising three distinct sections: lipid A anchored in the outer membrane, oligosaccharide core and O-antigen (O-Ag) polysaccharide. Concerning *L. pneumophila*, the O-Ag consists of a variable number of repeated saccharidic units (n = 18 to 40), identified as legionaminic acid, to form a polysaccharide layer that extends into the surrounding media.

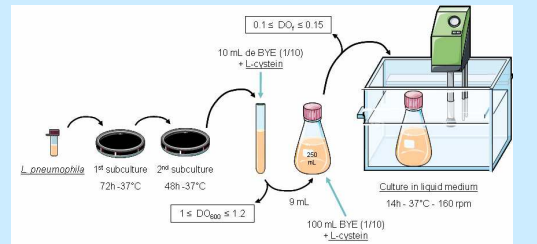


Materials & Methods :

1. Bacterial strains and culture condition

Clinical strains of <i>Legionella pneumophila</i>	
Mab 3/1 positive strains	Mab 3/1 negative strains
Lens	Bellingham
Knoxville	Olda
Allentown	Camperdown
Philadelphia	Oxford
Benidorm	Heysham

Bacteria were grown sequentially on BCYE 3 days, on BCYE 2 days and finally to early step of stationary phase in 100 ml BYE. Bacteria were pelleted (10 min., 5,000g), washed once with 1 mM KNO₃ and resuspended in 1 mM KNO₃ at ~10⁷ cfu/ml before electrophoretic measurements.



2. Electrophoretic measurements (Zetaphorometer IV, CAD Instruments)

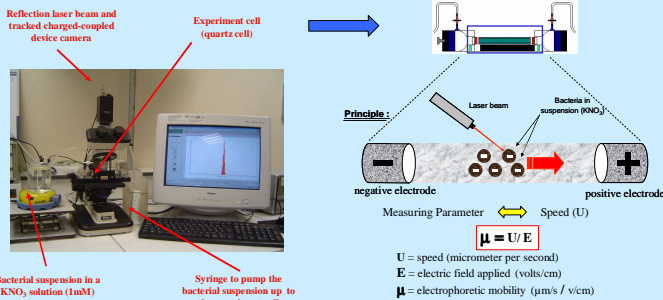
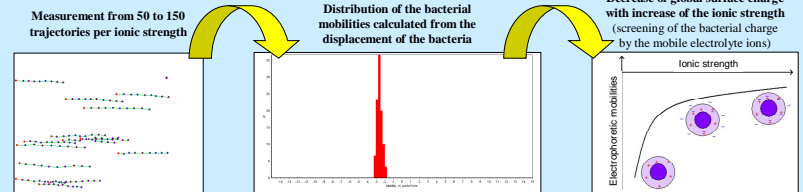
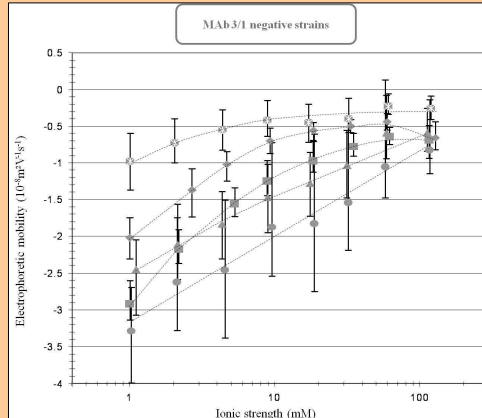
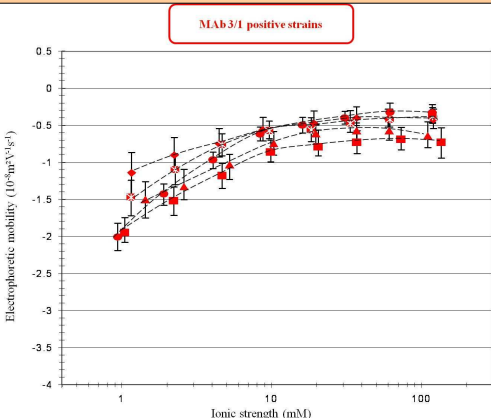


Image analysis software allowed recorded images to be processed in real time to calculate the electrophoretic mobilities from the displacement of bacteria subjected to a constant direct-current electric field (800 V/m). Different cycles were performed and 100 mean bacterial trajectory was obtained for each condition. All the experiments were carried out in natural pH (~6) and repeated threefold with different cultures.



Results :

Legend: Philadelphia (red square), Lens (red diamond), Knoxville (red triangle), Bellingham (red circle), Allentown (red inverted triangle), Oxford (red plus), Benidorm (red asterisk), Camperdown (red cross), Heysham (red dot), Olda (red x)



The ionic strength-mobility patterns of clinical strains which present 8 O-Acetyl groups in the side chain of the LPS were significantly different than those of bacteria devoid of 8 O-Acetyl groups.

Conclusions :

1. The LPS nature at the *L. pneumophila* surface contributes to the charge of the strains.
2. Among clinical strains, Mab 3/1 positive bacteria are mainly involved in community acquired infection whereas Mab 3/1 negative are rather source of nosocomial cases². From this fact, bacterial charge could explain differences in host cell association and also the infection efficiency.

Acknowledgments:

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References:

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