

(2008/8/31 2008/4/6)

Listeria monocytogenes

Legionella pneumophila

SDS

L.monocytogenes

20-

Study of the S-Layer Protein in *Legionella pneumophila* and *Listeria monocytogenes*

Amera M. Al-Rawi

Ghada A. Al-Taee

Mayada A. Al-Taee

*Department of Biology
College of Science
Mosul University*

ABSTRACT

The presence of surface layer was detected in each of *Legionella pneumophila* (gram negative) and *Listeria monocytogenes* (gram positive) by extraction of S-layer proteins then separated by using sodium dodecyl sulphate poly acrylamide gel electrophoresis (SDS-PAGE). Five proteins bands appeared for both types of bacteria.

The freezing effect on the S-layer protein of *L. monocytogenes* was studied by exposure to low temperature at -20C° for three months. Six protein bands were detected,,

.....

as well as their differences in approximate molecular weights for the freezing isolate in comparison with non-frozen isolate.

.(Beveridge and Graham, 1991)

crystalline arrays

Surface layers

.(Sleytr and Messner, 1983; Sleytr *et al.*, 1996) S-layers

S-layer

Pseudomurein

.(Konig, 1988; Engelhardt and Peters, 1998) (LPS)

.

stress

DNA

,

.(Dworkin and Blaser, 1996; Sara and Sleytr, 2000; Scholz *et al.*, 2001)

L.pneumophila

SDS-PAGE

(1995)

Zahringer

(Luneberg *et al.*, 1998)

SDS-PAGE

.

L.monocytogenes *Legionella pneumophila*

° 20-

L.monocytogenes

.

: :__

L.monocytogenes L.pneumophila

/ /

.

: :__

*L.pneumophila**L.monocytogenes*

.(1986) Johnson Ray

L.monocytogenes L.pneumophila

15

g×12000

% 0.05

³

1

SDS % 0.05

³

5

.(SDS) Sodium Dodecyl Sulphate

10

37

.

° 30

.

15

g×12000

(PSS) Protein Solubilizing Solution

³

0.5

³

1

³ 1 glycerol³

4

β-mercaptoethanol

300 :

. 8.0

.(0.02M) EDTA (0.2M)Tris

³

1

%20 SDS

:

:__

.(Ray and Johnson, 1986)

SDS

SDS

.....

.(SDS-PAGE) (1970) Laemmli
Transferrine Pepsine Trypsine Lysozyme :_____

150000 80000 45000 20000 14000 γ -Globuline

L.pneumophila

(1)

(1) SDS-PAGE

:1

()		()		
1.1	4.14	14000	Lysozyme	1
2.0	4.30	20000	Trypsine	2
3.9	4.6	45000	Pepsine	3
4.6	4.9	80000	Transferrine	4
6.3	5.17	150000	γ -Globuline	5

.(1) (2) 107151-16405

L.penumophila

16405

(1984) Iglewski Hindahl

.....

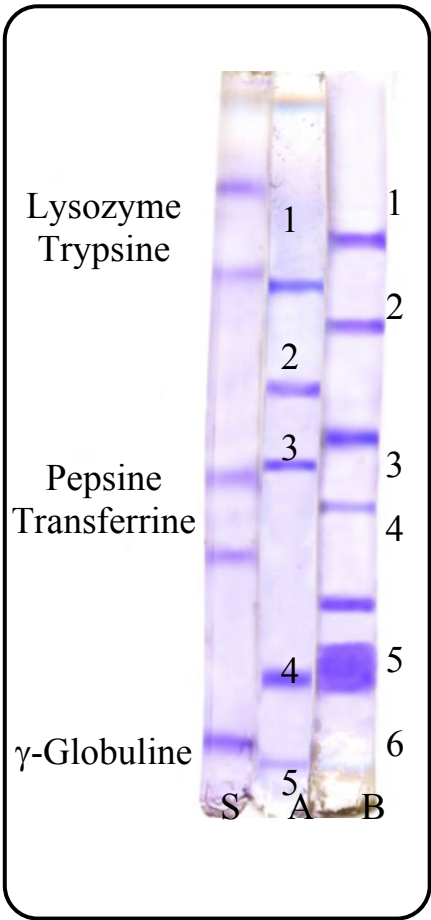
112201 17179

. (2) (3)

:3

L.monocytogenes

<i>L. monocytogenes</i>			<i>L. monocytogenes</i>		
()	()		()	()	
17179	1.6	1	20892	2.0	1
25409	2.4	2	33111	3.0	2
40738	3.4	3	45708	3.7	3
55590	4.1	4	117489	5.7	4
85113	5.0	5	169824	6.5	5
112201	5.6	6			



(2)

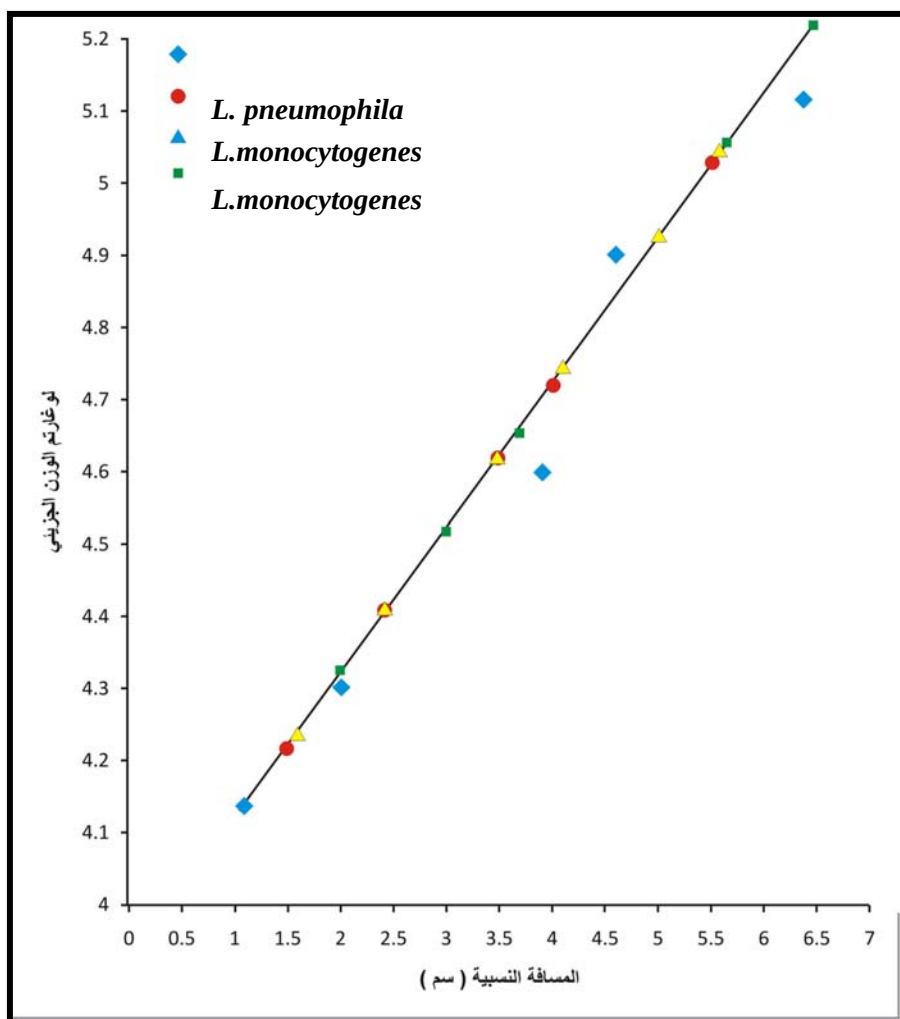
: S

: A

L.monocytogenes

: B

L.monocytogenes



:1

(Caps) Cold acclimation proteins

Proteases

.(Berger *et al.*, 1996 ; Hebraud *et al.*, 1994)

(Csps) Cold-Shock Proteins

.(Hebraud and Potier, 1999)

Mesophiles

• • • • •

L.monytogenes

33111

25409

(1997)

Domann

Internalin C

L.monytogenes

(1991)

Gaillard

30000

71000

80000

```
Int B   Int A
```

```
Int H   Int G   Int F   Int E   Int D
```

.(2001)

Vazquez-Boland

(1997)

Alvarez-Dominguez

90000 (Action polymerization protein) Act A

610

Act A

(1996) Berche Rouquette

```
.Int A
```

85113

L.monocytogenes

(1992)

Bubert

60000

P₆₀

P₆₀

(2001) Doyle

P₆₀

55590

112201 117489

(1999)

Pandiripally

104000

P₁₀₄

(97723-12445) (123026-19498)

L.monocytogenes *L.penumophila*

L.pneumophila

$$\cdot \left(\begin{array}{c} \\ \\ \end{array} \right) \quad \left(\begin{array}{c} \\ \\ \end{array} \right)$$

SDS-PAGE

Alvarez-Dominguez, C.; Roberts, R., and Stahl, P.D., 1997. Internalized *Listeria monocytogenes* modulates intracellular trafficking and delays maturation of the phagosome. J. Cell Sci. 110: pp.731-743.

Berger, F.; Morellet, N.; Menu, F., and Potier, P., 1996. Cold shock and cold acclimation proteins in the psychrotrophic bacterium *Arthrobacter glpfiformis* 5155. J. Bacterial. 178: pp.2999-3007.

Beveridge, T.J., and Grahahm, L.L., 1991. Surface layers of bacteria. Microbial. Rev. 55: pp.684-705.

Bubert, A.; Kuhn, M.; Goebel, W., and Köhler, S., 1992. Structural and functional properties of the P₆₀ proteins from different *Listeria* species. J. Bacteriol. 174: pp.8166-8171.

Domann, E.; Zechel, S.; Lingnan, A.; Hain, T.; Darji, A.; Nichterlein, T.; Wehland, J., and Chakraborty, T., 1997. Identification and characterization of a novel PrfA-regulated gene in *Listeria monocytogenes* whose product, IrpA, is highly homologous to internalin proteins, which contain Leucine-rich repeats. Infect. Immune. 65: pp.101-109.

Doyle, M.E., 2001. Virulence characteristics of *Listeria monocytogenes*. Food Research Institute, University of Wisconsin. Madison. E-mail: medoyle@facstaff.wisc.edu.

Dramsi, S.; Dehoux, P.; Lebrun, M.; Goossens, P.L., and Cossart, 1997. Identification of four new members of the internalin multigene family of *Listeria monocytogenes* EGD. Infect. Immune. 65: pp.1615-1625.

Dworkin, J., and M.J., Blaser, 1996. Generation of *Campylobacter fetus* S-layer protein diversity utilizes a single promoter on an invertible DNA Segment. Mol. Microbiol. 19: pp.1241-1253.

Engelhardt, H., and J., Peters, 1998. Structural research on surface layers: a focus on stability, surface layer homology domains, and surface layer-cell wall interactions. *J. Struct. Biol.* 124: pp.276-302.

Gaillard, J.L.; Berche, P.; Frehel, C.; Gouin, E., and Cossart, P., 1991. Entry of *Listeria momocytogenes* into cells is mediated by internalin, are peat protein reminiscent of surface antigens from gram-positive cocci. Cell. 65: pp.1127-1141.

Hebraud, M., and Porier, P., 1999. Cold Shock response and low temperature adaptation in psychrotrophic bacteria. *J. Mol. Microbiol. Biotechnol.* 1(2): pp.211-219.

Hebraud, M.; Dubois, E.; Potier, P., and Labadie, J., 1994. Effect of growth temperatures on the protein levels in a psychrotrophic bacterium, *Pseudomonas fragi*. J. Bacteriol. 176; pp. 4017-4024.

.....

- Hindahl, M.S., and Iglewski, B.H., 1984. Isolation and characterization of *Legionella pneumophila* outer membrane. J. Bacteriol. 159: pp.107-113.
- König, H., 1988. Archaeobacterial cell envelopes. Can. J. Microbiol. 34: pp.395-406.
- Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 227: pp.680-685.
- Luneberg, E.; Zahringer, U.; Knirel, Y.A.; Steinmann, D.; Hartmann M.; Steinmetz, L.; Rohde, M.; Kohl, J., and Frosch, M., 1998. Phase variable expression of lipopolysaccharide contribute to the virulence of *L.pneumophila*. J. Exp.Med. 188(1): pp.49-60.
- Pandiripally, V.K.; West brook, D.G.; Sunki, G.R., and Bhunia, A.K., 1999. Surface protein P₁₀₄ is involved in adhesion of *Listeria monocytogenes* to human intestinal cell line, Caco 2. J. Med. Microbiol. 48: pp.117-124.
- Raffelbauer, D.; Bubert, A.; Engelbrecht, F.; Scheinpflug, J.; Simm, A.; Hess, J.; Kaufmann, S.H.E., and Goebel, W., 1998. The gene cluster in LC₂DE of *Listeria monocytogenes* contains additional new internalin genes and is important for virulence in mice. Mol. Gen. Genet. 260: pp.144-158.
- Ray, B., and Johnson, M.C. 1986. Freeze-drying injury of surface layer protein and protection in *Lactobacillus acidophilus* cryoletters. Cryoletters. 7: pp.210-217.
- Rouquette, C., and Berche, P., 1996. The pathogenesis of infection by *Listeria monocytogenes*. Microbiologia. 12: pp.245-258.
- Sàra, M., and Sleytr, U.B., 2000. S-layer proteins. J. Bacteriol. 182: pp.859-868.
- Scholz, H.C; Riedmann, E; Witte, A; Lubitz, W, and Kuen, B., 2001. S-layer variation in *Bacillus stearothermophilus* PV72 is based on DNA rearrangements between the chromosome and the naturally occurring megaplasms. J. Bacterial. 183: pp.1672-1679.
- Sleytr, U.B; and Messner, P., 1983. Crystalline surface layers on bacteria. Annu. Rev. microbiol. 37: pp.311-339.
- Sleytr, U.B.; Messner, P.; Pum, D., and M., Sàra, 1996. Crystalline bacterial cell surface proteins. Landes Company, Academic Press Austin, Tex. 10: pp.911-916.
- Vazquez-Boland, J.A.; Kuhn, M.; Berche, P.; Chakrabarty, T.; Dominguez-Bernal, G.; Goebel, W.; Gonzalez-Zorn, B.; Wehland, J., and Kreft, J., 2001. *Listeria* pathogenesis and Molecular Virulence Determinants. Clin. Microbiol. Rev. 14: pp.584-640.
- Zahringer, U; Knirel, Y.A.; Linder, B.; Helbing, J.H.; Sonesson, A.; Marre, R., and Rietschel, E.T., 1995 The lipopolysaccharide of *L.pneumophila* serotype 1: chemical structure and biological significance. J.Lin Biol.Res 392: pp.113-139.