Journal of Industrial Microbiology, 8 (1991) 281–284
Elsevier

SIM 00377

Attachment of *Salmonella typhimurium* and *Listeria monocytogenes* to glass as affected by surface film thickness, cell density, and bacterial motility

J.S. Dickson and E.K. Daniels

United States Department of Agriculture, Agricultural Research Service, Roman L. Hruska U.S. Meat Animal Research Center, Clay Center, Nebraska, U.S.A.*

(Received 26 March 1991; revision received 3 September 1991; accepted 6 September 1991)

Key words: Biofilm; Contamination; Biofouling

SUMMARY

With equal cell densities, surface film thickness did not influence the numbers of *Salmonella typhimurium* and *Listeria monocytogenes* cells which attached to glass. Motile *L. monocytogenes* cells had a greater cell surface charge and generally attached in higher numbers than non-motile cells.

INTRODUCTION

Attachment is influenced by intrinsic properties of the bacterial cell, such as surface charge [3], hydrophobicity [4], and surface structures including extracellular polysaccharides [8]. Notermans and Kampelmacher [7] reported that non-motile *Salmonella* did not attach to broiler skin, while Lillard [6] found no significant difference in attachment between motile and non-motile cells. Extrinsic factors, such as high initial cell numbers in the inoculum result in higher numbers of attached cells [1]. The numbers of attached cells may also be affected by the thickness of the film of moisture on the surface to be colonized. With equal cell densities, a thin film of moisture may result in greater numbers of attached cells by allowing the cells closer physical proximity to the surface. Our intent was to study the effects of surface film thickness and cell motility on bacterial attachment using a static model system. This model was not intended to represent a specific ecosystem, but was simply a research tool.

Correspondence: J.S. Dickson, United States Department of Agriculture, Agricultural Research Service, Roman L. Hruska U.S. Meat Animal Research Center, P.O. Box 166, Clay Center, NE 68933, U.S.A.

* Mention of a trade name, proprietary product or specific equipment is necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

MATERIALS AND METHODS

Bacterial cultures

Strains of *Salmonella typhimurium* (ATCC 14028) and *Listeria monocytogenes* Scott A (Food and Drug Administration, Division of Microbiology, Cincinnati, OH) were grown and maintained in tryptic soy broth (TSB, BBL, Cockeysville, MD). Cultures of *L. monocytogenes* were inoculated 18 h prior to use and incubated at either 23 °C or 37 °C to produce both motile and non-motile cells [9], which was confirmed by microscopic examination. *S. typhimurium* was incubated at 37 °C. Cells were diluted 1 : 100 in Butterfield's Phosphate buffer [10] and initial populations were determined using tryptic soy agar (TSA, Difco, Detroit, MI) and the pour plate technique.

Attachment studies

A 1.0 × 1.0 cm area was marked on pre-cleaned microscopes slides (25 × 75 mm) using a 1.0 × 1.0 cm plastic template and a fine point indelible ink marker. A rapidly drying hydrophobic compound was applied using a Pap pen (Research Products International Corp., Mount Prospect, IL) to the area surrounding the 1.0 × 1.0 cm

area, to confine the inoculum. Table 1 describes the relationship of surface film thickness to each inoculum.

At 5, 10 and 20 min intervals, the slides were immersed in sterile phosphate buffer for 15 s quiescently. Cells remaining on the slide were considered to be attached. Slides were air-dried, heat-fixed, stained for 1 min in 1% (w/v) Crystal Violet (Difco), rinsed in distilled water and air-dried. Slides were viewed on a phase contrast microscope with a video display, and the cells in each field were counted by three observers. Ten fields were counted from each slide. The microscopic field was measured using a stage micrometer, and the number of attached cells per cm^2 was calculated [11]. Data from two independent replications were transformed to $\log_{10}$ values and analysed using the general linear model procedure of SAS [12].

Chromatography

Electrostatic interaction chromatography (ESIC) columns [8] were prepared using polystyrene columns (Bio-Rad, Richmond, CA) packed with a 1 : 1 (w/v) mixture of Dowex chloride form (1 by 8) (Sigma, St. Louis, MO; capacity 1.2 meq/ml) and phosphate buffer. Columns were washed with 30 ml of phosphate buffer and 1 ml of the cell suspension was adsorbed on to the column. Cells were eluted with 9 ml phosphate buffer, and populations of the initial and eluted suspensions were enumerated. The relative negative charge was expressed as r/e, where r was calculated as the difference between the initial (i) and eluted (e) samples.

RESULTS AND DISCUSSION

The inoculum levels allowed the comparison of effects of both surface film thickness and cell density (Table 1). Surface film thickness affected the numbers of *S. typhimurium* cells which attached to the glass slides only during the initial period of bacterial cell attachment (Table 2A). There was no significant difference in cell attachment for either film thickness after 10 min. As expected, the lowest numbers of attached cells were seen with the inoculum which had a cell density of half of the other two inocula (Table 2B). Since the surface film thicknesses were equal for the 50 + 50 and 100 μl inocula, the difference in attached cells is solely attributable to the difference in cell density of the inocula. Time was also a factor ($P < 0.05$) in attachment with all inoculum levels, with an increase in attached cells from 5 to 20 min. independent of film thickness.

The attachment of *L. monocytogenes* was similar to that of *S. typhimurium* for both motile (Table 3) or non-motile cells (data not shown). Surface film thickness did not influence attachment with equal cell densities (Table 3a). When the cell density of the inoculum was halved with equal film thickness, the numbers of attached cells with the lower cell density decreased. Significantly more motile than non-motile cells attached for each inoculum (Table 4). With inocula of equal cell densities, the numbers of non-motile cells which attached was generally only 50–60% of the numbers of motile cells which attached. However, this difference in attachment was partially mitigated by a reduction in cell density.

While motile cells can move freely through a drop of liquid medium and randomly encounter the surface, non-motile cells are only brought into the proximity of the surface by Brownian motion or gravitational forces (sedi-

TABLE 1

Relationship between cell numbers and surface film thicknesses for different inoculum levels

Inoculum	Cell density[a]	Surface film thickness[b]	Calculated film thickness[c]
50 μl	D	h	0.5
50 + 50 μl	0.5 D	2 h	1.0
100 μl	D	2 h	1.0

[a] Cell density (cfu/ml) of inoculum used to produce the surface film.
[b] Actual thickness of surface film.
[c] Calculated thickness in mm, using the formula $v = l \times w \times h$, where l and w are 10 mm and v is the inoculum volume in mm^3.

TABLE 2

Means of numbers of *Salmonella typhimurium* cells attached to glass as affected by (A) surface film thickness and (B) cell density

A. Inoculum	Cell density[a]	Surface film thickness[b]	Time (min)		
			5	10	20
50 μl	D	H	$5.04^{c,1}$	5.34^{2}	$5.44^{2,3}$
100 μl	D	2 H	5.35^{2}	$5.48^{2,3}$	5.55^{3}

B. Inoculum	Cell density[a]	Surface film thickness[b]	Time (min)		
			5	10	20
50 + 50 μl	0.5 D	2 H	$4.72^{c,1}$	5.15^{2}	$5.20^{2,3}$
100 μl	D	2 H	$5.35^{3,4}$	$5.48^{4,5}$	5.55^{5}

[a] Cell density (cfu/ml) of inoculum used to produce the surface film.
[b] Actual thickness of surface film.
[c] $\log_{10}$ cfu/cm^2; means with different superscripts are significantly ($P < 0.05$) different.

TABLE 3

Means of numbers of *Listeria monocytogenes* cells grown at 23 °C attached to glass as affected by (A) surface film thickness and (B) cell density

A. Inoculum	Cell density[a]	Surface film thickness[b]	Time (min)		
			5	10	20
50 µl	D	H	5.30[c,1]	5.50[2]	5.62[3]
100 µl	D	2 H	5.41[1,2]	5.43[2]	5.59[2,3]
B. Inoculum	Cell density[a]	Surface film thickness[b]	Time (min)		
			5	10	20
50 + 50 µl	0.5 D	2 H	4.92[c,1]	5.07[2]	5.20[3]
100 µl	D	2 H	5.41[4]	5.43[4]	5.59[5]

[a] Cell density (cfu/ml) of inoculum used to produce the surface film.

[b] Actual thickness of surface film.

[c] Log_{10} cfu/cm^2; means with different superscripts are significantly ($P < 0.05$) different.

mentation). The random motion of the motile cells may provide more frequent contact with the surface than non-motile cells, and thus may partially account for the higher levels of attachment. Alternately, since motility of *L. monocytogenes* can be altered by changes in growth temperature [9], it is likely that other characteristics of the bacterial cell wall may also be altered by growth temperature. There was a significant difference ($P < 0.01$) between the relative negative charge of the motile and non-motile cells (36.63 vs. 6.44, respectively). Bacterial cell surface charge has been positively correlated with attachment [3,5] and the observed difference in cell surface charge between motile and non-motile cells may also explain the lower attachment level of the non-motile cells. The difference in attachment, therefore, cannot be specifically related to the presence or absence of flagella.

TABLE 4

Means of numbers of *Listeria monocytogenes* cells attached to glass as affected by growth temperature of the cultures. Cells grown at 23 °C are motile, cells grown at 37 °C are non-motile

Inoculum	Growth temperature[a]	Time (min)		
		5	10	20
50 µl	23	5.30[b,1]	5.50[2]	5.62[3]
	37	5.01[4]	5.30[1]	5.32[1]
50 + 50 µl	23	4.92[1]	5.07[2]	5.20[3]
	37	4.89[1]	4.91[1]	5.02[1,2]
100 µl	23	5.41[1]	5.43[1]	5.59[2]
	37	5.14[3]	5.27[4]	5.30[1,4]

[a] Growth temperature in °C.

[b] Log_{10} cfu/cm^2; means with different superscripts are significantly ($P < 0.05$) different.

ACKNOWLEDGEMENTS

The authors wish to thank Ms. Jane Long for her technical assistance in the laboratory, Dr. Neal E. Woollen for the use of his microscope, and Ms. Carol Grummert and Ms. Marilyn Bierman for secretarial assistance with this manuscript.

REFERENCES

1 Chung, K.-T., J.S. Dickson and J.D. Crouse. 1989. Attachment and proliferation of bacteria on meat. J. Food Protect. 52: 173–177.

2 Dahlback, B., M. Hermansson, S. Kjelleberg and B. Norkrans. 1981. The hydrophobicity of bacteria — an important factor in their initial adhesion to the air–water interface. Arch. Microbiol. 128: 267–270.

3 Dickson, J.S. and M. Koohmaraie. 1989. Cell surface charge characteristics and their relationship to bacterial attachment to meat surfaces. Appl. Environ. Microbiol. 55: 832–836.

4 Fletcher, M. and G.D. Floodgate. 1973. An electron-microscopic demonstration of an acidic polysaccharide involved in the adhesion of a marine bacterium to solid surfaces. J. Gen. Microbiol. 74: 325–334.

5 Fletcher, M. and G.I. Loeb. 1979. Influence of substratum characteristics on the attachment of a marine pseudomonad to solid surfaces. Appl. Environ. Microbiol. 37: 67–72.

6 Lillard, H.S. 1986. Role of fimbriae and flagella in the attachment of *Salmonella typhimurium* to poultry skin. J. Food Sci. 51: 54–56.

7 Notermans, S. and E.H. Kampelmacher. 1974. Attachment of some bacterial strains to the skin of broiler chickens. Br. Poultry Sci. 15: 573–585.

8 Pederson, K. 1980. Electrostatic interaction chromatography, a method for assaying the relative surface charges of bacteria. FEMS Microbiol. Lett. 12: 365–367.

9 Peel, M., W. Donachie and A. Shaw. 1988. Temperature-dependent expression of flagella of *Listeria monocytogenes* studied by electron microscopy, SDS-PAGE and Western blotting. J. Gen. Microbiol. 134: 2171–2178.

10 Pertel, R. and N. Kazanas. 1984. Stains, reagents, and diluents. In: Bacteriological Analytical Manual, 6th. edn., pp. II.01–II.29, Association of Official Analytical Chemists, Arlington, VA.

11 Pusch, D.J., F.F. Busta, W.A. Moats, R. Bandler and S.M. Cichowicz. 1984. Direct microscopic count. In: Compendium of Methods for the Microbiological Examination of Foods (Speck, M.L., ed.), pp. 84–98. American Public Health Association, Washington, DC.

12 SAS. 1982. User's Guide: Statistics. SAS Inst., Cary, NC.