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APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Apr. 1989, p. 832–836
0099-2240/89/040832-05$02.00/0
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Vol. 55, No. 4

Cell Surface Charge Characteristics and Their Relationship to Bacterial Attachment to Meat Surfaces

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Received 23 September 1988/Accepted 12 January 1989

Cell surface charge and hydrophobicity of *Bacillus subtilis*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella typhimurium*, *Serratia marcescens*, *Staphylococcus aureus*, and *Staphylococcus epidermidis* were determined by hydrocarbon adherence, hydrophobic interaction, and electrostatic interaction chromatography. Surface charge and hydrophobicity were compared with the initial attachment values and rates of attachment of the bacteria to meat surfaces. There was a linear correlation between the relative negative charge on the bacterial cell surface and initial attachment to lean beef muscle (r^2 = 0.885) and fat tissue (r^2 = 0.777). Hydrophobicity correlated well with attachment to fat tissue only. The relative hydrophobicity of each bacterium was dependent on the specific method of determination, with wide variations noted between methods.

Bacterial attachment is influenced by cell surface charge (9), hydrophobicity (4), and structures, including extracellular polysaccharides (8) and flagella (15). There is disagreement over the role of surface structures, since nonfimbriated (14) and nonflagellated (13) cells have been reported to attach at rates similar to those of cells which possess these structures. However, other reports indicate that motile bacteria attach to surfaces more rapidly than nonmotile strains (2, 5). The actual role of flagella in attachment is probably dependent on the specific strain of bacterium as well as growth conditions.

Relative hydrophobicity of bacterial cells has been characterized by bacterial adherence to hydrocarbons (BATH) (20), hydrophobic interaction chromatography (HIC) (4, 12, 22) and contact angle (27). These methods have been reviewed by Rosenberg and Kjelleberg (20), and each has specific advantages and disadvantages. Although HIC has been used extensively, there is some concern that there may be a filtration effect or nonspecific binding of the bacteria by the column gel (16). Recently, BATH with hexadecane appears to have been validated by the use of bioluminescence (25). However, considerable variation in relative hydrophobicity has been reported, depending on the method of determination (6).

Bacterial cells have a net negative charge on the cell wall (3), although the magnitude of this charge varies from strain to strain. Cell surface charge of bacterial cells has been characterized by electrostatic interaction chromatography (ESIC) (10, 18). The problem of filtration or nonspecific binding to the resin, which has been associated with HIC, has not been reported with ESIC (18). The usefulness of each technique and its relationship to bacterial attachment is probably influenced by the substratum to which the bacteria are being attached.

Van Loosdrecht et al. (26, 27) characterized bacterial cells by measuring hydrophobicity (measured by water contact angle) and electrophoretic mobility. They concluded that cell surface hydrophobicity was the major determining factor in attachment to negatively charged polystyrene. However, as the relative hydrophobicity decreased, electrophoretic mobility had more influence on attachment. Hydrophobicity has also been identified as an important factor in bacterial attachment to human epithelial cells (21), soybean leaves (6), and air-water interfaces (4, 10).

Firstenberg-Eden et al. (7) determined an S value which was described as a measure of the relative strength of bacterial attachment to chicken and beef muscle surfaces. The S value measures the difference between bacteria which are physically attached to a surface and those which are loosely associated with a surface (e.g., trapped in a film of water covering the surface) [$S = \log_{10}$(physically attached bacteria) $- \log_{10}$(loosely attached bacteria)]. Farber and Idziak (5) also used S values in measuring the attachment of psychrotrophic bacteria to beef muscle. An increase in S value indicates an increase in the numbers of bacteria which physically attach to a surface under defined conditions. Using the principles of the S-value determination, we calculated an S_R value which represents the percentage of the total population of bacteria associated with the tissue surface which is physically attached to the surface [S_R = (physically attached bacteria)/(physically attached + loosely associated bacteria)].

The objective of this study was to determine the relationship between cell surface charge and bacterial attachment to meat surfaces. The bacteria selected represent both pathogenic and nonpathogenic strains which are associated with meat products.

MATERIALS AND METHODS

Bacterial cultures and media. Strains of *Bacillus subtilis* (ATCC 6851), *Escherichia coli* O157:H7 (Food Research Institute, Madison, Wis.), *Listeria monocytogenes* Scott A (Food and Drug Administration, Division of Microbiology, Cincinnati, Ohio), *Salmonella typhimurium* (ATCC 14028), *Serratia marcescens* (ATCC 8100), *Staphylococcus aureus* (ATCC 25923), and *Staphylococcus epidermidis* (ATCC 12228) were grown and maintained in tryptic soy broth (Difco Laboratories, Detroit, Mich.). Cultures were transferred 18 h prior to use and were incubated at 37°C. The cells were harvested by centrifugation (3,000 × g for 10 min at 5°C), and the pellets were suspended in Butterfield phos-

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TABLE 1. Relative hydrophobicity of bacteria determined by HIC, contact angle, and adherence to hydrocarbons (BATH)[a]

Bacterium	HIC	Contact angle	BATH	
			Hexadecane	Xylene
Bacillus subtilus	0.371 (4)	29.00 (3)	1.086 (7)	0.986 (4)
Escherichia coli	0.203 (2)	32.00 (2)	0.995 (4)	0.959 (3)
Listeria monocytogenes	0.278 (3)	26.50 (4)	0.997 (5)	1.046 (5)
Salmonella typhimurium	0.392 (5)	26.42 (5)	0.936 (2)	1.088 (7)
Serratia marcescens	−0.130 (NA)	33.25 (1)	1.043 (6)	1.058 (6)
Staphylococcus aureus	0.085 (1)	15.56 (7)	0.284 (1)	0.504 (1)
Staphylococcus epidermidis	0.750 (6)	18.25 (6)	0.974 (3)	0.557 (2)

[a] Numbers in parentheses indicate ranking from most to least hydrophobic. NA, Not applicable.

phate buffer (18). The harvested cells were washed in phosphate buffer and were suspended in sterile buffer for the chromatography experiments.

BATH. Hydrocarbon adherence was tested by the method of Sweet et al. (23). For each strain, 2 4-ml volumes of washed cells were added to separate 13-mm-diameter test tubes. Two 4-ml volumes of phosphate buffer were added to two other test tubes. For both the bacterium and the buffer, one tube was used as a control and the other was used for the assay. A 1-ml volume of xylene (Fisher Scientific Co., Pittsburgh, Pa.) or hexadecane (Sigma Chemical Co., St. Louis, Mo.) was added to each assay tube. The tubes were allowed to equilibrate at 37°C for 10 min in a water bath, were vortexed for 15 s, and were incubated at 37°C for 30 min. After incubation, the aqueous layer was carefully removed from each assay tube and was transferred to a separate tube. Any excess hydrocarbon which was transferred was removed by bubbling air through the tubes for 1 min, at a flow rate of approximately 3 ml/s. The A_{540} was then measured on a Spectronic 20 (Bausch & Lomb., Inc., Rochester, N.Y.). The spectrophotometer was zeroed by using the control phosphate buffer, and the ratio of the absorbance of the bacterium assay to the absorbance of the bacterium control was calculated. The adjustment factor for the phosphate buffer assay tube was very small, averaging less than 0.005 absorbance units.

Chromatography. HIC and ESIC columns were prepared in manners similar to those described by Dahlback et al. (4) and Pedersen (18), respectively. Pasteur pipettes (5-mm diameter) were plugged with glass wool and were washed with sterile phosphate buffer. The hydrophobic interaction columns were packed with a 1:1 (vol/vol) mixture of phenyl Sepharose CL-4B (Pharmacia, Uppsala, Sweden) and phosphate buffer to produce a gel height of 30 mm. The ESIC columns were packed with 1 ml of a 1:1 (wt/vol) suspension of the ion-exchange resin and phosphate buffer. Dowex chloride form (1 by 8) (Sigma; capacity, 1.2 meq/ml) was used for the anion resin, and Dowex hydrogen form (50 by 8) (Bio-Rad Laboratories, Richmond, Calif.; capacity, 1.7 meq/ml) was used for the cation resin. The mesh size was 100 to 200 for both resins.

Prior to use, all columns were flushed with 10 to 15 ml of phosphate buffer. A 0.1-ml sample of the cell suspension was added and adsorbed onto the columns with 0.2 ml of phosphate buffer, while the cells in 1.0 ml of the suspension were simultaneously enumerated. The cells were eluted in the initial experiments with 3 5-ml volumes of phosphate buffer. However, it was found that >99.9% of the eluted bacteria were recovered in the first 10 ml. In all of the subsequent experiments, the cells were eluted with a single 10-ml wash. The initial cell suspensions and the eluted samples were plated on tryptic soy agar (Difco) by the pour plate technique (1). The number of bacteria bound to the columns was calculated as the difference between the initial and eluted samples after those results were adjusted for the dilution factors (0.1 for the initial, 10 for the eluted). The relative hydrophobicity was expressed as g/e, while the relative ion values were expressed as r/e, with g or r representing the number of bacteria retained by the columns and e representing those eluted.

Contact angle. Contact angles were determined by the sessile drop technique, using the method described by J. F. James (Program Abstr. 5th Int. Pathog. *Neisseria* Conf., abstr. no. V119, 1986). Cells were collected on nitrocellulose filters, and 100 μl of phosphate buffer was dropped on the filter. At the moment of contact, the drop was photographed with a macro lens, and contact angles were measured from the resulting photographs.

Cell surface area. Wet mounts of the washed cells were prepared and photographed through a phase-contrast microscope. A stage micrometer was photographed under identical conditions, and all photographs were enlarged to an identical size. A conversion factor was determined from the photographs of the stage micrometer, and then six cells from each culture were measured. Surface areas were calculated as πd^2 for cocci and $2\pi r^2 + \pi d^2$ for bacilli. These calculations assume that the cells are perfect spheres or cylinders with spherical ends. While it is recognized that individual cells do not conform exactly to those equations, the calculated surface areas do provide a relative index of the actual surface areas.

Attachment experiments. Lean beef muscle or fat tissue was cut into 0.5-cm slices and stored in sterile plastic bags at −10°C. Prior to use, the slices were aseptically cut into strips (1 by 2 cm; surface area, 7 cm^2) and were thawed at room temperature. A 2-ml sample of harvested bacteria was diluted in 18 ml of phosphate buffer in a sterile beaker, and the tissue strips were inoculated in this mixture for 5 min.

The samples were aseptically transferred to 99-ml bottles of phosphate buffer at the specified time interval, and the bottles were gently inverted 25 times in a period of 15 s. The bacteria in the buffer were enumerated by the pour plate technique, and this population was described as loosely attached bacteria. The buffer was decanted, and the samples were transferred to 99 ml of phosphate buffer in stomacher bags and were homogenized for 2 min in a Stomacher 400 (Tekmar Co., Cincinnati, Ohio). The bacteria were enumerated as described above, and this population was described as strongly attached bacteria. The S_R values were calculated as (strongly attached bacteria)/(loosely + strongly attached bacteria). The S_R value essentially represents the percentage of the total bacterial population which is strongly attached.

TABLE 2. Surface areas and relative surface charges of bacteria

Bacterium	Surface area, $10^{-6}/mm^2$	ESIC	
		r/e (−)	r/e (+)
Bacillus subtilus	973.01	85.79	14.00
Escherichia coli	162.50	1.33	0.16
Listeria monocytogenes	51.42	37.73	−0.44
Salmonella typhimurium	93.49	9.47	4.78
Serratia marcescens	119.31	5.35	12.25
Staphylococcus aureus	32.77	9.57	3.02
Staphylococcus epidermidis	29.76	30.34	7.16

RESULTS

Relative hydrophobicity. Bacterial hydrophobicity varied greatly, depending on the method of measurement (Table 1). The negative HIC value for *Serratia marcescens* reflects the lack of precision of this method, since a negative value indicates that more cells were eluted than were added to the column. In addition, values in excess of 1.00 with the BATH test indicate cell lysis. Although the relative hydrophobicities as determined by each method cannot be directly compared, ranking the bacteria from most hydrophobic to least hydrophobic is a useful means of comparison. Each method of determination resulted in a completely different ranking. The best correlations between methods were for contact angle and xylene BATH (linear, $r^2 = 0.774$) and for HIC and contact angle (exponential, $r^2 = 0.809$).

Surface area and relative charges. All of the bacterial strains tested exhibited greater negative charges than positive charges, with the exception of *Serratia marcescens* (Table 2). As with HIC, negative values are a reflection of the lack of precision of the method. There was some linear correlation between total charge (positive plus negative) and surface area ($r^2 = 0.750$). *B. subtilis* had the largest surface area and greatest total charge, although *E. coli* had the second largest surface area but the least total charge.

Attachment to meat surfaces. A higher percentage (greater S_R value) of gram-positive bacteria attached to both fat and lean tissues in 5 min than of gram-negative bacteria (Table 3). A higher percentage of *Salmonella typhimurium* and *S. epidermidis* attached to lean surfaces than to fat surfaces, although the other five bacterial strains attached preferentially to fat surfaces.

Relationship between hydrophobicity, surface charge, and attachment. The correlation coefficients for attachment and hydrophobicity and surface charge (Table 4) indicate that, with the exception of BATH (hexadecane) and attachment to fat tissue, there was little apparent linear relationship between hydrophobicity and attachment. While polynomial regressions could be fitted to the data in some cases with slightly higher *r* values, graphical presentation of the data did not indicate that these were justified. The greatest correlation was seen between BATH (hexadecane) and attachment to fat tissue, with an *r* value of 0.811, after *S. aureus* had been removed from the data set (Fig. 1). The greatest correlation between hydrophobicity and attachment to lean tissue was seen with HIC, although the correlation was only 0.690. The relative negative charge as determined by ESIC correlated well with attachment to both tissue types, with *r* values of 0.885 and 0.777 for lean (Fig. 2) and fat (Fig. 3) tissue, respectively. The correlation for lean tissue dropped from 0.885 to 0.754 when *S. epidermidis* was included in the data set.

TABLE 3. S_R values of bacteria attached to lean muscle and fat tissue after 5 min

Bacterium	Gram reaction	S_R value[a] for tissue	
		Lean	Fat
Bacillus subtilus	+	0.386	0.397
Escherichia coli	−	0.118	0.183
Listeria monocytogenes	+	0.168	0.252
Salmonella typhimurium	−	0.170	0.139
Serratia marcescens	−	0.128	0.251
Staphylococcus aureus	+	0.229	0.302
Staphylococcus epidermidis	+	0.382	0.288

[a] S_R = [(strongly attached bacteria)/(loosely + strongly attached bacteria)].

TABLE 4. Correlation of hydrophobicity and surface charge with bacterial attachment to lean muscle and fat tissue

Parameter	Correlation coefficient (*r*) for tissue	
	Lean	Fat
Hydrophobicity		
Contact angle	0.477	0.247
HIC	0.690	0.098
BATH (hexadecane)	0.299 (0.040)[a]	0.811 (0.087)[a]
BATH (xylene)	0.485	0.359
Surface charge		
ESIC (+)	0.497	0.564
ESIC (−)	0.885 (0.754)[b]	0.777

[a] *r* values in parentheses include *S. aureus*.
[b] *r* value in parentheses includes *S. epidermidis*.

DISCUSSION

The HIC and ESIC data for *Salmonella typhimurium* (Tables 1 and 2) were within the range of values reported by Hermansson et al. (10), who evaluated several smooth and rough fimbriated and nonfimbriated strains of the bacterium. However, our data for *Serratia marcescens* did not correspond to their reported values. The differences could be attributed to variations between strains or to differences in the methodology. Hermansson et al. (10) used radiolabeled cells and based measurements on radioactivity retained in the gel and resins. For valid comparisons, the methodologies, particularly the physical dimensions of the columns as well as the flow rate, must be identical (19).

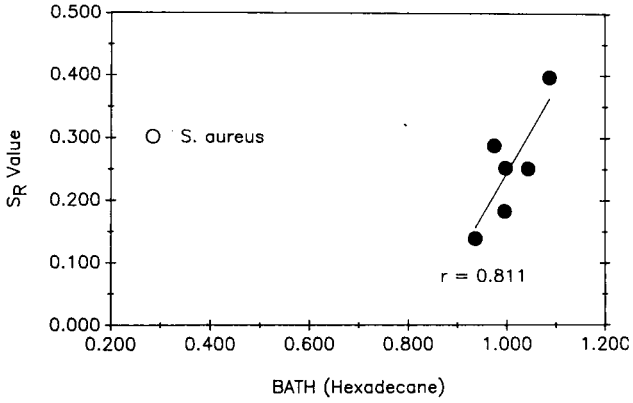

FIG. 1. Relationship between hydrophobicity (BATH hexadecane) and bacterial attachment to fat tissue.

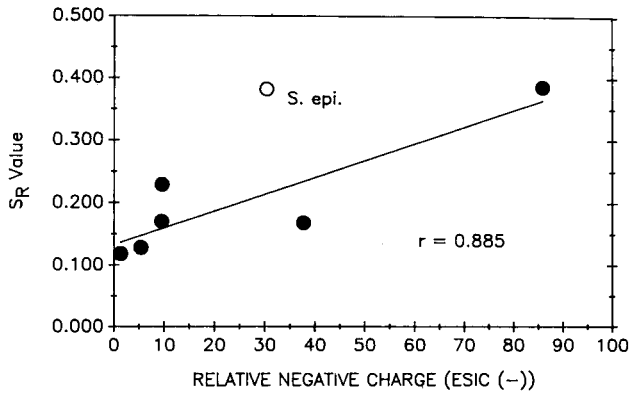

FIG. 2. Relationship between relative negative charge (ESIC −) and bacterial attachment to lean muscle surfaces. S. epi., *S. epidermidis*.

The major contributing factor to attachment to lean tissue was the net negative charge on the bacterial cell (Fig. 2). The general lack of correlation between hydrophobicity and attachment was surprising, given the information in the literature. However, the different surface charges between substrata may well account for these differences. Most of the previous work has measured attachment to surfaces with defined charges, i.e., negatively charged polystyrene (9, 26, 27). The sarcolemma of a muscle fiber is a complex of protein, mucopolysaccharide, collagen, and fibronectin (17). Because of this, the surface charge would be expected to contain both positive and negative charges in different magnitudes, in many ways similar to the surface charge on a bacterial cell wall. Therefore, there would be attraction and repulsion by the positive and negative charges on both the bacterium and the substratum. Thomas and McMeekin (24) reported that *Salmonella* spp. attached primarily to collagen fibers in chicken muscle which had been immersed in water or physiological saline, and there may be a similar selective attachment to portions of lean beef tissue.

Although fat tissue is primarily hydrophobic lipid material, this material is contained within cell membranes, which are similar to the sarcolemma. However, the method of preparation of the tissue would have resulted in rupture of some of the cells, and this lipid material may have coated much of the tissue surface. This would make the surface hydrophobic and may explain the correlation between hydrophobicity and attachment. While this does complicate the interpretation of the data, it is not unrealistic in regard to real-life situations. The rupture of the fat cells and subsequent lipid coating of surfaces commonly occur at all stages of meat processing, from the initial removal of the hide from the carcass through the final preparation into retail cuts. There was relatively little difference between the correlations of BATH and ESIC (−) with attachment, although BATH did correlate better than ESIC.

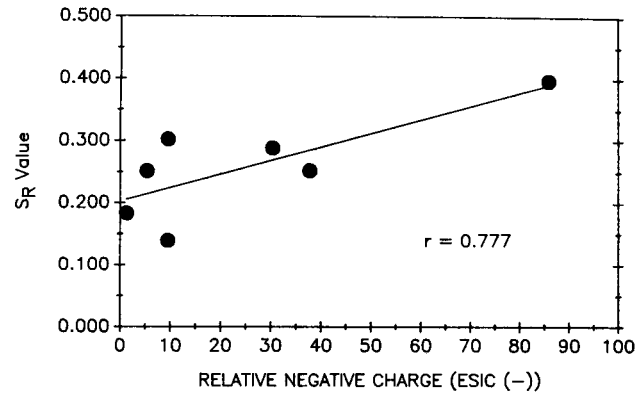

FIG. 3. Relationship between relative negative charge (ESIC −) and bacterial attachment to fat tissue surfaces.

Van Loosdrecht et al. (27) indicated that attachment increased as both negative charge (electrophoretic mobility) and hydrophobicity (contact angle) increased. Our results are similar to these in that an increase in attachment to fat tissue surfaces correlated with an increase in both negative charge (ESIC; Fig. 2) and hydrophobicity (BATH hexadecane; Fig. 3). While we could not correlate hydrophobicity with attachment to lean tissue, this may be related to the surface charge on the different substrata (polystyrene versus lean tissue).

Bacterial attachment to any surface is related to surface charges on both the cells and the substratum. The surface charges on lean muscle and fat cells are undoubtedly as complex as those on bacterial cells, and bacterial attachment to these cells is related to the interaction of these surface charges. The effects of the surface charges on substratum cells should be evaluated in terms of their effects on bacterial attachment. We have established that the magnitude of the bacterial cell surface charge is an important factor in attachment to meat.

ACKNOWLEDGMENTS

We thank Terri Alberts and Tammy Steuhm for their technical assistance with this research.

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