Comparison of Poultry Processing Equipment Surfaces for Susceptibility to Bacterial Attachment and Biofilm Formation

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ABSTRACT During processing of poultry meat products, broiler carcasses come in contact with many solid surfaces. Bacteria from the carcasses can attach to wet equipment surfaces, form biofilms, and provide a source of cross-contamination for subsequent carcasses. In this study an array of common equipment surface materials was compared for susceptibility to bacterial attachment and biofilms. To model mixed microbial populations relevant to poultry processing, samples were taken directly from the processing line and exposed to the surface materials. Whole carcasses were rinsed with phosphate-buffered saline (100 mL), and the rinse was diluted in nutrient broth. Absorbance values (412 nm) of the suspensions at varying dilutions containing test surfaces were compared hourly with controls without test surfaces. The kinetics of bacterial attachment and biofilm formation on test surfaces were determined under the influence of pH, time, and bacterial cell density, and the elemental composition of the surface materials was determined by energy-dispersive X-ray analysis. Our results showed that surfaces vary in affinity for bacterial attachment and biofilm formation. Analysis by spectrophotometry and scanning electron microscopy confirmed that attachment to stainless steel, polyethylene, and belting was not significantly different from controls. Attachment to picker-finger rubber was significantly less than attachment to stainless steel and the other surfaces. In fact, picker-finger rubber inhibits bacterial contamination. An increased understanding of bacterial attachment and biofilm formation will assist in the development of interventions to counteract these processes and, thereby, enhance plant sanitation and pathogen control.

(Key words: biofilm, bacteria, pathogen, poultry processing, stainless steel)

INTRODUCTION

Reduction of bacterial contamination of poultry products during processing is of major concern among processors and those concerned with food safety, because of the frequent incrimination of products such as chicken, turkey, and eggs in outbreaks of food-borne illness (Gibbs et al., 1978; Adams and Mead, 1983; Franco et al., 1995; Smith and Fratamico, 1995). Mechanical equipment has vastly increased the number of carcasses processed by a single plant each day. The addition of equipment to increase automation has resulted in the presentation of new surface areas for carcasses to contact repeatedly and, thus, new opportunities for bacterial attachment and cross-contamination (McEldowney and Fletcher, 1988). Understanding the conditions conducive to bacterial attachment and biofilm formation will provide important information for successful development of hazard analysis critical control point (HACCP) plans for poultry production.

Previous research has examined the attachment phenomena with pure cultures of single species of pathogens and other bacteria. In studies of the dairy processing environment, cells of Pseudomonas fragi attached and extended appendages to surfaces of stainless steel and glass (Zoltai et al., 1981). Listeria monocytogenes attached to stainless steel and other surfaces within 20 min of contact (Mafu et al., 1990). Listeria grew on stainless steel, Teflon®, nylon, and polyester for 7 to 18 d, whereas its biofilm formation was supported at 21 C but was reduced at 10 C (Blackman and Frank, 1996). Gram-negative and Gram-positive bacteria, including Pseudomonas and Klebsiella, were isolated from samples of steel, rubber, and cast iron chips positioned on floor drains and food contact surfaces in several food-processing environments (Helke et al., 1993; Zottola, 1994). Comparative studies between attached bacteria and planktonic bacteria (those not attached) showed that when microorganisms attached to surfaces, they became more resistant to the chemicals...
used for plant sanitation (Krysinski et al., 1992; Mosteller and Bishop, 1993). The tolerance for chlorine and heat treatments of *Listeria* spp. and other microorganisms in biofilms was increased after attachment (Mead and Adams, 1986; Frank and Koff, 1990; Wirtanen and Mattila-Sandholm, 1992; Oh and Marshall, 1995). *Salmonella enteritidis* cells that were attached were more than twice as resistant to heat treatment as planktonic cells (Dhir and Dodd, 1995). Attached cells of *Campylobacter jejuni*, *Escherichia coli* O157:H7, *L. monocytogenes*, and *Salmonella typhimurium* were more resistant to trisodium phosphate treatment than were their corresponding planktonic cells (Somers et al., 1994).

Substantial bacterial contamination of the poultry processing environment, (e.g., carcasses and plant surfaces) depends on the attachment over time of many different species of microbes to other microbes, debris, and inert surfaces. Bacteria are attracted to the interfaces between surfaces (Arnold and Shimkets, 1988a; Hermanowicz et al., 1995), and biofilms occur on solid surfaces in contact with liquid (Costerton et al., 1987; Zottola, 1994). Organic and inorganic material in the liquid can sediment to the solid surface. Subsequently, biologically active microorganisms will be attracted to these conditioned surfaces and adhere. When bacterial cells adhere or attach to a surface, they produce extracellular polymers that anchor the cells and provide a favorable environment for growth and subsequent attachment of more microorganisms and sediment (Steinberg and Poole, 1981; Arnold and Shimkets, 1988a). This complex community forms a biofilm.

Although past studies have provided valuable information about bacterial attachment and biofilms by using pure cultures, there is little information on the environmental conditions that enhance bacterial attachment and biofilm formation from mixed populations of bacteria that exist in a poultry processing facility. Research on the physical and chemical characteristics of bacterial attachment presents the opportunity for reduction of pathogens and spoilage organisms by preventing the formation or buildup of biofilms. Food safety could be enhanced by increasing the use of materials that prevent attachment of microorganisms prone to develop into biofilms while decreasing the use of materials that encourage bacterial attachment. Prevention of biofilm development could also reduce the use of chemicals in food plant sanitation, thereby lowering the costs to produce a safe, wholesome product.

The key to the study of biofilm formation is the detection and analysis of factors influencing production and distribution of the biofilm in the processing plant. The primary goal of this study was to compare common equipment surface materials for their susceptibility to bact-

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**FIGURE 1.** Changes in absorbance values of mixed population bacteria in broiler carcass rinses exposed to surface materials. Values for stainless steel (▲), polyethylene (●), belting (—), and the control without test material (○) were not different from each other (\( P > 0.05 \)). Values for picker-finger rubber samples (■) were less than the other surfaces (\( P < 0.05 \)) after an initial lag phase of 2 to 3 h for bacterial metabolic recovery after inoculation. Each value represents the mean of three trials.

**MATERIALS AND METHODS**

**Sample Preparation**

New York-dressed carcasses were collected from a commercial broiler processing plant, placed on ice, and transported to the laboratory. The time for transport until processing was about 30 min. Each carcass was bagged, weighed, and then rinsed with 100 mL phosphate-buffered saline. Over three trials, aerobic plate counts showed that the average number of bacteria present in the suspensions was \( 2.12 \times 10^6 \)/mL rinse. Aliquots of the rinse were diluted with nutrient broth in duplicate, 10-fold series for further testing. Test surfaces (4 cm) were materials, not previously used, which included stainless steel, conveyor belting, polyethylene, and picker-finger rubber. The surfaces were washed briefly in 1% Micro cleaning solution, rinsed in distilled water, and sonicated for 30 min before being added to test tubes containing the carcass rinse. Surfaces were added to tubes containing diluent for negative controls, and tubes with carcass rinse and no surfaces were used as positive controls. The pH was measured for all samples before and after assay. The test intervals and length of time that the test materials were held in the rinse suspensions varied for each experiment as shown in the Results section and Figures 1 to 4. Changes in turbidity were measured by spectrophotometry during these incubation times, and then the surfaces...
BACTERIAL ATTACHMENT TO PROCESSING SURFACE MATERIAL

FIGURE 2. Inhibition of microbial growth by picker-finger rubber. Absorbance values of carcass rinse suspensions containing rubber (▲) were less than the control (●) during the assay (P < 0.05). Samples were incubated at 37 C, 80 to 85% RH, to imitate conditions in a picker room.

were removed and prepared for microscopy as described below.

**Turbidimetric Assays**

Assays were performed with mixed microbial populations obtained from the whole carcass rinse. Initially, the rinses were diluted with nutrient broth for absorbance readings. Absorbance values of the carcass rinse suspensions were measured by a Beckman DU640 spectrophotometer equipped with Peltier temperature controller and auto cell holder.8 Samples were incubated at 37 C, 80 to 85% RH, during the experiments. The temperature and humidity chosen represent conditions during early processing, for example, the picker room. Prior to the experiments, wavelength scans from 350 to 750 nm at 1,200 nm/min were made of the carcass rinse suspensions to determine maximum response. During the experiments, spectrophotometric readings of the suspensions were taken at 412 nm (maximum response during the scans). Absorbance values of carcass rinse suspensions containing test surfaces were compared with values of carcass rinse suspensions without test surfaces (controls). During the course of the experiments, loss of turbidity in the suspensions was monitored to estimate reduced numbers of bacteria relative to controls. Numerical data were evaluated by the pooled-variance t-test (Shott, 1990) and ANOVA to compare the differences observed between sample and control. Probability values are given where applicable for each experiment. Differences were determined between surfaces within individual trials and for all trials combined.

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9Sigma Chemical Co., St. Louis, MO 63178.
10Tousimis Research Corp., Rockville, MD 20851.
12JEOL, USA, Peabody, MA 01960.

**Scanning Electron Microscopy**

The test surfaces used in the assay described above were examined by scanning electron microscopy (SEM). The surfaces were removed from the test tubes, rinsed with 0.1 M sodium cacodylate buffer9 to remove unattached bacteria, and fixed for 2 h at room temperature with a mixed fixative of 1% glutaraldehyde10 and 1% paraformaldehyde10 in 0.1 M sodium cacodylate buffer.10 Then, samples were rinsed in 0.1 M sodium cacodylate buffer,10 dehydrated in ascending concentrations of ethanol, critical-point dried in a Samdri 814 critical point dryer,10 mounted on aluminum stubs, and sputter-coated with gold-palladium in a Samsputter 2-A.11 The samples were examined in a JEOL 6400V SEM,12 at an accelerating voltage of 5 kV.

**Energy Dispersive X-Ray Analysis**

Test materials were analyzed for elemental composition using energy-dispersive X-ray analysis with ISIS v.3.0 software11 on a JEOL 6400V SEM.12 These samples were mounted on carbon stubs and were carbon-coated. Initial analyses at accelerating voltages of 10, 15, and 20 kV were performed to determine elements present and the best accelerating voltage for resolving the elemental peaks and minimizing charging effects. Elemental X-rays were detected by a Si(Li) spectrometer detector11 with a 7.5 mm thick beryllium window, an active area of 10 mm2, with the specimen at a 0 degree tilt angle. Subsequent elemental analyses were at 15 kV. Spectra were also collected using the thin window position of the Oxford detector11 to determine if any significant light elements could be found in trace amounts.

**RESULTS**

**Influence of pH**

Before and after each trial, the pH was measured separately for the carcass rinse suspensions, diluent, and car-

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**FIGURE 3.** Absorbance values from 10-fold series of carcass rinse dilutions after 22 h of exposure to test surfaces at 37 C. Readings for picker-finger rubber (●) were less than stainless steel (▲), control (■), or other test surfaces (not shown) for all dilutions.
cass rinse suspensions containing test surfaces. The mean pH values for suspensions with test surfaces during three trials ranged from $6.9 \pm 0.1$ to $7.1 \pm 0.2$. Individual measurements ranged from 6.8 to 7.4, but there were no significant pH changes in the assay medium after exposure to the test surfaces during the trials. At approximately 7.0, the pH values were favorable for bacterial growth throughout the experiments.

**Influence of Time**

Wavelength scans were performed separately for the carcass rinse suspensions and the suspensions containing test surfaces. The scans indicated that the maximum absorbance value occurred at 412 nm for all samples, and the initial addition of test surfaces did not cause shifts in the spectra of any of the samples. Hourly comparisons of the samples at 412 nm from zero to 6 h showed that bacterial numbers for each of the suspensions increased over time (Figure 1). An initial lag period was observed during the first several hours, as would be expected, allowing for metabolic recovery of the bacteria from the transition to the diluted media. In some experiments, the density of the stainless steel suspension exceeded that of the controls, but over five trials, the stainless steel was not significantly different from the control ($P > 0.05$). The attachment activity of polyethylene and belting was not significantly different from controls ($P > 0.05$). The absorbance values of the suspension containing the picker-finger rubber were significantly less than the absorbance values of the suspensions containing the other test surfaces ($P < 0.05$). In separate experiments (Figure 2) picker-finger rubber inhibited an increase in bacterial numbers when compared with controls every 15 min for 6 h after exposure to the rinse.

**Influence of Cell Density**

Spectrophotometric readings (absorbance values at 412 nm) of the carcass rinse suspensions containing the test surfaces were compared with controls for each of the serial, 10-fold dilutions. Initially, succeeding samples in the dilution series showed relative decreases in absorbance levels as the suspensions became correspondingly more dilute. During incubation, each of the dilutions showed relative increases in absorbance levels each hour. The original samples were diluted with media so that the increase in absorbance value was due to bacterial growth, not an increase in animal cells or dissolved organics from the carcass rinse. No evidence of these cells or materials was observed with SEM. Neither lysis nor enzyme activity seemed to be a problem in the diluted samples over the brief time of the assay. Bacterial cells remained intact when viewed by light microscopy and SEM. Figure 3 shows the absorbance readings of the dilution series for picker-finger rubber, stainless steel, and control after 22 h. The data for belting and polyethylene were omitted to simplify the graph for visual clarity, because they were not different from the control or stainless steel. Changes in cell density did not affect bacterial attachment to the

**FIGURE 4.** Differences in bacterial adherence and biofilm formation on test surfaces as shown by scanning electron microscopy. The mixed population of bacteria were present in broiler carcass rinse. Adherence was prolific on all surfaces as shown by stainless steel after 2 to 3 h (a, bar = 10 $\mu$m) except picker-finger rubber (b, bar = 3 $\mu$m). After 6 h, biofilm formation was evident on all surfaces as shown by stainless steel (c, bar = 10 $\mu$m), except picker-finger rubber (d, bar = 10 $\mu$m).
surfaces. The absorbance values of the picker-finger rubber suspension were less at the end of every time period during the experiment and were significantly less than the suspensions \((P < 0.05)\) with the other test surfaces at each of the serial dilutions.

**Confirmation by SEM**

The accumulation of bacterial cells on solid surfaces and the increase in cell density during the aggregation into a biofilm were examined with SEM. The test surfaces for SEM were duplicates of the spectrophotometry samples, i.e., they were from the same batch of surfaces. From 0 to 2 h, cells were dispersed and infrequent on all the surfaces. Figure 4 shows the differences between bacterial interaction with stainless steel and the picker-finger rubber. Cell density was highest on the stainless steel. The numbers and dispersion of bacteria on the stainless steel surface typified the other surfaces tested. After 2 to 3 h, clumps of attached cells became larger and more frequent on all of the surfaces (Figure 4a) except the picker-finger rubber (Figure 4b). The attached cells on all but the rubber were too numerous to count at the given times. Also, when cells began to clump during biofilm formation, many cells were hidden from view and could not be counted accurately. Microorganisms of various types were shown to be intimately associated with or attached to the surfaces, and the mixed population of bacterial species was readily apparent by the variation in sizes and shapes of the rods and cocci (Figure 4a). Extracellular fibrils connected many cells to each other and to the surfaces. After 6 h or more, biofilm formation was evident on all surfaces (Figure 4c) except the picker-finger rubber (Figure 4d). Most of the cells were arranged in large clumps (Figure 4c); within the clumps, most of the cells were aligned side by side. An extracellular matrix covered and obscured many individual cells. Bacteria did not exhibit attachment properties or form biofilms on the picker-finger rubber at any of the time points tested (Figure 4b,d). No bacterial cells were found on the negative controls.

**Elemental Composition of Surface Materials**

To determine whether there is any correlation between the ability of the bacteria to adhere to the surface of the test materials and the elemental composition of the material, we analyzed the materials by energy-dispersive X-ray analysis. Samples from the same batches used for the spectrophotometry and SEM were used for the X-ray analysis. Table 1 shows the elements determined for each of the test materials. The method is semi-quantitative; therefore, the order of quantity, from greatest to least, can be determined but not the exact percentages or quantity of each element.

**DISCUSSION**

Bacterial attachment to surfaces such as metals, rubber, plastics and poultry skin presents a formidable obstacle for surface sanitizing and cleaning treatments (Taylor, 1970; Wirtanen and Matilla-Sandholm, 1992). When bacterial cells initially attach to a surface, they can produce extracellular fibrils that form a complex matrix conducive to growth and subsequent attachment of more bacteria, other microbes, and debris (Steinberg and Poole, 1981; Arnold and Shimkets, 1988b). The ultimate composite is a biofilm that is resistant to cleaners and sanitizers and is extremely difficult to remove.

Identification of factors that play a role in bacterial attachment is a necessary step toward determining the relative importance to food safety of biofilms found in the poultry processing plant. Previous studies have used cultures of one or two bacterial species to test bacterial attachment and biofilm properties (Leriche and Carpenter, 1995; Wik and Breitholtz, 1996). These single or dual cultures may not behave or react like the mixed population found in a natural environment. The behavior of a single strain or species can change when mixed with a population with different traits (Arnold and Shimkets, 1988b; Okabe et al., 1995). In this work, mixed microbial populations were prepared by rinsing broiler carcasses taken directly from the processing line and used to study their formation of biofilms on equipment surface materials.

The nature and rate of bacterial attachment to solid surfaces depends upon the bacterial species, cell density, and surface properties, as well as environmental conditions such as pH and contact time (Notermans et al., 1975; Rosenberg et al., 1977; Arnold and Shimkets, 1988a; Smith, 1995). In this study, the effects of these factors on the rates of attachment to various surface materials were examined. Throughout the experiment, the controls were equivalent and stable as shown by the maxima and spectra of the wavelength scans, kinetics, and pH values. Wavelength scans showed the same maximum absorbance values for the controls and the samples containing each of the test surfaces. All samples attained maximum absorbance values within a 4-nm range, with a mean of 412 nm, suggesting that the test surfaces did not cause chemical changes that would interfere with the

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**TABLE 1. Analytical composition of elements in surface materials by x-ray analysis**

<table>
<thead>
<tr>
<th>Surface material</th>
<th>Bacterial adhesion</th>
<th>Element</th>
<th>Primary</th>
<th>Trace &lt;4%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stainless steel</td>
<td>+</td>
<td>Fe, C, Cr, F, Ni</td>
<td>Mn, Si, O</td>
<td></td>
</tr>
<tr>
<td>Belting</td>
<td>+</td>
<td>Ca, Cl, Ti, Si, S</td>
<td>Zn, Al</td>
<td></td>
</tr>
<tr>
<td>Polyethylene</td>
<td>+</td>
<td>S, Zn, Cl, Si, Fe, Cu</td>
<td>Al, Ca, Ti, K</td>
<td></td>
</tr>
<tr>
<td>Rubber</td>
<td>–</td>
<td>S, Zn, Si</td>
<td>Cl, Ti, Ca, Fe, K, Cu</td>
<td></td>
</tr>
</tbody>
</table>
spectra of the sample suspensions. Hourly comparisons of the absorbance values of the samples at 412 nm showed that, after a lag period, the turbidity of all the suspensions increased over time as would be expected from the increase in planktonic bacterial numbers due to growth, replication, and division. Likewise, an expected decrease in the absorbance values of the samples occurred in serial dilutions of the rinse, due to the decreased bacterial cell density at the beginning of the experiment. The test surfaces did not cause changes in the acidity or alkalinity of the sample liquid for the duration of the assay. Thus, differences in bacterial affinity for the surfaces could not be attributed to changes in pH. Consequently, differences in changes in the suspensions with test surfaces relative to controls without surfaces during the progress of the experiment would be expected to be generated by the interaction of the microorganisms with components of the test surfaces. Thus, fewer bacteria in the suspensions with test surfaces than in suspensions without surfaces would indicate that bacteria were attaching to the surfaces and were no longer in suspension. The bacterial attachment and formation of biofilms on the test surfaces were shown well by SEM (Figure 4a,c). However, at the concentrations of bacterial cells in this experiment, the bacterial attachment on test surfaces that were shown by SEM could not be differentiated by spectrophotometry. Spectrophotometry was useful for monitoring increases in cell numbers by turbidity and by measuring decreases for the rubber samples but was not sensitive enough to distinguish differences in bacterial attachment between samples other than the rubber.

Historically, the use of rubber fingers on mechanical pickers to remove feathers from broiler chickens after scalding has been considered a major contributor to cross-contamination (Lillard, 1986; Dodd et al., 1988). Contaminating bacteria can come from the birds, with microorganisms attached to or entrapped in the skin (McMeekin et al., 1984; Kim et al., 1993). During the defeathering process, which usually entails scalding and picking, the intact skin can be damaged, allowing bacteria to become lodged underneath and proliferate (Thomas and McMeekin, 1980; Lillard et al., 1987). The results in this study have shown that picker-finger rubber inhibits microbial contamination. The increase in absorbance values of the suspension containing picker-finger rubber was less than the control during the course of the experiment indicating inhibition of bacterial growth. Further, the kinetics of growth in the presence of the picker-finger rubber were less than for the controls or other surfaces. The SEM showed that the picker-finger rubber inhibited attachment of bacteria and biofilm formation. However, after rubber picker fingers have become worn, cracked, or covered with dirt and feces, they could provide a favorable growth substrate for bacteria in the heat and moisture of the defeathering machinery (Kim and Doores, 1993). Bacteria readily grow on organic detritus and could easily be buried in the crevices and ground into the skin or flesh of a carcass.

Surface composition can control the reactivity of the surface (Hochella et al., 1989), influencing the binding of substrates including bacterial extracellular polymers. Some of the elements in the materials that were susceptible to bacterial attachment (Table 1), such as iron, manganese, and calcium, are commonly known to enhance bacterial attachment. Sulfur and zinc, primary elements in rubber, are common antimicrobials. However, microbial adhesion cannot be explained entirely by the comparison of these elements because the polyethylene not only contained sulfur and zinc as primary elements but also appeared to be as susceptible to bacterial attachment as the belting and stainless steel tested. Some other inhibitory factor exuding from the rubber may contribute to the inhibition.

This study demonstrated biofilm development and attachment properties of a mixed population of bacteria taken from broiler carcasses on a poultry processing line. A protocol was developed to assess the kinetics of attachment of the bacteria to test samples of various equipment surface materials. A time course of the bacterial cell types and surface characteristics during the initiation and development of biofilms on the test surfaces was shown by SEM. As bacteria accumulated on the test surfaces, they exhibited an increasingly complex extracellular matrix of fibrils connecting individual cells, and many bacteria aligned side to side. Picker-finger rubber resisted bacterial attachment and inhibited bacterial growth and biofilm formation.

The use of mixed microbial populations from the poultry processing environment to study bacterial interactions with surfaces and the discovery of the inhibition of attachment and growth of bacteria by picker-finger rubber are novel. Ongoing work in an actual processing environment shows that this initial work was worthwhile and should have an impact on industry. The resistance of rubber picker fingers during processing may last even longer than expected.

Improvements are needed in the food industry for sampling biofilms and for identification, enumeration, and detection of pathogens within a biofilm. The relative importance of each species, especially pathogens, to the initiation, buildup, penetrability, and longevity of a biofilm is unknown. Understanding these processes will assist in developing methods to prevent bacterial attachment and biofilm formation, which will ultimately improve sanitation practices and pathogen control.

ACKNOWLEDGMENTS

The authors thank Manju Amin, Ruth Goldwire, Jerrie Robinson, and Allan Savage at Russell Research Center, Athens, GA 30604, for technical assistance.

REFERENCES


