APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Apr. 2003, p. 2166–2171 Vol. 69, No. 4
0099-2240/03/$08.00+0 DOI: 10.1128/AEM.69.4.2166–2171.2003

Survival of *Yersinia pestis* on Environmental Surfaces

Laura J. Rose,* Rodney Donlan, Shailen N. Banerjee, and Matthew J. Arduino

Division of Healthcare Quality Promotion, National Center for Infectious Diseases, Centers for Diseases Control and Prevention, Atlanta, Georgia 30333

Received 9 September 2002/Accepted 6 January 2003

The survival of two strains of *Yersinia pestis* (avirulent A1122 and virulent Harbin) on the surfaces of four materials was investigated. Viability was evaluated with epifluorescence microscopy by using the metabolic stain cyanoditolyl tetrazolium chloride and plate counts. Small numbers of cells suspended in phosphate buffer survived 2 to 4 h after visible drying on stainless steel, polyethylene, or glass and beyond 48 h on paper. Cells suspended in brain heart infusion broth (BHI) persisted more than 72 h on stainless steel, polyethylene, and glass. Small numbers of cells suspended in BHI were still viable at 120 h on paper. These data suggest that *Y. pestis* maintains viability for extended periods (last measured at 5 days) under controlled conditions.

Concern about use of several bacterial species as potential agents of bioterrorism has prompted the development of rapid methods for detection and emergency response capacity building. One of these potential agents is *Yersinia pestis*. In the event that *Y. pestis* is used as a terrorist weapon, either in a covert or overt manner, a response and remediation team would need to know about survival and persistence of the organism in the environment. In addition, information regarding survival of this species on surfaces in health care environments would be beneficial to health care workers. Knowledge of surface interactions, adhesion, and desiccation would assist in risk assessment and cleanup or remediation efforts.

Several early studies investigated the survival of potential human pathogens on environmental surfaces. Early work by McDade and Hall (13) demonstrated that survival of *Escherichia coli* O:126, *Morganella morganii*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, and *Salmonella enterica* serovar Derby deposited onto glass, three types of tile, and stainless steel was dependent upon relative humidity (RH). Viable cells were never recovered from surfaces at 48 h when the RH was maintained at 53%, but all strains demonstrated survival to at least 7 days when 11% RH was maintained. Nakamura (18) investigated the viability of *Shigella sonnei* on cotton, glass, wood, paper, and metal at five different temperatures. When incubated on cotton and wood at 15°C, *S. sonnei* survived for 11 to 27 days, depending upon the strain tested. If glass or metal surfaces were inoculated with *S. sonnei* and incubated at 15°C, survival was between 2 and 10 days. Methicillin-resistant *Staphylococcus aureus* survived on dry hospital mops up to 56 days (19). Three common hospital pathogens, *S. aureus*, *E. coli*, and *P. aeruginosa*, survived for 3 to 6 months on three surfaces commonly seen in health care environments (23).

Early studies of *Y. pestis* focused on the viability of airborne suspensions (11, 26). Survival of *Y. pestis* and other bacteria deposited on stainless steel coupons from airborne suspensions and held at several temperatures and humidity levels was studied by Wilkinson (30). Several factors that influence the ability of bacteria to survive desiccation include chemical and physical characteristics of the substrate material (26, 29), air temperature and RH (12–14, 30), the organism's growth phase (11), and cell surface characteristics (17, 30). Survival data for desiccation of *Y. pestis* are conflicting due to variations in experimental design, substrate, and environmental conditions (17).

The objective of this study was to assess the viability of *Y. pestis* while it was drying on four surfaces of differing characteristics that may be seen in health care, food service, or office environments—stainless steel, glass, paper, and polyethylene—as a function of time of exposure to a controlled 54 to 60% RH environment. Organisms that have been subjected to adverse conditions, such as starvation, desiccation, or treatment with disinfectants or antimicrobials, are capable of entering a viable but nonculturable state (15, 21, 22). Since desiccation of bacteria on a surface may induce such a viable but nonculturable state, making growth on laboratory media an unreliable detection method, we used cyanoditolyl tetrazolium chloride (CTC; Polysciences, Inc., Warrington, Pa.) as an epifluorescence method for detection of respiratory activity of the cells, along with total plate counts.

MATERIALS AND METHODS

Test surfaces. Coupons measuring 1 by 1 cm were prepared at the Centers for Disease Control and Prevention (CDC) from stainless steel (S-180 grade, T-304; Stewart Stainless Supply, Inc., Suwanee, Ga.), low-density polyethylene (1/16 in. thick; Curbell Plastics, Inc., Hiram, Ga.), glass (1/8 in. thick, Lillie Glassblowers, Inc., Smyrna, Ga.), and paper (brown manila envelope). The materials were analyzed for roughness and contact angle by using a profilometer (Tencor AS500; KLA-Tencor, San Jose, Calif.) and contact angle goniometer (model number 100-00; Rame-Hart, Inc., Mountain Lakes, N.J.). Standard methodology for each instrument was followed (1). Surface characteristics were also visualized by environmental scanning electron microscopy (Phillips XL30 ESEM, FEI Co., a subsidiary of Phillips, Hillsboro, Oreg.).

New stainless steel, polyethylene, and glass coupons were thoroughly cleaned with a nonbactericidal detergent (Versa-Clean; Fisher Scientific, Pittsburgh, Pa.), ultrapure, endotoxin-free reverse osmosis water (RO), and 70% ethanol. Stainless steel, glass, and paper coupons were autoclaved for 15 min at 121°C, and the polyethylene coupons were sterilized by UV light, 20 min each side (Phillips Sterilamp G36T6L, approximately 107 μW-s/cm^2), before inoculation with the bacterial suspension.

Bacterial strains and suspension preparation. *Y. pestis* A1122 (avirulent) and *Y. pestis* Harbin 35 (virulent) were grown under biosafety level 3 conditions on

* Corresponding author. Mailing address: Centers for Disease Control and Prevention, 1600 Clifton Rd., C-16, Atlanta, GA 30333. Phone: (404) 639-2161. Fax: (404) 639-3822. E-mail: lrose@cdc.gov.

TABLE 1. Surface characteristics of materials

Material	Contact angle[a] (degree)			Roughness (Ra)[b]			Roughness (TIR)[c]		
	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*
Stainless steel	92.3	6.3	15	2,248.3 Å	112.1 Å	2	1.5 μm	0.1 μm	2
Polyethylene	97.6	0.6	15	1,145.6 Å	72.6 Å	2	1.9 μm	1.7 μm	2
Glass	31.7	12.1	17	118.9 Å	0.9 Å	2	6,755.6 Å	495.4 Å	2
Paper	103.7	4.5	15	4.0 μm	0.1 μm	2	23.5 μm	1.9 μm	2

[a] As measured by goniometer, an indicator of surface free energy (surface tension).
[b] Ra, average roughness as measured by profilometer, arithmetic average deviation of the absolute value of the roughness profile from the mean line or centerline.
[c] TIR, total indicator runout. Measured by profilometer, TIR is defined as the difference between the highest and lowest points within the sampling length.

Trypticase soy agar with 5% sheep's blood (TSA II; Becton Dickinson Microbiology Systems, Cockeysville, Md.) at 26°C in a Low Temperature Biological Oxygen Demand incubator (Lab-Line Instruments, Inc., Melrose Park, Ill.) for 48 h. Cells were harvested by using a sterile swab (Dacron tip) and were suspended in 9 ml of a nonnutritive buffer, Butterfield buffer (3 mM KH_2PO_4, pH 7.2, BB; Becton Dickinson). The suspensions were vortexed within a biosafety cabinet for 1 min and were then adjusted to a 0.5 McFarland standard with a turbidity meter (Dade Behring, Inc., West Sacramento, Calif.), resulting in a suspension of 10^8 cells per ml. An additional trial was conducted by using *Y. pestis* Harbin, since it is of more concern as a pathogenic organism: cells were grown in a brain heart infusion broth (BHI; Difco, Inc.), a recommended growth medium for *Y. pestis*, for 48 h. The broth cultures were then adjusted to a 0.5 McFarland standard in BHI.

Inoculation and humidity control. A 20-μl aliquot of the suspension was placed in the center of each coupon, and the coupons were placed in a humidity chamber maintained at 54 to 60% RH for 2, 3, 4, 5, 6, 7, 24, and 48 h (six coupons per time period). The humidity was maintained by placement of a saturated solution of $MgNO_3$ inside a small desiccator cabinet (catalog no. 08-647-20; Fisher Scientific, Atlanta, Ga.) as described by Foarde et al. (9). A set of control time zero (t_0) coupons were inoculated and immediately processed, as described below, without placement in humidity chambers, to verify initial concentrations.

Recovery and viable cell counting. Three coupons were retrieved from the humidity chamber at the end of each time period and were placed in polyethylene tubes with 3 ml of BB. The coupons were alternately vortexed for 30 s and were then sonicated (FS 20, a 40-KHz sonic cleaner; Fisher Scientific) for 30 s; the cycle was repeated two more times. Preliminary tests were conducted to confirm that sonication did not reduce viable cell counts. The coupons were removed from the BB with sterile forceps and were placed inoculated side up in sterile petri dishes. Three 100-μl aliquots of BB supernatant were removed and spread on each of two plates of TSA II. A fourth 100-μl aliquot was used for serial dilutions; each dilution was plated in duplicate (100 μl for each plate) onto TSA II.

Staining and fixation. The remaining 2.6-ml BB with dislodged cells was vacuum filtered through a black polycarbonate 25-mm, 0.2-μm-pore-size membrane (Millipore Corp., Bedford, Mass.). The membranes were transferred to absorbent pads saturated with CTC (Polysciences, Inc., Warrington, Pa.) dissolved in 50% Reasoners 2 agar (R_2A) broth without phosphate (0.25 g each of yeast extract, proteose peptone no. 3, Casamino Acids, and dextrose; 0.15 g of sodium pyruvate; and 0.025 g of $MgSO_4$ per liter) as described by Pyle et al. (20). The coupons were flooded with 1.25 mM CTC and were dissolved in 50% R_2A broth without phosphate. The coupons and membranes were allowed to incubate at room temperature with the CTC-R_2A for 2 h. The membranes were lifted, and 100 μl of 37% formaldehyde was added to the absorbent pads, and the membranes were replaced and the cells on the membranes were fixed for 15 min. The membranes were placed back on the filter apparatus, and 2 ml of 10-μg/ml 4′,6-diamidino-2-phenylindole (DAPI; Sigma-Aldrich, St. Louis, Mo.) was placed on top of the membranes and allowed to incubate at room temperature for 15 min before removal by vacuum filtration. The membranes were allowed to dry and stored overnight at 5°C. The coupons were tilted to remove the CTC-R_2A, 100 μl of 5% formaldehyde was dropped on the surfaces, and any adhered cells were fixed for 15 min. The coupons were tilted to remove the 5% formaldehyde and were then incubated 15 min with 100 μl of DAPI. The DAPI was removed, and the coupon was dipped in sterile RO water, allowed to dry, and stored overnight at 5°C.

Extended survival confirmation. The limit of detection for the enumeration of viable cells by epifluorescence microscopy on membranes was 4×10^2 cells; therefore, the three remaining inoculated coupons were processed for spread plate recovery when low numbers of viable cells were expected. The three coupons were placed into individual polyethylene tubes with 3 ml of BB and were alternately vortexed and sonicated for 30 s each; the cycle was repeated three times. The coupons were removed from the tubes with sterile forceps and were placed in Trypticase soy broth (BBL Inc.), incubated at 26°C. The tubes were examined visually for characteristic growth over the next 7 days, and a 100-μl aliquot was plated to TSA II at day 7. Serial dilutions (1:10) of the recovered cells in BB were performed as needed, or if low viable counts were expected, the entire 3-ml volume was plated (0.5 ml per plate) directly on TSA II and incubated at 26°C. Colonies were counted after 3 days and again after 5 days, and results were recorded in numbers of CFU.

Epifluorescence microscopy and enumeration. The membranes were mounted on microscope slides with low fluorescence immersion oil and were observed directly without a coverslip. A Zeiss epifluorescence microscope (Zeiss, Thornwood, N.Y.) was used to observe and obtain direct counts of the viable and total bacteria on the membranes and those remaining on the coupons by counting cells within a field defined by an ocular grid. Twenty fields were enumerated if the counts were 50 or greater per field. When low numbers of viable cells were observed, as many as 100 fields were counted. It was determined that the area of the membrane was equivalent to the area of 4×10^4 fields. The level of detection was calculated on the basis of 1 viable cell in 100 fields (1 cell/100 fields $\times$ 4×10^4 fields/membrane = 400 cells per membrane). The microscope was fitted with light filters for DAPI (Zeiss filter set 702) or CTC (Zeiss filter set 709).

Electron microscopy. Paper coupons with dried bacteria (dried 5 days at 55% RH) were placed in a sealed desiccator with a vial containing 1.0 ml of osmium tetroxide, and the desiccator was placed in a chemical fume hood. The samples were exposed to the osmium vapors at room temperature for 24 h. The coupons were mounted on aluminum stubs with silver paint, sputter coated with 25 nm of gold, and observed with a Phillips XL 30 ESEM (FEI Co., a subsidiary of Phillips, Hillsboro, Oreg.).

Statistical analysis. The data were transformed to the $\log_{10}$ scale for smoothing and analysis purposes. To account for some zero values, we added 1 to all data points before converting to $\log_{10}$ scale. The mean and standard deviation of logarithmic CFU for all the coupons for each combination were calculated. Bar charts and *xy* plots were drawn to show these mean values and their dispersion over time. Shapiro-Wilk statistic W or the Kholmogorov D statistic was used to test the normality of the data. Mean values of $\log_{10}$ (CFU + 1) were compared (24) to test if they had significantly changed over time or over any other combination of parameters (i.e., suspension media or substrate material). Student's *t* test or Wilcoxon's rank sum test was appropriately used to compare the means (SAS/STAT User's Guide, version 8, SAS Institute, Cary, N.C.).

RESULTS

The surface properties of the materials employed in these tests were characterized as shown in Table 1. These are characteristic properties for the materials. Glass is the smoothest, as indicated by the Ra roughness value (118.9 Å) and also the most hydrophilic, as indicated by the lowest contact angle (31.7°). Paper was the roughest and most hydrophobic (Ra, 4.0 μm; and contact angle, 103.7°).

The survival times of *Y. pestis* A1122 in BB on stainless steel can be seen in Fig. 1. Similar results were seen for *Y. pestis* A1122 on polyethylene and glass (results not shown). Fibers from paper coupons interfered with acquiring accurate counts of CTC-stained cells recovered from paper. Actively respiring

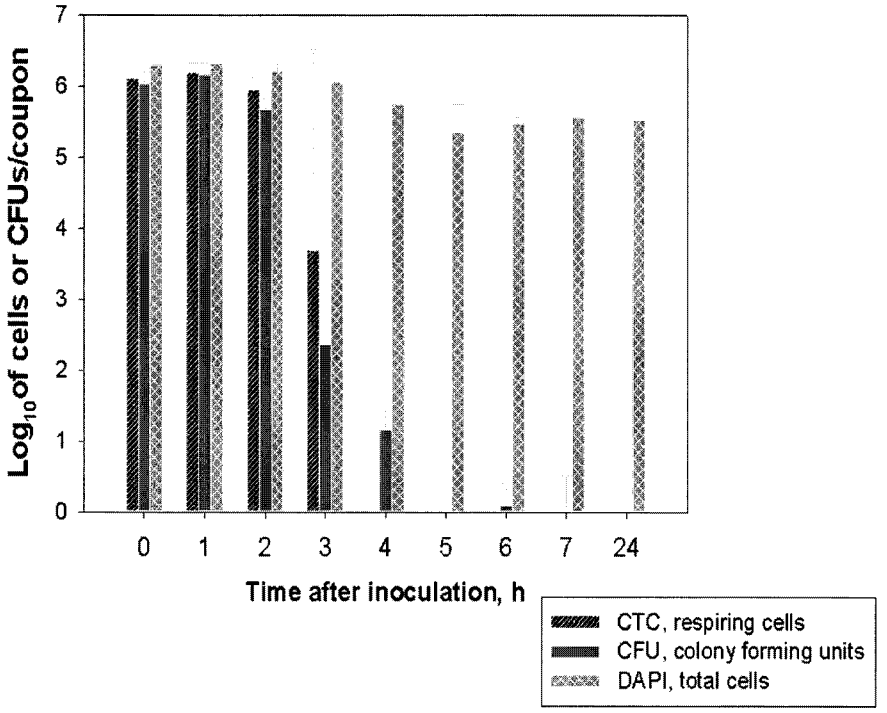

FIG. 1. Survival of *Y. pestis* A1122 on stainless steel over time. *y* axis numbers represent $\log_{10}$ cells as counted under epifluorescence microscopy (CTC- and DAPI-stained cells, mean of three to nine coupons) or in CFU (mean of 3 to 18 coupons).

cells (CTC positive) were not detected (limit of detection, 10^2/coupon) after 3 h. Low numbers of CFU were detected after 4, 6, and 7 h. The total number of cells (DAPI stained) on the coupon surface fluctuated less than 1 log during the entire 24-h period, indicating that significant numbers of cells remained even though none was viable after 7 h. Because the level of detection of the CTC assay did not allow enumeration of low numbers of cells, this assay was not continued for *Y. pestis* Harbin.

The viability of both strains in BB remained fairly constant for the first 2 h after inoculation on polyethylene and paper ($P > 0.1$) (Table 2). Inocula began to dry on these coupon surfaces between 2.5 and 3.0 h. If the inoculum was visibly wet, the viability was not statistically different from that at t_0. The variability in drying time is reflected in the high standard deviation values after the 3-h period. This is more pronounced for the avirulent A1122 strain (Table 2). After 3 h, the number of viable organisms declined rapidly. Although low numbers of both strains (0.1 to 1.8 $\log_{10}$) were able to survive 6 h after inoculation (3 h after the inoculum appeared visibly dry), by 24 h most stainless steel, polyethylene, and glass coupons contained only nonviable cells. Paper coupons, however, demonstrated an ability to support the organism more than 48 h when suspended in BB (Table 2). *Y. pestis* A1122 inoculated on paper retained 100 viable cells after 48 h, a 4-log reduction from the time zero concentration (Table 2). *Y. pestis* Harbin on paper was less robust in BB than the avirulent A1122 strain, having a 6-log reduction within 24 h with no viable cells remaining at 48 h (Table 2).

Survival of *Y. pestis* Harbin was enhanced when the bacteria

TABLE 2. Recovery of viable *Y. pestis* in phosphate buffer at 55% relative humidity as measured in number of CFU

Time[a] (h)	Mean $\log_{10}$ CFU/coupon (SD) for different surfaces and *Y. pestis* strains (n = 3–9)							
	Stainless steel		Polyethylene		Glass		Paper	
	A1122	Harbin	A1122	Harbin	A1122	Harbin	A1122	Harbin
0	6.05 (0.32)	6.32 (0.11)	6.27 (0.30)	6.59 (0.12)	6.40 (0.14)	6.28 (0.21)	6.00 (0.40)	6.60 (0.05)
2	5.67 (0.16)	6.29 (0.19)	6.14 (0.13)	6.89 (0.16)	4.89 (2.05)	6.24 (0.14)	6.17 (0.22)	6.57 (0.08)
3	2.37 (2.77)	6.39 (0.12)	5.43 (2.06)	6.25 (0.40)	3.26 (2.43)	2.25 (0.65)	6.33 (0.23)	6.50 (0.14)
4	0.79 (0.92)	0.19 (0.45)	0.44 (0.71)	2.79 (0.55)	1.35 (1.21)	1.69 (0.17)	4.64 (0.51)	5.18 (0.34)
5	0.22 (0.50)	0.00 (0.00)	0.72 (1.12)	2.35 (0.32)	1.29 (1.13)	1.14 (0.60)	3.96 (0.21)	5.64 (0.26)
6	0.08 (0.26)	0.11 (0.32)	0.94 (0.86)	1.81 (0.34)	0.00 (0.00)	1.66 (0.88)	3.29 (0.67)	4.00 (0.25)
7	0.29 (0.60)	0.00 (0.00)	0.00 (0.00)	1.55 (0.36)	0.00 (0.00)	0.93 (0.61)	3.34 (0.25)	3.52 (0.13)
24	0.00 (0.00)	0.00 (0.00)	0.04 (0.17)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	2.81 (0.57)	0.65 (0.87)
48	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	1.99 (0.46)	0.00 (0.00)

[a] Time since inoculation.

TABLE 3. Recovery of viable *Y. pestis* Harbin in BHI at 55% RH as measured in number of CFU

Time[a] (h)	Mean $\log_{10}$ CFU/coupon (SD) (n = 3–9) for different materials			
	Stainless steel	Polyethylene	Glass	Paper
0	5.81 (0.19)	5.73 (0.20)	5.83 (0.19)	5.78 (0.16)
24	3.30 (0.10)	2.36 (0.60)	2.37 (0.47)	2.74 (0.88)
48	2.17 (0.25)	1.37 (1.00)	1.19 (0.55)	2.70 (0.50)
72	1.42 (0.43)	0.74 (0.92)	0.35 (0.52)	2.06 (0.35)
96	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.57 (0.61)
120	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.09 (0.27)

[a] Time since inoculation.

were grown in BHI and inoculated onto materials while suspended in BHI. Table 3 shows that over 100 viable organisms survived on paper after 72 h when grown in BHI but that less than 10 organisms remained at 24 h when suspended and inoculated in BB (Table 2). Some *Y. pestis* Harbin also survived on polyethylene and stainless steel past 72 h (Table 3) when grown and inoculated in BHI, but none were able to survive 24 h when suspended in BB.

The avirulent strain A1122 survived desiccation significantly better than did the Harbin strain when suspended in BB and then inoculated onto stainless steel (at 7 h, $P < 0.01$) and paper (at 24 and 48 h, $P < 0.01$). The *Y. pestis* Harbin strain survived desiccation better than did the A1122 strain on glass when suspended in BB (at 7 h, $P < 0.01$). The strains showed no statistically significant difference in surviving desiccation when inoculated on polyethylene (at 24 h, $P = 0.39$).

Scanning electron micrographs of the paper coupons (Fig. 2) show the paper fibers and a substance strung between the fibers, which is most likely a sizing, a common paper treatment to improve rigidity and water resistance of the paper. Figure 3 is a scanning electron micrograph of a suspension of *Y. pestis* Harbin in BHI that was inoculated onto paper and allowed to dry at 55% RH 4 days.

DISCUSSION

Comparison of the two viable cell assays was as expected, with CTC-enumerated cells being equivalent or slightly higher than CFU counts on stainless steel at t_0 and 1, 2, and 3 h. If the CTC-stained cells remain equivalent or slightly higher at t_4 and later, they would not be seen, since the level of detection was determined to be 400 cells per membrane. The total cell counts (DAPI stained) decreased less than 1 log over the 24-h time period; this decrease may be attributed to the aerosolization of some cells during transfer from the chamber to the tube, lysis and fragmentation of cells upon desiccation, and adherence of some cells to the coupon. The coupons were stained and observed for adhered cells, but enumeration was difficult, since the remaining cells were deposited in a ring at the edge of what was the drop of inoculum and were not evenly distributed, as was needed for accurate counting.

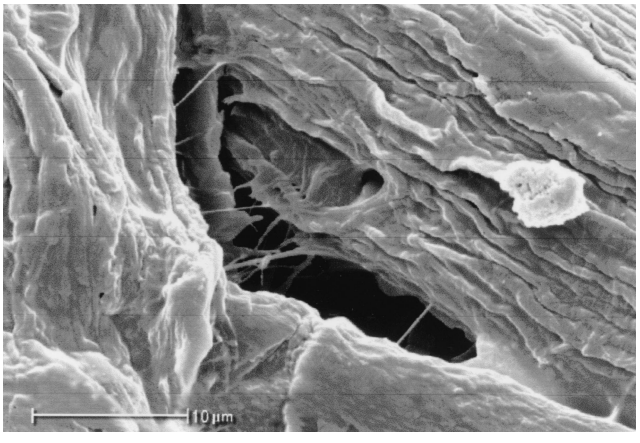

FIG. 2. Paper coupon without bacteria.

Two strains of *Y. pestis* (A1122 and Harbin) demonstrated a significantly greater survival capability on paper than on the other materials tested in this study. The physical properties of each material, such as free energy and electrostatic charges, may contribute to these differences. The interactions between substrate surfaces and bacterial surfaces are complex and still being explored, but bacterial surfaces and substrate properties do influence bacterial adhesion and colonization (3, 7, 8). These properties may also influence survival.

The contact angle is one indicator of free energy and hydrophobicity of the material. Attachment occurs more readily to hydrophobic materials, such as polyethylene (8), than to hydrophilic substances, such as glass. Studies of biofilm development have shown that attachment to surfaces can induce expression of genes related to morphology (3), synthesis of proteins (2), and synthesis of extracellular substances, such as alginate or polysaccharides (4, 5). Other factors, such as nutrient availability, high osmolarity, and oxygen tension, can also trigger production of extracellular substances that can enhance attachment to surfaces (4). Increased survival of *Y. pestis* on paper may be explained by the high contact angle, indicating initial hydrophobicity, and the high roughness values seen in Table 1. The deep peaks and valleys between paper fibers may provide a microenvironment for bacteria to be protected from desiccation. Paper may also contain a sizing for increased rigidity and water resistance. Sizing can be synthetic, but it often is a starch made from wheat, corn, rice, or potato. A starch sizing, seen in Fig. 2 as strands between the larger paper fibers, would provide some nutrients for the bacteria, allowing for extended longevity.

The rapid decline of A1122 on glass when suspended in BB is likely due to the low contact angle and low roughness of the material. The inoculum was able to spread over a larger surface area, allowing more rapid drying. In addition, the high-energy, hydrophilic properties of the glass were not conducive to bacterial adhesion, and therefore, survival could not be enhanced by the mechanisms seen in biofilm development upon attachment, such as polysaccharide or alginate production.

Variability in survival exists among strains of the same species on different surface materials. *Y. pestis* Harbin survived longer than *Y. pestis* A1122 on glass, while *Y. pestis* A1122 survived longer than *Y. pestis* Harbin on paper and stainless steel. The A1122 strain is a high-passage laboratory reference strain that has lost the 70-kb calcium dependent V plasmid for virulence from repeated lab transfers. This repeated laboratory passage may also have allowed adaptation to survive a wider range of conditions.

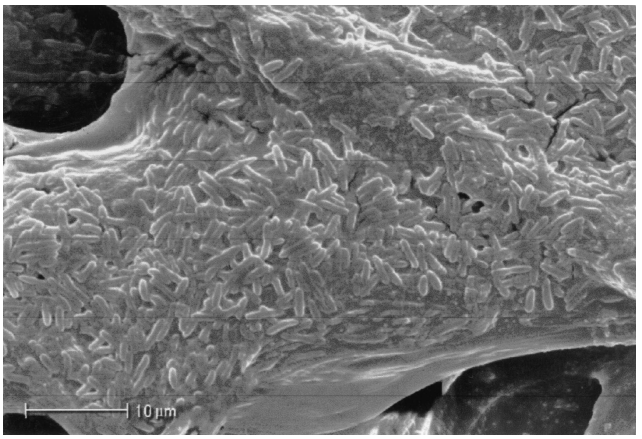

FIG. 3. *Y. pestis* Harbin dried on paper coupon for 5 days at 55% RH.

To compare the results of this study to a previous similar study, Wilkinson (30) reported a 4-log reduction in viable cells of *Y. pestis* A1122 suspended in broth in 5.3 h when held at 52% RH. The present study reports a 5.8-log reduction in viable cells *Y. pestis* A1122 suspended in BB but only a 2.5-log reduction of *Y. pestis* Harbin suspended in broth in 24 h on stainless steel. Wilkinson used a similar broth (heart infusion broth from Difco) for growth and atomizing fluid. Wilkinson's work (30) differed in that the cells were suspended in broth and deposited by aerosolization and settling within a chamber. The differences in survival times between the two studies may be due to the strains used, methods of deposition, types of stainless steel, or the slight differences in temperatures. A large difference was observed in survival of the organisms at 30°C (4-log reduction in 14 min) from that at 22°C (4-log reduction in 5.3 h) in Wilkinson's work. The present study's chambers were held at a room temperature that varied between 18 and 22°C. The method of deposition in this study may account for the longer survival times. Organisms in a droplet would stay moist and protected longer that cells that are separated from each other immediately, as would occur in creating an aerosol. Small droplets of cells would allow more uniform, quicker drying of cells than if deposited in a 20-μl drop on a surface.

This experiment was conducted at 55% RH. Other studies have reported that the most rapid decline in viability occurs at higher levels of RH (12–14, 26). Wilkinson (30) reported that *Pasteurella* (*Y.*) *pestis* A1122, when deposited by aerosolization in growth media on metal surfaces, survived more than 3 days at 11% RH but less than 2 days at 100% RH. Webb (26) proposed that water bound to proteins in the cell membrane was removed more quickly in a low-humidity environment, allowing less distortion of the protein structure and, therefore, less damage to the cell membrane. Slow drying as occurs in higher RH environments allows distortion of membrane protein structure. Variability in membrane protein content of different species and strains may explain the variability in response to desiccation. Death of bacteria by desiccation when in an airborne state is influenced by addition of amino acids and protein derivatives to the suspension. Webb (27) found that addition of Bacto Peptone, proteose peptone, and Bacto Protone reduced death rates of *Serratia marcescens* when aerosolized. He reasoned that compounds that could replace water molecules associated with proteins bound to cell membranes would enhance survival during desiccation (27, 28). This may be a factor in the enhanced survival of *Y. pestis* Harbin suspended in BHI, which survived at least two additional days on all four materials tested.

All tests in this study were conducted on cells grown 2 days on TSA II at 26°C. Under these growth conditions, we believe that the resulting suspension would be a mixture of cells in log-phase and stationary-phase growth. Goodlow et al. (11) demonstrated that cells in the lag phase of growth are more resistant to desiccation stress (in aerosol) than are those in log-phase growth.

Since the reported airborne infective dose of *Y. pestis* is estimated to be 100 to 20,000 organisms (6, 10, 25), a paper inoculated with *Y. pestis* and held 5 days under humidity ranges similar to those of this study may still pose a threat of human infection. This study did not address reaerosolization of the organism after drying or if the organisms retain their virulence factors after 24 to 72 h. Some studies have shown that chlorine-stressed pathogenic organisms lost or reduced their virulence while still viable (16). Animal testing would be required to determine if the Harbin strain still retained its infectiousness after drying.

ACKNOWLEDGMENTS

This work is the result of collaboration with Richard Meyer, Bioterrorism Preparedness Response Program, National Center for Infectious Diseases, CDC. We extend our gratitude to Janice Carr, Department of Healthcare Quality Promotion, National Center for Infectious Diseases, CDC, for her invaluable scanning electron microscope work and to Nicholas Pyenson, Emory University, Atlanta, Ga., for his laboratory assistance.

Use of trade names and commercial sources is for identification purposes only and does not imply endorsement by the Public Health Service of the Department of Health and Human Services.

REFERENCES

1. **American Society of Mechanical Engineers Standards Committee B46.** 1996. ASME B46.1–1995. Surface texture (surface roughness, waviness, and lay): an American national standard. American Society of Mechanical Engineers, New York, N.Y.
2. **Brozel, V. S., G. M. Strydom, and T. E. Cloete.** 1995. A method for the study of *de novo* protein synthesis in *Pseudomonas aeruginosa* after attachment. Biofouling **8:**195–201.
3. **Dalton, H. M., L. K. Poulsen, P. Halasz, M. L. Angles, A. E. Goodman, and K. C. Marshall.** 1994. Substratum-induced morphological changes in a marine bacterium and their relevance to biofilm structure. J. Bacteriol. **176:** 6900–6906.
4. **Davies, D. G., and G. G. Geesey.** 1995. Regulation of the alginate biosynthesis gene *algC* in *Pseudomonas aeruginosa* during biofilm development in continuous culture. Appl. Environ. Microbiol. **61:**860–867.
5. **Davies, D. G., A. M. Chakrabarty, and G. G. Geesey.** 1993. Exopolysaccharide production in biofilms: substratum activation of alginate gene expression by *Pseudomonas aeruginosa*. Appl. Environ. Microbiol. **59:**1181–1186.
6. **Ehrenkranz, N. F., and K. F. Meyer.** 1955. Studies on immunization against plague. VIII. Study of three immunizing preparations in protecting primates against pneumonic plague. J. Infect. Dis. **96:**138–144.
7. **Fletcher, M.** 1996. Bacterial attachment in aquatic environments: a diversity of surfaces and adhesion strategies, p. 1–24. *In* M. Fletcher (ed.), Bacterial adhesion, molecular and ecological diversity. Wiley-Liss, Inc., New York, N.Y.
8. **Fletcher, M.** 1980. Adherence of marine microorganisms to smooth surfaces, p. 345–374. *In* E. H. Beachey (ed.), Bacterial adherence, receptors and recognition, ser. B, vol. 6. Chapman & Hall, London, United Kingdom.
9. **Foarde, K., E. Cole, D. Van Osdell, D. Bush, D. Franke, and J. Chang.** 1992. Characterization of environmental chambers for evaluating microbial growth on building materials, p. 185–189. *In* IAQ 92 environments for people. Proceedings of the American Society for Heating, Refrigeration and Air-Conditioning Engineers, Inc., Atlanta, Ga.

10. **Franz, D. R., P. B. Jahrling, A. M. Frielander, D. J. McClain, D. L. Hoover, and W. R. Bryn.** 1997. Clinical recognition and management of patients exposed to biological warfare agents. JAMA **278:**399–411.
11. **Goodlow, R. J., and F. A. Leonard.** 1961. Viability and infectivity of microorganisms in experimental airborne infection. Bacteriol. Rev. **25:**182–187.
12. **Lidwell, O. M., and E. J. Lowbury.** 1950. The survival of bacteria in dust. II. The effect of atmospheric humidity on the survival of bacteria in dust. J. Hyg. **48:**21–27.
13. **McDade, J. J., and L. B. Hall.** 1964. Survival of gram-negative bacteria in the environment. I. Effect of relative humidity on surface-exposed organisms. Am. J. Hyg. **80:**192–204.
14. **McDade, J. J., and L. B. Hall.** 1963. Survival of Staphylococcus aureus in the environment. I. Exposure on surfaces. Am. J. Hyg. **78:**330–337.
15. **McFeters, G. A., F. P. Yu, B. H. Pyle, and P. S. Stewart.** 1995. Physiological assessment of bacteria using fluorochromes. J. Microbiol. Methods **21:**1–13.
16. **McFeters, G. A., and M. W. Chevallier.** 2000. Chemical disinfection and injury of bacteria in water, p. 255–275. *In* R. R. Colwell and D. J. Grimes (ed.), Nonculturable microorganisms in the environment. ASM Press, Washington, D.C.
17. **Mitscherlich, E., and E. H. Marth.** 1984. Microbial survival in the environment: bacteria and rickettsiae important in human and animal health. Springer-Verlag, New York, N.Y.
18. **Nakamura, M.** 1962. The survival of Shigella sonnei on cotton, glass, wood, paper, and metal at various temperatures. J. Hyg. **60:**35–39.
19. **Oie, S., and A. Kamiya.** 1996. Survival of methicillin-resistant *Staphylococcus aureus* (MRSA) on naturally contaminated dry mops. J. Hosp. Infect. **34:** 145–149.
20. **Pyle, B. H., S. C. Broadaway, and G. A. McFeters.** 1995. Factors affecting the determination of respiratory activity on the basis of cyanoditolyl tetrazolium chloride reduction with membrane filtration. Appl. Environ. Microbiol. **61:** 4304–4309.
21. **Rollins, D. M., and R. R. Colwell.** 1986. Viable but nonculturable stage of *Campylobacter jejuni* and its role in survival in the natural aquatic environment. Appl. Environ. Microbiol. **52:**531–538.
22. **Roszak, D. B., and R. R. Colwell.** 1987. Metabolic activity of bacterial cells enumerated by direct viable count. Appl. Environ. Microbiol. **53:**2889–2893.
23. **Smith, S. M., R. H. Eng, and F. T. Padberg, Jr.** 1996. Survival of nosocomial pathogenic bacteria at ambient temperature. J. Med. **27:**293–302.
24. **Snedecor, G. W., and W. G. Cochran.** 1989. Statistical methods, 8th ed. Iowa State University Press, Ames.
25. **Speck, R. S., and H. Wolochow.** 1957. Studies on the experimental epidemiology of respiratory infections. VIII. Experimental pneumonic plague in Macacus rhesus. J. Infect. Dis. **100:**58–68.
26. **Webb, S. J.** 1959. Factors affecting the viability of airborne bacteria. I. Bacteria aerosolized from distilled water. Can. J. Microbiol. **5:**649–669.
27. **Webb, S. J.** 1960. Factors affecting the viability of airborne bacteria. II. The effect of chemical additives on the behavior of airborne cells. Can. J. Microbiol. **6:**71–87.
28. **Webb, S. J.** 1960. Factors affecting the viability of airborne bacteria. III. The role of bonded water and protein structure in the death of airborne cells. Can. J. Microbiol. **6:**89–105.
29. **Webb, S. J.** 1963. The effect of relative humidity and light on air-dried organisms. J. Appl. Bacteriol. **26:**307–313.
30. **Wilkinson, T. R.** 1966. Survival of bacteria on metal surfaces. Appl. Microbiol. **14:**303–307.