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FEATHER-DEGRADING BACTERIA DO NOT AFFECT FEATHERS ON CAPTIVE BIRDS

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Abstract.—Attention has recently been focused on microbes that occur in the plumage of wild birds and can degrade feathers under laboratory conditions and in poultry-waste composters. In particular, *Bacillus licheniformis*, a soil bacterium, was found in the plumage of many birds netted in eastern North America, and poultry feathers were rapidly broken down when incubated in a suspension of this bacterium (Burtt and Ichida 1999). If feather-degrading microbes affect wild birds under normal conditions, they may have played an important role in the evolution of molt, plumage color, and sanitation behavior, such as sunning and preening. We performed the first test on whether a feather-degrading bacterium can degrade feathers of live birds housed outdoors under seminatural conditions. We found no evidence that *B. licheniformis* degraded wing feathers of Northern Cardinals (*Cardinalis cardinalis*) when applied twice (with a two-week interval) during the winter, despite the fact that it degraded Northern Cardinal feathers when incubated in our laboratory. In a second experiment, we found no evidence that *B. licheniformis* degraded feathers of European Starlings (*Sturnus vulgaris*) when applied twice (with a one-week interval) during the summer, despite the fact that birds were housed in humid conditions that should have favored the growth of *B. licheniformis*. *Received 16 February 2003, accepted 12 September 2004.*

Key words: *Bacillus licheniformis*, *Cardinalis cardinalis*, European Starling, feather-degrading bacteria, keratin, Northern Cardinal, plumage, *Sturnus vulgaris*.

Las Bacterias que Degradan Plumas no Afectan las Plumas de Aves en Cautiverio

Resumen.—Recientemente se ha prestado atención a los microbios que habitan en el plumaje de las aves silvestres y que pueden degradar las plumas bajo condiciones de laboratorio y en lugares de descomposición de los desechos de criaderos de aves de corral. En particular, *Bacillus licheniformis*, una bacteria del suelo, fue hallada en el plumaje de varias aves atrapadas con redes en el este de América del Norte, y plumas de aves de corral que fueron incubadas en una suspensión de esta bacteria se degradaron rápidamente (Burtt and Ichida 1999). Si los microbios degradadores de plumas afectan a las aves silvestres bajo condiciones normales, éstos pueden haber jugado un rol importante en la evolución de la muda, el color del plumaje y los comportamientos sanitarios como los baños de sol y el acicalamiento. Realizamos una primera prueba para determinar si las bacterias pueden degradar plumas de aves vivas mantenidas a la intemperie en condiciones semi-naturales. No encontramos evidencia de que *B. licheniformis* degradara las plumas del ala de *Cardinalis cardinalis* tras ser aplicada dos veces durante el invierno (con un intervalo de dos semanas), a pesar de que degradó las plumas de *C. cardinalis* cuando fue incubada en nuestro laboratorio. En un segundo experimento, no encontramos evidencia de que *B. licheniformis* degradara plumas de

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Sturnus vulgaris tras ser aplicada dos veces durante el verano (con un intervalo de una semana), a pesar de que las aves fueron albergadas en condiciones húmedas que deberían haber favorecido el crecimiento de *B. licheniformis*.

The recent report that the soil bacterium *Bacillus licheniformis* degrades feathers in poultry waste (Williams et al. 1990), and the subsequent discovery that this bacterium is present in the plumage of many North American birds and rapidly degrades poultry feathers in the laboratory (Burtt and Ichida 1999), raises the question of whether this and other keratin-degrading microbes have any effect on the feathers of live birds. Burtt and Ichida (1999) found fewer incidences of birds with feather-degrading microbes after prebasic molt, which suggests the possibility that avian molt functions, in part, to reduce the load of feather-degrading microbes, and that those microbes could have played a role in the evolution of molt. In addition, they reported that birds in contact with the ground or water were more likely to test positive for feather-degrading microbes, which is consistent with the idea that plumage may be contaminated by soil-living feather-degrading microbes that then proliferate during warm, moist episodes. Of the many interesting effects feather-degrading microbes may have on avian behavior and evolution, perhaps the most provocative suggestion thus far is that feather melanin functions to slow degradation by microbes, and that Gloger's Rule (darker birds in warm, humid climates) may be the result of increased selection for resistance to feather-degrading microbes in habitats more favorable to microbes (Burtt 1999, Goldstein et al. 2004).

Although there are many reasons to suspect that feather-degrading microbes play an important ecological role in the lives of birds, the fact that the best-known of those organisms, *B. licheniformis*, requires high humidity and temperature (~45°C) to thrive raises the question of whether the plumage of live birds provides a suitable habitat. The present study is the first attempt to find out whether the feather-degrading bacterium *B. licheniformis* can degrade feathers *in situ*.

Methods

We captured wild birds, applied a concentrated suspension of feather-degrading bacteria to their wing feathers, and allowed the bacteria time in which to degrade the feathers in an outdoor aviary. Before releasing the birds, we removed the treated feathers and a number of antibiotic-treated or saline-treated control feathers for examination at high magnification. Experiment 1 was carried out approximately four months after the prebasic molt, under cold and dry conditions. Experiment 2 was carried out just before the prebasic molt, under warm, humid conditions. The other important differences between the experiments were that in experiment 1, we used Northern Cardinals (*Cardinalis cardinalis*) and allowed bacteria four weeks in which to degrade feathers; whereas in experiment 2, we switched to the more darkly pigmented European Starling (*Sturnus vulgaris*) and allowed bacteria only two weeks in which to degrade feathers, because conditions were more favorable for bacterial growth. Another difference in design was that in experiment 1, control feathers came from the same birds as the bacteria-treated and antibiotic-treated feathers; whereas in experiment 2, we attempted to reduce the chance of contamination by using different birds as controls. Otherwise, methodological differences were slight and are noted in the description of experiment 2 below.

Experiment 1.—The experiment was carried out in winter, approximately four months after the prebasic molt, at the time of year when feather-degrading bacteria are most likely to be found in the plumage of wild birds (Burtt and Ichida 1999). We captured 16 Northern Cardinals using mist nets and treadle traps in an early-successional deciduous forest at the edge of the College of William and Mary campus, Williamsburg, Virginia, between 24 October and 16 December 2001. Birds were acclimatized in a large outdoor cage (3.0 m [length] × 7.2 m [width] × 2.1 m [height]). Four birds, all adult males, died of injuries almost immediately, probably because of overcrowding of this aggressive species in the acclimatization cage. The remaining four males (two hatch-year, two adult) and eight females (four hatch-year, four adult) were maintained throughout the study on an *ad libitum* diet of safflower (*Carthamus tinctorius*) and sunflower

(*Helianthus annuus*) seeds and vitamin–mineral-supplemented water and grit. Generous quantities of fresh blueberries (*Vaccinium corymbosum*), frozen corn (*Zea mays*) and peas (*Pisum sativum*), and live mealworms (*Tenebrio* sp. larvae) were added daily. Fresh-cut evergreen trees and numerous natural and artificial perches were also provided.

On 27 December, we divided birds into two groups of six and moved them into two halves of a large outdoor cage, divided by a transparent plastic partition. Each cage half was an L-shaped enclosure of galvanized wire with two rectangular areas measuring 4.2 × 2.4 × 2.1 m and 3.0 × 2.4 × 2.1 m for a total of 6 m^3 per bird. The cage itself provided only limited protection from wind and rain, because it had wire mesh walls and roof. Each group contained birds of each sex–age class and had similar exposure to the sun; each group had the same number and locations of perches, roosts, and food and water bowls.

Northern Cardinals have nine primaries and nine secondaries, which are replaced annually after breeding (June–October). We used secondaries 7 and 8 for treatments, with primaries II and III treated as back-ups in case secondaries were missing at the end of the study (required for only one bird). Each bird served as its own control, because we treated feathers on the right wing with the feather-degrading bacterium *B. licheniformis* and those on the left wing with the broad-spectrum antibiotic chloramphenicol. As a control for bacteria and antibiotic treatment, we applied sterile saline to secondary 4 on the left wing.

To induce the growth of fresh feathers, we plucked feathers on 27 December or 8 February (2002), two weeks before each wing received its first treatment. That was done with secondaries 5 and 8 and primary II. Adjacent feathers (secondaries 4 and 7, primary III) were left in place to serve as worn feathers.

Half of the subjects were first treated, on the right wing, with bacteria; whereas the other half, caged separately, received antibiotic on the left wing on the same date (Table 1). Applications of either *B. licheniformis* or chloramphenicol were repeated two weeks apart (9 and 24 January). Two weeks after the second application, each treated feather was plucked and frozen at –80°C. Each bird that had received bacteria on the right wing received antibiotic treatments on the left wing two and four weeks later (treated on 22 February and 8 March); conversely, those that had initially received antibiotics on the left wing were treated with bacteria on the right wing on those dates. Thus, feathers from one wing on each bird were included in each treatment group. Birds housed together in the same half of the cage were treated identically, thereby reducing the chances of contamination across treatments. The cage was thoroughly sanitized with 10% bleach between the two treatments.

To treat feathers, we pipetted 150 μL of the bacterial or antibiotic suspension onto a sterile cotton swab and applied that to one side of the feather with a steady back-and-forth motion for 10 s. That was repeated for the other side of the feather, with an additional 150 μL of suspension on a new swab. As an antibiotic, we used chloramphenicol in 0.9% sterile saline (20 μg mL^{-1}). A bacterial suspension was made from strain 138B of *B. licheniformis* (ATCC#55768, generously provided by E. H. Burtt, Jr., and J. M. Ichida). The day before each application, we added a loopful (~10 μL) of bacteria from an overnight culture to 100 mL of nutrient broth with 7.5% NaCl to favor *B. licheniformis*. That was grown overnight at 45°C with constant aeration (200 rpm). Culture was harvested by centrifugation (5,000 × gravity), washed in distilled water, and resuspended in 0.9% sterile saline (45 mL) for a resulting suspension that contained approximately 3.79 × 10^5 bacteria per microliter, based on optical density at 600 nm.

The worn feathers that we pulled to induce new feather growth were used for an additional *ex vivo* treatment (Table 1). We glued secondary 8 and primary II feathers by the rachis to a sheet of styrofoam and suspended them from the side of the cage containing the subjects, with dorsal surface up and rachis oriented parallel to the ground. They were treated exactly as the worn feathers on the birds, and thus served as

TABLE 1. Summary of treatments for feathers from each subject in experiment 1.

Treatment	Bacteria			Antibiotic			Saline		
Feather wear	Fresh	Worn	Worn	Fresh	Worn	Worn	Fresh	Worn	Worn
Location	*in vivo*	*in vivo*	*ex vivo*	*in vivo*	*in vivo*	*ex vivo*	*in vivo*	*in vivo*	*ex vivo*

a control for any effect of being attached to the live bird. The feathers were exposed to the same ambient sunlight, temperature, and rainfall as the feathers on the live birds, but received no abrasion from the cages, no body warmth, and no preening or other behavior that might alter the microenvironment for feather-degrading bacteria.

Feathers were plucked from subjects or removed from the styrofoam sheet after each treatment was completed (8 February or 22 March), placed into a sealed plastic bag, and frozen at –80°C. Primary feathers, which had been treated for use as back-ups, were not immediately frozen. Instead, we cultured them to determine whether putative *B. licheniformis* was still present on the birds. To do that, we suspended the distal half of each feather in nutrient broth containing 7.5% NaCl and incubated for 24 h, with constant aerobic agitation at 45°C. If the broth remained clear (indicating no significant bacterial growth), we classified the feather as uncolonized. If the broth was turbid (indicating growth of bacteria), we classified the feather as colonized.

After freezing, all feathers were transferred into new plastic bags coded so that their identity was known only to D.A.C. (who did none of the microscopy). J.L.A. (who did all of the microscopy in experiment 1) then removed feathers from the freezer and cut three samples from each using a razor. Cuts were made across the vane at 1.2-cm intervals to produce three pieces ~0.6 cm^2 each, originating (1) near the distal tip, (2) near the midpoint of the feather, and (3) near the superior umbilicus. Pieces were mounted on metal stubs and coated with a 20-nm layer of gold–palladium in a Hummer sputter-coater set at 80 mtorr. The entire dorsal surface was then examined for lesions at 100× on an Amray 1810 scanning electron microscope (Bedford, Massachusetts). All samples were quantified twice in a row, and the average was recorded. To determine the amount of variation produced by observer error, the observer requantified 4–12 samples from a previous day each time the microscope was used ($n = 40$ blind recounts).

A lesion was defined as any area of the vane with one or more barbules partially or completely missing. Preliminary examination of feathers incubated with *B. licheniformis* had indicated that this was the type of lesion most closely correlated with length of time exposed to the bacteria. Scarring of the rachis was also correlated, as well as the total number of missing barbules (including more than one adjacent barbule). However, for brevity, we present only one of those three highly correlated assays (Cristol et al. unpubl. data).

To determine the size of each feather sample, we measured their surface areas using a light microscope attached to a video image analysis system running National Institutes of Health imaging software. Density of lesions for each feather was calculated from all three pieces by dividing mean number of lesions by mean surface area, and that value was arcsine-transformed to better approximate a normal distribution for analyses. Because the same individuals provided feathers for each treatment, we used an ANOVA model analogous to repeated measures, with subject mean squared used for calculating F ratios. Means of untransformed data are presented with standard deviation (SD) in figures.

To determine whether the suspension of *B. licheniformis* we were using degraded Northern Cardinal feathers under ideal conditions, we inoculated pieces of feathers removed from our subjects with the same suspension used to inoculate them on the live birds, and incubated them alongside uninoculated control feather pieces. Specifically, we dipped 36 pieces of rectrice feathers (~2 cm^2) from our subjects for 10 s in either a suspension containing *B. licheniformis* (see above) or sterile saline. We suspended each feather piece above 25 mL of distilled water in a 50 mL centrifuge tube, so that it was not immersed but remained humid. Tubes were then incubated at 45°C; at every 24 h (to 216 h), two experimental and two control tubes were removed and frozen to stop further bacterial degradation. Those samples were then examined for barbule lesions under the electron microscope as described for other samples.

Experiment 2.—We captured 26 adult European Starlings (12 males, 14 females), using walk-in traps, between 1 May and 1 June 2003. Birds were group-housed in the large outdoor cages used in experiment 1 and maintained throughout the study on an *ad libitum* diet of turkey starter mash without antibiotics. On 6 June, we divided birds into two groups of 13 and moved one group (saline-treated controls) into an L-shaped portion of the aviary with two rectangular areas measuring 4.2 × 2.4 × 2.1 m and 3.0 × 2.4 × 2.1 m for a total of 3 m^3 per bird.

Controls were group-housed because of space limitations and because contamination was not an issue for saline-treated controls. Birds in the other group (experimentals) were separated and housed individually (unlike in experiment 1) in cages measuring 3.0 × 2.4 × 2.1 m for a total of 9 m^3 per bird. Each group contained almost equal numbers of males and females and had similar numbers of perches, roosts, and food and water bowls. Unlike in experiment 1, the roof of each cage was half-covered with black polyethylene sheeting to reduce sunlight and increase humidity. To increase humidity further, cages were sprayed continuously with a mist of water from irrigation hoses located above the roof. Although birds could roost outside of the direct spray, they could not get food without being misted, and the cement floors of all cages were continuously covered with running water. Food bowls were sanitized daily to curb mold growth, and no health problems developed despite continuous humidity approaching 100%.

European Starlings have nine primaries and nine secondaries, and our subjects were just starting prebasic molt (of the primaries) during the experiment. We used secondary 7 feathers for treatments, and treated secondary 5 feathers as back-ups in case a feather was lost during the treatments (which never happened). On each bird, we treated feathers on one wing (randomly selected) with *B. licheniformis* and those on the other wing with chloramphenicol. Unlike in experiment 1, birds received treatments on both wings simultaneously, rather than sequentially. Controls were treated in the same way, but with sterile saline containing no antibiotic or bacteria.

To induce growth of fresh feathers, we plucked secondaries 5 and 7 on 12 June, 21 days before the first treatment. Unlike in experiment 1, we did not treat a set of worn European Starling feathers or place feathers on the sides of the cage for *ex vivo* treatment (Table 1).

Each feather received two applications of either antibiotic or feather-degrading bacteria 7 days apart (unlike in experiment 1, in which treatments were 14 days apart). Seven days after the second application (17 July), treated feathers were plucked and frozen at –80°C. The solutions of antibiotic and bacteria that we used, as well as the application technique, were identical to those in experiment 1. Because antibiotic- and bacteria-treated feathers were from two wings of the same subject, we compared the arcsine-transformed density of lesions on them using a paired *t*-test.

Frozen feathers were coded and cut by D.A.C., and then a technician with no knowledge of the treatments quantified damage in the same manner as in experiment 1. Unlike in experiment 1, two (rather than three) pieces of feather vane were quantified. Pieces came from opposite sides of the rachis, spanned the width of the vane, measured ~1 cm^2 each, and were centered on the midpoint of the vane lengthwise. Because we were using the identical bacterial solution as in experiment 1, we did not repeat our analysis of *in vitro* feather degradation.

We used a 1-cm^2 piece of vane from the middle of the feathers to determine whether *B. licheniformis* was still present at the time the feather was collected. To do that, we incubated feather pieces in 5 mL of nutrient broth containing 7.5% NaCl for 72 h with constant aerobic agitation at 50°C. If the broth remained clear, we classified the feather as uncolonized. If the broth was turbid, we classified the feather as colonized. Those cultures showing growth were (1) plated on solid agar in such a way that individual colony morphology could be observed as well as their ability to hydrolyze the milk protein casien at 35°C, and (2) harvested by centrifugation for subsequent genomic DNA extraction for *kerA* polymerase chain reaction (PCR) as described below. Casein hydrolysis is indicative of the bacterium's ability to degrade the protein in feathers. Despite the selective culturing, colony morphology indicated that few of the broth cultures contained a single bacterial species.

The PCR primers were designed to amplify a 900-base-pair region of the *kerA* gene, which encodes the protein that enables *B. licheniformis* to degrade β-keratin, the main constituent of feathers. Although *kerA* is 98% similar to a gene found in a closely related species, *B. subtilis*, primers were designed to amplify only the sequence from *B. licheniformis*. The PCR reaction consisted of the manufacturer's buffer (Applied Biosystems, Foster, California), 2.1 mM $MgCl_2$, 0.3 mM dNTP suspension, 400 ng of the *kerA* reverse primer (5′ CGGACTTGTGAAGCTGAAAG 3′), and 400 ng of the *kerA* forward primer (5′ CAGGAGTGAAAACCGCATCT3′). That was combined with 5 μL of the genomic DNA isolated from the bacteria grown on each feather using RediLyse lysozyme (Epicentre, Madison, Wisconsin). We brought up the volume to 50 μL

with sterile deionized water. We accomplished PCR using 35 cycles of 94°C for 30 s, 50°C for 30 s, 72°C for 90 s. Genomic DNA from *B. licheniformis* PWD-1, which produces the *kerA* amplicon, was used as a positive control. Amplification products were resolved on 1% agarose gels.

Results

Experiment 1.—There was no effect of treatment ($F = 0.60$, df = 2 and 11, $P = 0.57$) or feather wear ($F = 0.06$, df = 1 and 11, $P = 0.81$) on density of lesions for feathers treated *in vivo* (Fig. 1), and there was no interaction between those factors ($F = 0.62$, df = 2 and 11, $P = 0.55$). Worn feathers that were suspended on styrofoam from the side of the cage had a higher density of lesions than worn feathers on the birds ($F = 9.74$, df = 1 and 11, $P = 0.01$); but that effect was not dependent on treatment with feather-degrading bacteria, because there was no treatment effect or interaction of location and treatment (treatment: $F = 0.10$, df = 1 and 11, $P = 0.75$; interaction: $F = 0.16$, df = 1 and 11, $P = 0.70$).

After the experiment was completed, we incubated the fresh primary feathers from bacteria- and antibiotic-treated wings in a solution favoring the growth of *B. licheniformis*. We classified 8 of the 12 feathers from bacteria-treated wings as colonized, and none of those from the antibiotic-treated wings.

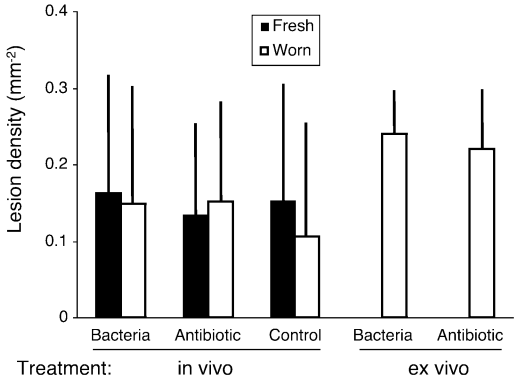

Fig. 1. Microscopic damage (number of barbule lesions per millimeter squared of feather vane [mean ± SD]) on fresh and worn feathers of Northern Cardinals treated with feather-degrading bacteria, antibiotics, or saline control either on live birds (*in vivo*) or on plucked feathers suspended from the side of the cage (*ex vivo*).

The bacterial solution we applied to the birds degraded Northern Cardinal rectrices when incubated in a humid container at 45°C (Fig. 2). Density of lesions on the rectrices was significantly related to length of time incubated (linear regression: lesion density = 0.006 * time + 0.17, $r^2 = 0.83$, $F = 33.0$, df = 1 and 7, $P = 0.0007$; Fig. 2), with the first damage appearing between 48 and 72 h after the onset of incubation. Uninoculated control feathers were not degraded by the experimental conditions in ≤216 h, and pre-existing degradation was minimal (lower line in Fig. 2).

Observer error was minimal. While gathering data during experiment 1, the observer recorded 493 lesions on the 40 samples subjected to blind retesting. Although those samples were recounted on different days, the average sample differed by 4.1%, with 10 having more and 6 having fewer lesions. That high level of repeatability was not surprising, because lesions were prominent and unambiguous at the magnification we used, so we did not assess observer reliability for experiment 2.

Experiment 2.—Damage to feathers was minimal and did not differ between bacteria-treated and antibiotic-treated European Starling feathers (paired $t = 0.31$, $P = 0.76$). Untreated

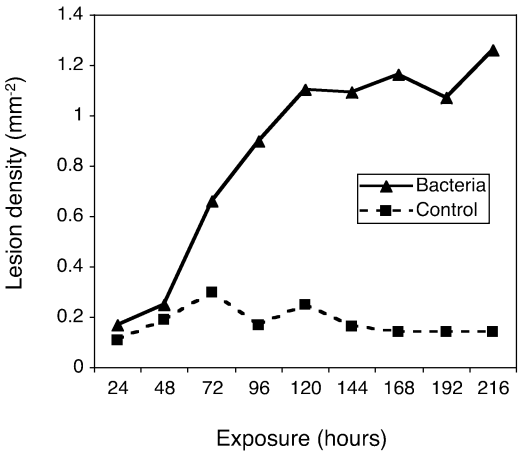

Fig. 2. Microscopic damage (number of barbule lesions per millimeter squared of feather vane [mean ± SD]) on feathers of Northern Cardinals treated *in vitro* with *B. licheniformis* and incubated at 45°C with high humidity for ≤216 h. Control feathers were incubated in sterile saline. Each data point represents the mean of two feathers.

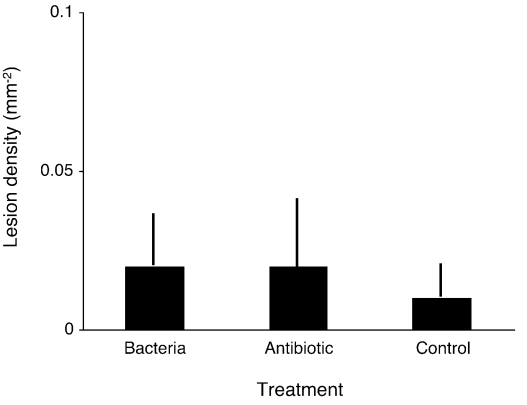

FIG. 3. Microscopic damage (number of barbule lesions per millimeter squared of feather vane [mean ± SD]) on worn feathers of European Starlings treated *in vivo* with feather-degrading bacteria, antibiotics, or saline controls. Note that the *Y*-axis differs from that in Figure 1.

control feathers had a similarly low level of damage (Fig. 3). Overall, density of lesions on European Starling feathers was approximately an order of magnitude lower than that on fresh Northern Cardinal feathers in experiment 1.

When we cultured pieces of feathers to determine whether *B. licheniformis* was still present at the end of the experiment, we cultured colonies whose morphology was consistent with that of *B. licheniformis* from none of the 26 antibiotic-treated or control feathers, and from 7 (54%) of the 13 bacteria-treated feathers. The PCR revealed the presence of *B. licheniformis* on most of the bacteria-treated feathers and some additional feathers (bacteria: 61.5%; antibiotic: 23.1%; control: 15.4%; Table 2). Thus, it appears that bacteria applied to feathers survived the duration of the experiment on most feathers; and some feathers that we had not treated with bacteria were contaminated with *B. licheniformis* from other feathers on the birds, from the cage environment, or from the hands of researchers during processing of the feathers.

DISCUSSION

The strain of *B. licheniformis* that we used has previously been shown to severely degrade isolated feathers under laboratory conditions (Burtt and Ichida 1999). In our lab, it produced detectable damage on Northern Cardinal tail feathers when incubated in a humid environment for >48 h. However, in experiment 1, when we applied it twice over a four-week period to the feathers of 12 wild-caught Northern Cardinals held outdoors in winter, we detected no damage to either fresh or worn feathers. Secondary feathers swabbed liberally on both surfaces with a suspension containing many millions of bacteria had no more lesions than fresh or worn feathers swabbed with an antibiotic, or fresh or worn feathers left untreated. In experiment 2, we repeated the treatment of wing feathers with a solution containing *B. licheniformis*; but we applied the bacteria at a seven-day interval to European Starlings and housed the birds outdoors under warm, extremely humid conditions in summer to favor the growth of microbes. Still, the wing feathers treated with *B. licheniformis* in experiment 2 suffered almost no damage and did not differ from antibiotic-treated feathers. Interestingly, we found fewer lesions, by an order of magnitude, on the European Starling feathers than on the Northern Cardinal feathers, including the untreated controls. Because even the untreated control European Starling

TABLE 2. Results of bacterial culture for 72 h, growth on agar plates infused with casein, and subsequent PCR amplification of plated colonies of bacteria from European Starling feathers collected at the end of experiment 2.

	Turbidity (<72 h) [a]	Hydrolyzes casein [b]	Morphology consistent [c]	PCR confirms
Antibiotic	10	3	0	3
Control	12	2	0	2
Bacteria	13	8	7	8

[a] Turbidity indicates bacterial growth.
[b] Casein (milk protein) hydrolysis indicates the potential to degrade feathers.
[c] Shape and properties of colonies consistent with *B. licheniformis*.

feathers had little degradation, and all fresh feathers were approximately the same age when plucked (Northern Cardinals = 42 days, European Starlings = 35 days), our results suggest that European Starling feathers are inherently more resistant to wear. The blackish-brown European Starling feathers may be more resistant, presumably because of higher melanin content, to all types of degradation than the pale reddish-yellow Northern Cardinal feathers (Goldstein et al. 2004). However, because multiple factors differed between experiments 1 and 2, that is speculation.

Experiment 1 was carried out in winter, several months after the Northern Cardinals' prebasic molt, and at the time of year when wild birds are most likely to be carrying *B. licheniformis* (Burtt and Ichida 1999). Our experimental subjects lived in an outdoor cage and were subjected to several extended periods of rainy weather (daily average rainfall was 0.21 cm for the entire period). However, for most of the time, they were dry, and air temperatures, though always above freezing (monthly average temperature was 6.8°C in January and 8.0°C in February), never approached the optimal temperature for the bacterium (~45°C). We do not know the temperatures experienced by the bacteria in the Northern Cardinal's plumage, but wing feathers probably approached ambient, because they are underlain by insulative down.

It is possible that the bacteria we applied in experiment 1 were unable to reproduce themselves or degrade feathers under the cool, dry environmental conditions they experienced. However, it is also possible that bacteria began to degrade feathers and reproduce, but that the birds eliminated them behaviorally. Subjects had ample opportunity to bathe (six pans of fresh water were always available) and may thus have mechanically rid themselves of bacteria or significantly reduced bacterial loads. They appeared to spend a lot of time sunning on the many perches provided, so a considerable proportion of the bacteria applied may have been killed by ultraviolet light. The recent finding that avian preen-gland oil suppresses growth of this strain of *B. licheniformis* (Shawkey et al. 2003) suggests another way in which birds may reduce damage from feather-degrading bacteria. If birds were behaviorally reducing bacterial degradation, it becomes relevant that our subjects were in captivity, where they may have had more time than free-living counterparts for maintenance behavior.

That feathers suspended from the side of the cage showed higher levels of degradation would have supported the idea that birds controlled bacteria behaviorally (e.g. by staying dry or by preening). However, antibiotic-treated feathers suspended from the cage exhibited approximately the same level of damage, which suggests that it was actually the result of increased exposure to the environment, rather than unchecked *B. licheniformis* degradation. One limitation of experiment 1 is that we did not confirm the presence of *B. licheniformis* on the feathers at the end of the experiment, a shortcoming that was rectified in experiment 2.

Experiment 2 was motivated by the concern that the cold, dry conditions during experiment 1 may have suppressed or killed bacteria, thus preventing feather damage that we would have detected under warm, humid conditions. We had carried out experiment 1 in winter, several months after the prebasic molt, because that is apparently the time of year when birds are most likely to be carrying feather-degrading bacteria (Burtt and Ichida 1999). But it seemed possible that higher prevalence in winter might not result in degradation until conditions improved. Thus, we carried out experiment 2 at the warmest time of year (mean temperatures 3–17 July = 26.2°C), and we elevated humidity to near saturation by continuously misting from above and running water constantly across cage floors. Despite what seemed like more favorable conditions for bacterial growth, we found no evidence of degradation on treated feathers. Culturing and PCR amplification confirmed that *B. licheniformis* had survived on most of the treated feathers. Thus, the microbes either survived in such low quantities that they produced no detectable damage, or they entered the inactive spore stage of their life cycle until revived during *in vitro* cultivation. Our conclusion is that if there are conditions under which *B. licheniformis* can affect the plumage of a live bird, those are very specialized, such as in nocturnal or cavity-dwelling species that are exposed to less ultraviolet light, or on certain feathers that are not protected behaviorally or chemically (e.g. a down feather on the back of the neck that

lacked melanin and could not be treated with uropygial oil). Further experiments with live birds are required, perhaps in environmental chambers where exposure to light and moisture can be closely regulated.

Illuminating the interactions between environmental conditions, avian maintenance behavior, and the rich microbial community of the plumage presents a ripe target for interdisciplinary experimental ornithology. The diversity of potential feather-degrading microbes found in soil is greater than previously suspected (Lucas et al. 2003). *Bacillus licheniformis* has recently been isolated from a higher proportion of wild birds than originally reported in 1999 by Burtt and Ichida (Whitaker et al. 2005). The presence of feather-degrading microbes could have important behavioral or evolutionary consequences, such as being one of the selective forces responsible for feather molt or dark pigmentation (Burtt and Ichida 1999, Goldstein et al. 2004). However, our negative findings suggest that the presence of *B. licheniformis* on the plumage of wild birds may have little effect on the birds' lives and could be nothing more than harmless incidental contamination from soil. Perhaps bacteria remain on the plumage after they are molted and degrade feathers on the ground. Because we tested only wing feathers on two species and at two times of year, it is certainly possible that other species or other types of feathers are more susceptible to degradation, or that longer periods of warm, humid weather are required. Much remains to be learned about the putative ecological role of feather-degrading microbes, with the obvious next step being to determine whether there are any conditions under which they can significantly degrade the feathers of live birds.

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