Molecular Analysis of Shower Curtain Biofilm Microbes
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Households provide environments that encourage the formation of microbial communities, often as biofilms. Such biofilms constitute potential reservoirs for pathogens, particularly for immune-compromised individuals. One household environment that potentially accumulates microbial biofilms is that provided by vinyl shower curtains. Over time, vinyl shower curtains accumulate films, commonly referred to as “soap scum,” which microscopy reveals are constituted of lush microbial biofilms. To determine the kinds of microbes that constitute shower curtain biofilms and thereby to identify potential opportunistic pathogens, we conducted an analysis of rRNA genes obtained by PCR from four vinyl shower curtains from different households. Each of the shower curtain communities was highly complex. No sequence was identical to one in the databases, and no identical sequences were encountered in the different communities. However, the sequences generally represented similar phylogenetic kinds of organisms. Particularly abundant sequences represented members of the α-group of proteobacteria, mainly Sphingomonas spp. and Methylobacterium spp. Both of these genera are known to include opportunistic pathogens, and several of the sequences obtained from the environmental DNA samples were closely related to known pathogens. Such organisms have also been linked to biofilm formation associated with water reservoirs and conduits. In addition, the study detected many other kinds of organisms at lower abundances. These results show that shower curtains are a potential source of opportunistic pathogens associated with biofilms. Frequent cleaning or disposal of shower curtains is indicated, particularly in households with immune-compromised individuals.

Cases of opportunistic infections in humans have increased steadily over the past decade, and often the source of infection remains unidentified (6, 13, 20, 25, 27). The expanding case-loads of opportunistic infections correspond to a rising number of immune-compromised patients, many of whom self-medicate (20, 25, 37, 43). Potential or adventitious pathogens in households pose a particular threat to such patients (24). Households provide many environments in which microorganisms can thrive, often with the formation of biofilms. Bacteria have been cultured from many environments in and around homes, particularly in moist settings such as those involving water pipes, toothbrushes, and spas (12, 14, 29, 30, 38). Although evidence of microbial growth and biofilm formation is ubiquitous in households, little is known about the diversity and complexity of the organisms that make up household microbial communities.

Several studies have shown that domestic water supplies can be a source of opportunistic infectious agents, and household plumbing accumulates numerous microorganisms (5, 55). Potentially infectious agents such as Mycobacterium spp. and Legionella spp. have been detected in water systems and may serve as reservoirs for infection (10, 24, 32, 33). One water-related setting that may provide a persistent reservoir for pathogenic microorganisms is shower curtain biofilms, although there is little information on the makeup of the microbial communities that compose such biofilms.

In order to identify the kinds of organisms that colonize shower curtain biofilms, we undertook a molecular survey of several such communities. In our study, we isolated microbial-community DNA from shower curtains and used a PCR-based molecular survey to determine the phylogenetic diversity of 16S rRNA gene sequences from these communities. Numerous studies have utilized 16S rRNA sequences to assess the nature of microbial organisms in the environment without the requirement for culture (1, 2, 22, 39). The rRNA sequence collection constitutes a rough census of this particular community. The phylogenetic identification of the constituent microbes can provide some insight into their natures by comparison with available cultured organisms.

MATERIALS AND METHODS

Samples. DNA was extracted from five different samples taken from four different vinyl shower curtains, all in use for more than 6 months in Boulder, Colo. These samples consisted of (i) two samples from the same shower curtain, one from the bottom section (whitish pink flakes) of a dry shower curtain that had been stored at 25°C for about 1 week subsequent to wetting (SC1A) and one sample from a pink film on a corner of the curtain that was folded over and constantly wet (SC1B); (ii) one sample of white flakes from a dry curtain that had been stored at 25°C for about 1 week subsequent to wetting (SC2); (iii) one sample of pinkish flakes from the bottom section of a dry curtain that had been stored at 25°C for about 1 week subsequent to wetting (SC3); and (iv) one sample of a pinkish orange biofilm from a wet shower curtain that was often used and essentially continuously moist (SC4). Dry biofilm flakes or moist bio-
films were scraped from shower curtains and stored at −80°C pending extraction of DNA.

Epifluorescence microscopy. Biofilm (“shower scum”) was hydrated with sterile phosphate-buffered saline (0.01 M) dispersed by vortex mixing, and a smear was dried onto a microscope slide, stained with 10 μg of 4′,6-diamidino-2-phenylindole (DAPI; Sigma, St. Louis, Mo.)/μl and mounted with antifade (Citifluor Ltd., Leicester, England). Slides were examined with an epifluorescence microscope (Eclipse E1000; Nikon Instruments Inc., Melville, N.Y.) with a SPOT camera and software (Diagnostic Instruments Inc., Sterling Heights, Mich.).

DNA extraction. Samples from shower curtains were dispensed in sterile purified water (Becton Dickinson Corp., Franklin Lakes, N.J.) and sonicated for approximately 1 h. After sonication, colonies were re-suspended in purified water and dispensed. The DNA was isolated using the ARB program (http://www.arb-home.de) and considering secondary structure. The sequencing reaction mixtures were prepared using the ARB program (http://www.arb-home.de) and considering secondary structure. The sequencing reaction mixtures were prepared using the ARB program (http://www.arb-home.de) and considering secondary structure.

RESULTS

Vinyl shower curtains over time accumulate a film, flakey when dry, that is popularly referred to as “soap scum.” Examination of this material from shower curtains by epifluorescence microscopy, as shown in Fig. 1, revealed that the “soap scum” is in fact a lush bed of microbes, generally imbedded in a biofilm matrix. In order to survey rRNA gene sequences, DNA was purified from biofilms scraped from four different shower curtains, including two different patches from one curtain, one dry and one continuously wet. All of the DNAs extracted from shower curtain samples produced PCR products with bacteria-specific 16S rRNA gene (rDNA) primers.

None of the extraction controls showed detectable amounts of amplified rDNA. PCR products were cloned, and unique sequences were identified by RFLP analysis and sequenced (22). In the course of the study, 337 clones were screened and 117 unique rRNA sequences were determined.

The sequences obtained from the shower curtain microbes were compared to each other and to sequences in GenBank. Results of phylogenetic analyses for the Sphingomonas- and Methylobacterium-related sequences are presented in Fig. 2. There were no identical sequences between different shower curtain samples. Instead, as generally occurs with environmental samples, members of phylogenetic clusters of closely related sequences were seen. Several of the clone DNA sequences were closely related to cultured organisms. The resolution of the trees was fairly low in several places, likely because the data set was limited (only 670 positions). Despite the limitations of the data set, the relevant relationships were supported with high MP and NJ bootstrap values (>70%).

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The pie chart diagrams in Fig. 3 illustrate the diversity and abundance of organisms found in the various shower curtain samples. The sequences represented are only the most abundant of the sequences encountered. Approximately 15% of the clones analyzed on the basis of RFLP or sequence were observed only once, so we did not nearly exhaust the diversity of organisms that comprise the communities. Most of the clones we sequenced were >95% identical to sequences already in GenBank. Only 38 out of 337 clones screened (11.2%) were less than 95% identical (genus-level relatedness) to known sequences in GenBank. Few clones had less than 90% identities to known sequences. Although the specific sequences associated with the different shower curtains differed in detail, the phylogenetic comparisons revealed common themes. The
most prevalent types of organisms found in all of the samples belonged to the phylogenetic group α-proteobacteria. Sphingomonas spp. and Methylobacterium spp. prevailed in three of the four shower curtains in dry (SC2 and SC3) and moist (SC4) samples. Methylobacterium and Sphingomonas related clones found in the shower curtains spanned the diversity of these genera, and several of the clones were closely related to cultured species (Fig. 2). Although there were types of organisms consistently associated with all of the curtains, each of the samples contained significantly different proportions of specific organismal types. Indeed, one of the shower curtain communities (SC1) (Fig. 3) did not contain Methylobacterium spp.

the abundance of rRNA gene clones detected in this study, such organisms likely are responsible for the pink color of some shower curtain biofilms. We acknowledge, however, that the abundance of rRNA gene clones does not directly indicate the relative abundances of the corresponding cells. Different species of organisms contain different numbers of rRNA genes per cell, and potential biases in the recovery of different rRNA genes by PCR techniques must be recognized. Nevertheless, we believe that the relative frequencies of the rRNA genes detected represent some rough reflection of the environmental abundance of the corresponding organisms.

In addition to Sphingomonas spp. and Methylobacterium spp., the shower curtains also contained other representatives of the Proteobacteria phylogenetic division, particularly α-pro-
Proteobacteria. Proteobacteria are diverse in physiology and ubiquitous in water environments, ranging from deep seawater to waste and drinking water (8, 48, 49). The sources of carbon and energy for these biofilm communities are unclear. Potential food resources include soap products, sloughed-off human debris, and bath area volatiles. Biofilms provide a supportive and protective environment in which many kinds of metabolism can thrive. Sphingomonads, for instance, use a broad range of carbon compounds, including complex organics such as dibenzofuran (18) and hexachlorocyclohexane (23). Some isolates from the deep subsurface have been shown to metabolize aromatic compounds such as toluene, naphthalene, and others, although laboratory strains have not shown these properties (17). Sphingomonads colonize new environments readily and adhere to surfaces through the production of exopolysaccharides, such as gellan, and welan (41).

Potential pathogens. In this limited survey, we did not encounter any known specific pathogens. However, several sphingomonads are known to be opportunistic pathogens. For instance, Sphingomonas paucimobilis has a history of infecting immune-compromised patients or persons with predisposing conditions (21). Infection with S. paucimobilis can lead to intravascular catheter-related bacteremia, urinary tract infections, pneumonia, cutaneous infections, and visceral abscesses (7, 21, 45). In hospitals, sources of S. paucimobilis infections have been traced to fluid in humidifiers and tap water (21, 31, 40).

Methylobacterium spp. also are known to cause infections in immune-compromised patients or patients with other diseases that render them prone to infection (15, 16, 20, 25, 35, 47). We found several clones representing organisms closely related to Methylobacterium extorquens and Methylobacterium zatmanii (Fig. 2B), both known to cause illness in immune-compromised patients (20, 25). We also encountered abundant M. mesophilicum in a 16S rDNA clone library prepared from humidifier filter samples (data not shown). In patients, M. mesophilicum has been detected in blood, peritoneal fluid, and ascitic fluid and has been known to cause pneumonia, skin ulcers, empyema, keratitis, and bacteremia (25, 35, 47). Based on the rising number of cases, it has been proposed that Methylobacterium spp. infections could increase dramatically in the future (16, 19, 20, 42, 47).

In addition to the consistent presence of Sphingomonas spp.

FIG. 2. Results of phylogenetic analyses with Sphingomonas spp.-related (A) and Methylobacterium spp.-related (B) 16S rRNA sequences obtained in this study. The analyses performed were based on alignments of approximately 670 nucleotide positions. The alignments included cultured Sphingomonas and Methylobacterium spp. and outgroup γ-proteobacteria (Escherichia coli L10328), β-proteobacteria (Nitrospira mutiformis L35509), and α-proteobacteria (Roseobacter denitrificans M59063). The phylogenetic trees shown were estimated by using ML, MP, and NJ analyses converged on very similar tree topologies. Filled circles indicate both MP and NJ bootstrap support exceeding 70%, and open circles indicate bootstrap support exceeding 50%. (See Materials and Methods for details on the phylogenetic and bootstrap analyses.)
and Methylobacterium spp., we also uncovered a number of other bacterial species at low frequencies. Some of these species are closely related to known opportunistic pathogens, including Nocardia spp. and Gordonia spp. (high-G+C gram-positive bacteria). Infections with Gordonia terrae can invade wounds and result in bacteremia and brain abscess (34). Patients with such infections usually have underlying diseases predisposing them to opportunistic infections, but infections of healthy patients have been reported (9). There have been reports of severe infections in immune-compromised patients by several Nocardia species, which often are misidentified by standard culture methods (11). Other microorganisms detected in samples include close relatives of the known opportunistic pathogens Afipia felis (36) and Moraxella osloensis (4).

Our results suggest that shower curtains harbor potential opportunistic pathogens that can threaten immune-compromised or otherwise ill patients. For immune-compromised people, consistent exposure to sources of infection, such as shower curtains, is a public health problem. Exposure can be minimized by regular cleaning or by changing shower curtains.

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