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Prevalence of microbial biofilms on selected fresh produce and household surfaces

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Abstract

Investigations of biofilms in domestic environments are sparsely represented in the literature. In this study, samples of various household surfaces, including food, laundry and kitchen items, were analyzed for evidence of biofilm presence. Visualization of the surfaces was carried out using cryostage scanning electron microscopy (CSEM) and light microscopy. Qualitative evidence of the presence of biofilm formation was obtained from all of the sample groups analyzed, suggesting the widespread existence of microorganisms in biofilms on domestic surfaces. This suggests that biofilms may be important in household hygiene, and highlights the need for standardized, approved biofilm methods suitable for consumer products testing.

Keywords: Biofilm; Domestic surfaces; Vegetable; Laundry; Produce; Testing; Sanitizer; Disinfectant

1. Introduction

Generally, biofilm literature relating to domestic environments, including laundry and vegetable surfaces is sparse. Defined as microbial cells adherent to a surface or interface and covered with a layer of microbially produced exopolymeric substance layer, biofilms are often a significant mode of bacterial growth in natural and pathogenic ecosystems (Costerton et al., 1994). Biofilms are generally far more resistant to disinfection than the suspended cells or use-dilution tests commonly used to support disinfection claims (Le Chevallier et al., 1988). This suggests that there is a need to demonstrate the relevance of sanitizer test systems to the real-world microbial targets for which the products are designed.

In the USA, foodborne illness causes an estimated 9000 human deaths and affects 6–80 million people every year (Altekruse et al., 1997). The ability to recognize how pathogens survive on foods is therefore an important area for focus. Literature regarding healthy home environments indicates recent changes in the public awareness of microbial hazards in the home (Kurtzweil, 1995; McDonough, 2000). Consumer concerns are focusing more on the microbiologic safety of food than on the presence of pesticide or

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antibiotic residues (Bruhn, 1997). The perception of microbiological contamination is largely focused on meat and poultry products, with 98% of respondents recognizing that harmful bacteria could be present. In contrast, only 48% of respondents were aware of the risk posed by bacteria present on fruits and vegetables (Collins, 1997). Foodborne disease outbreaks have occurred with *Escherichia coli* O157:H7 due to contaminated lettuce and apple cider (Tauxe, 1997); with *Cyclospora cayetanensis* on raspberries (Herwaldt et al., 1997); and *Salmonella* on tomatoes (Torok et al., 1997).

Several disinfectant sprays for hard surfaces, and anti-microbials for use on fresh produce are now commercially available, making anti-microbial claims against a range of microorganisms. Products designed to sanitize surfaces should be tested under conditions that will realistically determine their efficacy. If biofilms are present on the surfaces to be sanitized, pathogenic microbes of concern may be either deposited onto or into that pre-existing biofilm, or may have grown there as a member of the biofilm, and it would therefore be reasonable to expect that efficacy tests for sanitizing products include biofilm assays. The majority of the anti-microbial products do not appear to have been tested against biofilm organisms, largely due to inherent technical difficulties and the lack of a suitable consensus method. Although one standardized biofilm method has been accepted by the American Society for Testing and Materials (http://www.astm.org ASTM E2196-02), there are no such methods that have been approved or endorsed by the regulatory agencies.

Food processing surfaces have been reported to support the surface growth of a range of microorganisms (Zottola and Sashara, 1994; Hood and Zottola, 1997), indicating that the potential for bio-transfer exists within food processing plants, where the microorganisms present on equipment surfaces may contaminate the finished product (Wirtanen et al., 1996). Once introduced, pathogens may grow on the surfaces or merely persist there, and become surrounded by biofilm material from non-pathogen growth. Environments where food is processed prior to packaging, exposed awaiting sale or prepared before consumption are at particular risk for the development of biofilms due to the regular presence of moisture and nutrients. The problem of food-contamination is not limited to large-scale facilities. Household sponges, cutting boards, dishcloths, counter tops and other surfaces represent areas where microbial pathogens can be deposited and thus the question arises as to whether such organisms are likely to encounter, or form, biofilms on these surfaces.

The majority of reports in the literature concerning habitats such as kitchen surfaces, attempt to simulate the transfer potential of microbes from work surfaces or cutting boards to food products via artificial inoculation of surfaces with planktonic cultures, yet fail to demonstrate the relevance of such approaches (Anon, 1993; Ak et al., 1994; Park and Cliver, 1996). High-surface areas and repeated exposure to moisture and nutrients make kitchen sponges ideal habitats for the concentration, growth and potential spread of bacteria. Raloff (1996) described a study by Enriquez et al. who found that the majority of 75 dishrags and 325 sponges included in their study harbored *E. coli*, *Salmonella* spp., *Pseudomonas* spp. and *Staphylococcus* spp. In another study involving 213 houses, 21% tested positive for *Listeria monocytogenes*, with dishcloths and various other utensils and structural surfaces being the most highly contaminated items and locations. *Listeria* spp. were isolated from dishcloths and cleaning brushes in numbers as great as 10^4 colony forming units (CFU) per object, while kitchen sinks, vegetables compartments in refrigerators and toothbrushes supported approximately 10^3 CFU per object (Beumer et al., 1997). Generally, little consideration has been given to the presence of biofilms per se, although the excellent method development work of Harris et al. (2001), addresses the issue of cross-contamination superimposed recently onto endemic microbiota. For various other contamination scenarios, questions remain regarding whether pathogens are generally exposed on the surface, or whether they persist in protected association within biofilms. The answer is of great consequence for testing methodologies, yet the question has rarely been addressed in the context of food and household surfaces, and the presence of biofilms has not been broadly demonstrated on these surfaces.

The primary aim of this study was to image a range of household and fresh produce surfaces using a combination of light microscopy and cryo-

stage scanning electron microscopy (CSEM), in order to assess the prevalence of biofilm on these surfaces.

2. Materials and methods

2.1. Samples

Common salad vegetables were purchased from the fresh produce section of a local supermarket, namely: tomatoes (Catlin-organic, Eurofresh, and Roma); carrots (bagged and bulk); lettuce (Iceberg, leaf and bagged chopped mesculin); and mushrooms (brown, white and Portabello). Supermarket-acquired vegetables were chosen with the aim of obtaining samples that had undergone normal storage and processing prior to their presentation to the consumer. Used laundry samples (damp and dry towels, damp and dry socks) were provided by the Procter and Gamble, and had been exposed to a range of normal wear and storage conditions. Used wooden cutting board and kitchen sponge samples were acquired from volunteers at Montana State University, and had been in active household use for undetermined durations. Appropriate baseline blanks were also prepared in order to observe inherent surface structures, and included: tomato, lettuce and carrot skin washed with bactericidal soap under running water, followed by surface spraying with 75% and 95% ethanol and air-drying in a sterile petri-dish; new unworn socks and new unused kitchen sponges. The samples were analyzed using CSEM and light microscopy combined with Alcian blue staining to provide visual evidence of biofilm formation.

2.2. Cryostage scanning electron microscopy (CSEM)

Cryostage SEM involves sample freezing in liquid nitrogen at temperatures below -200 °C, which are then maintained at temperatures below -140 °C throughout the imaging process. This technique theoretically eliminates the structural artifacts inherent in other chemical fixation protocols and maintains the structural integrity of the sample. All the samples were prepared in exactly the same manner. Each individual sub-sample was approximately $5-8 \times 8$ mm (1–3 mm in thickness). The individual samples were attached to a beveled brass coupon ($\approx 13 \times 28 \times 3$ mm thickness) using O.C.T. compound (TissueTek). Liquid carbon was then applied to two or three points along the edges of the sample to provide a conductive bridge between the sample surface and the coupon surface to prevent sample charging under exposure to the electron beam. From this point in the protocol, all samples were handled in an identical manner. The brass coupons with mounted samples were then attached to a threaded rod and dipped in liquid nitrogen. After approximately 2 min, the coupon and samples were quickly removed from the liquid nitrogen and, via the rod and cap assembly, introduced to the first of two dovetailed cryostages where coating was carried out according to the manufacturer's instructions. Imaging was carried out with a JEOL JSM-6100 scanning microscope. All images were collected at an accelerator voltage of 8.0 kV and a filament current of 3.2 A and recorded using Polaroid Type 665 (pos/neg B&W Instant Pack; ISO 80/20) film. The working distance varied between 11 and 39 mm. All images were digitized with a PC platform using a Hewlett-Packard Scan Jet 4c as high-resolution TIFF files (635 dpi) which were then converted to high quality JPEG files using Photoshop 5.0 software.

2.3. Light microscopy and Alcian blue staining

Light microscopy was used in conjunction with Alcian blue staining to confirm the presence of biofilm matrix. Alcian blue stains acidic polysaccharides often present in the biofilm matrix (exopolymeric substance—EPS) (Fassel and Edmiston, 1999). Staining was carried out by the direct addition of an aqueous solution of 0.1% Alcian blue 8GX (Sigma) onto the surface of the sample. Thin sections of tomato, carrot, mushroom, lettuce or sponges, or fibers from the towels or socks were incubated at room temperature with the dye (cutting board samples were not analyzed using this technique). After 20 min, samples were gently flushed with 0.45 μm filter-sterilized water to remove excess dye. The stained samples were then mounted in two to three drops of sterile water, and a coverslip was applied (when sample thickness allowed). All microscopic examinations were carried out using an Olympus BH-2 (RFCA) microscope and

images were recorded using Image Pro-Plus software (Version 3.0, Media Cybernetics).

3. Results

All of the samples examined exhibited evidence of biofilm presence. The images presented here are representative of the overall observations made for each sample type.

3.1. Tomatoes

All of the types of tomatoes examined (Catlin-organic, Eurofresh, and Roma) exhibited evidence of bacterial colonization and biofilm formation, often with extensive production of exopolymeric substance (EPS) visible on Alcian blue-stained samples observed under phase contrast microscopy (Fig. 1).

3.2. Carrots

Both the bagged and bulk loose carrots had patchy biofilms present on their surfaces (Fig. 2). Several different microcolony morphologies were observed. Both individual cells and clumps of bacterial cells could be distinguished. There was also evidence of fungal colonization as indicated by the presence of hyphae. The bagged carrots exhibited a higher density of bacterial colonization than the bulk loose carrots.

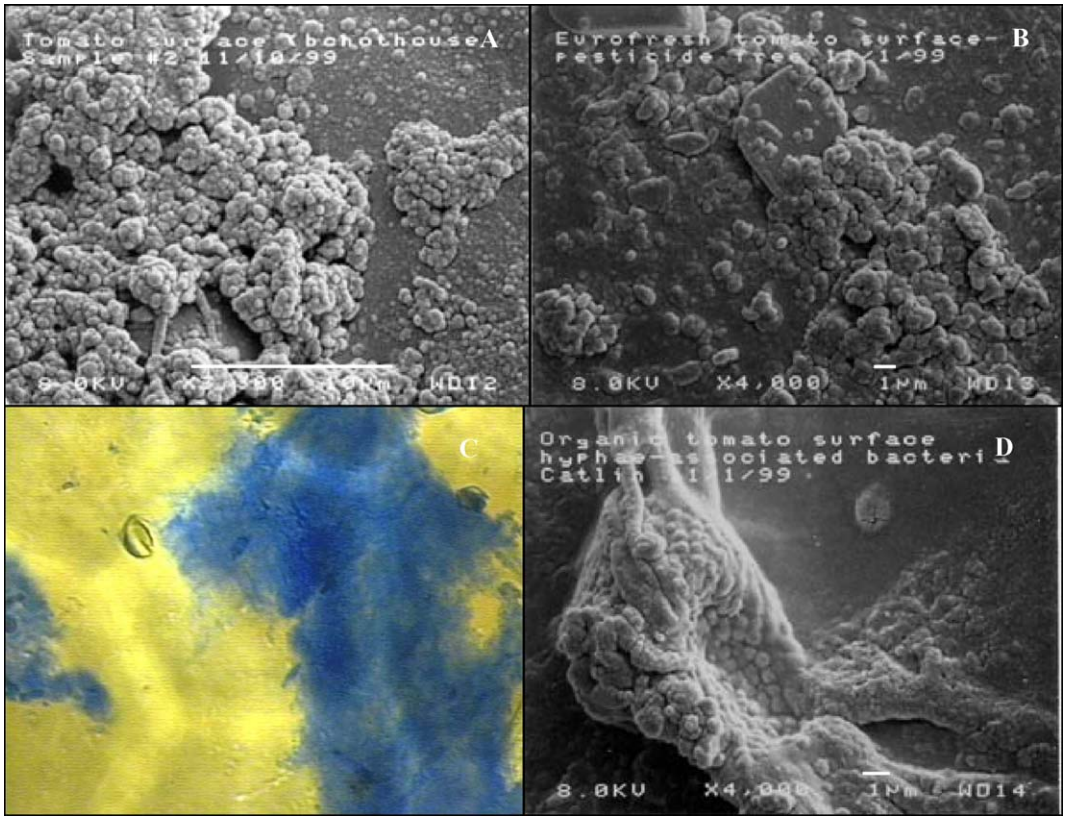

Fig. 1. Images of biofilm on the surface of tomatoes: (A) BC hothouse tomato (CSEM); (B) Eurofresh tomato (CSEM); (C) Catlin organic tomato (Alcian blue): 40× magnification; (D) Catlin organic tomato (CSEM).

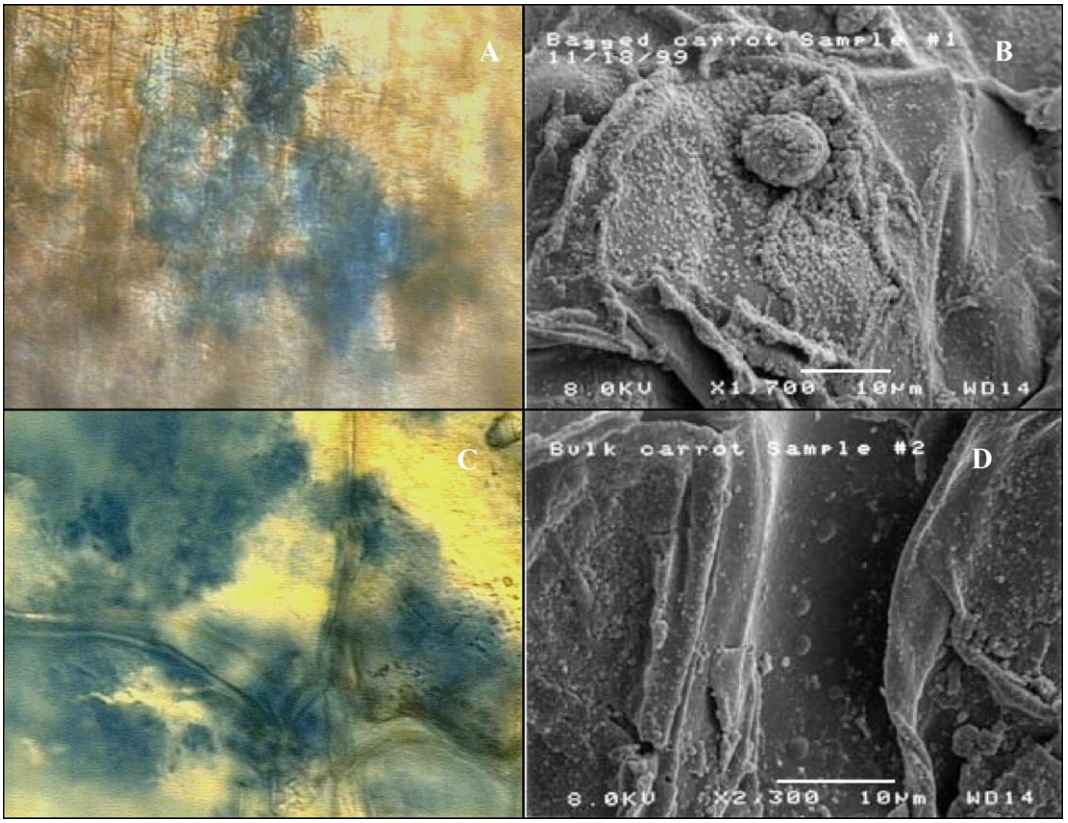

Fig. 2. Images of biofilm on carrot samples. (A) Bagged carrot (Alcian blue): 60× magnification; (B) bagged carrot (CSEM); (C) bulk carrot stained (Alcian blue): 60× magnification; (D) bulk carrot (CSEM).

3.3. Mushrooms

Mushrooms had considerable amounts of biofilm present on both the upper cap surface and in the gill areas. Large amounts of Alcian blue-stained EPS were observed, indicating that large amounts of biofilm were present (Fig. 3). In Fig. 6, individual bacterial cells are clearly visible at the edge and in the center of the biofilm cluster, appearing white against the darkly stained EPS matrix. Visual observations were used to rank the mushroom types in order of decreasing quantity of biofilm colonization: brown mushrooms> white mushrooms>portabello mushrooms.

3.4. Cutting boards

All of the eight used domestic cutting board surfaces tested were wooden and all exhibited bacterial biofilm growth. The biofilms present on cutting boards samples possessed considerable masses of EPS (Fig. 4). Fungal growth, however, was minimal or absent. In most cases, the observed biofilms were present as isolated areas within deep crevices/cut marks in the wood.

3.5. Kitchen sponges

New, unused baseline sponges showed little evidence of bacterial colonization, with the exception of occasional small patches of individual bacterial cells, lacking EPS. All of the 14 used domestic kitchen sponges demonstrated evidence of bacterial and, in some cases, fungal growth. The presence of EPS material was often apparent (Fig. 5), and in some cases, the EPS layer obscured the morphology of the underlying cells. Varying microcolony morphologies

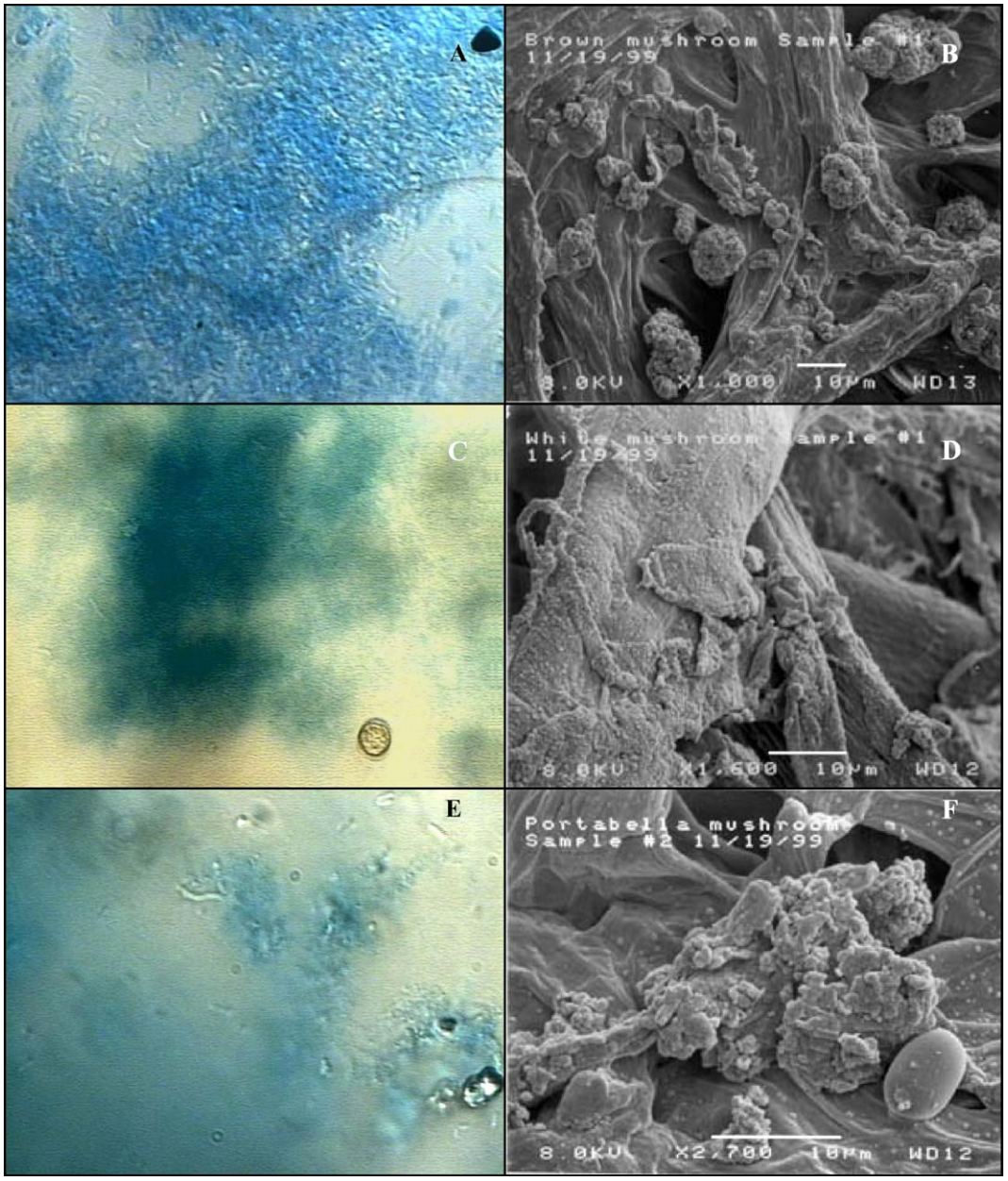

Fig. 3. Biofilm on the surface of mushrooms. (A) Brown bulk mushroom (Alcian blue): 60 × magnification; (B) brown bulk mushroom (CSEM); (C) white mushroom (Alcian blue): 60 × magnification; (D) white mushroom (CSEM); (E) Portabello mushroom (Alcian blue): 60 × magnification; (F) Portabello mushroom (CSEM).

suggested the presence of different bacterial species. Due to the heavy background staining of the sponge with the Alcian blue dye, this staining technique was not of use in enhancing biofilm visualization for this sample type.

3.6. Damp and dry socks

Damp, worn sock samples showed especially large amounts of blue-stained biofilm-associated EPS, adherent to, and between fibers (Fig. 6). Conversely,

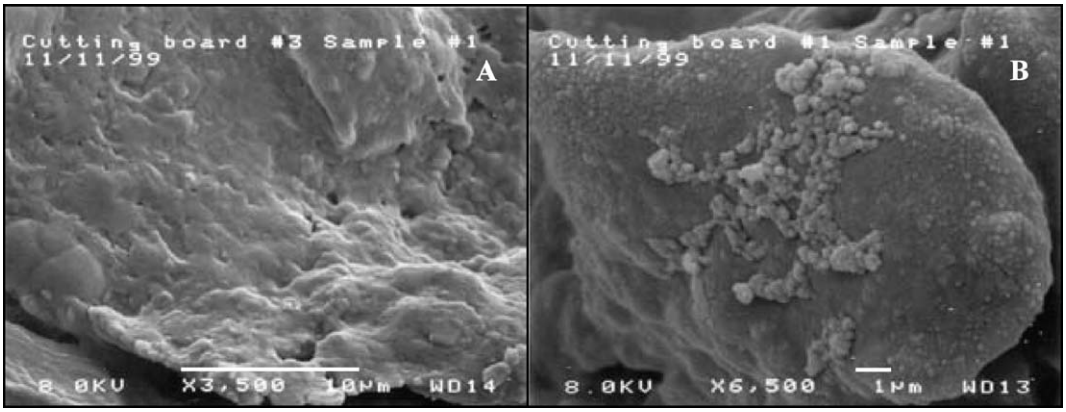

Fig. 4. CSEM images of used cutting board samples.

minor amounts of blue-stained EPS were noted for the new unworn sock (i.e. baseline/negative control). On the worn but dry socks, bacterial growth was observed on both internal and external sock surfaces, however it appeared that microbial colonization was greater on the inner sock surfaces (i.e. adjacent to the skin). Biofilm microcolony and cell morphologies were somewhat varied and encompassed four distinct types. The dried sock images were all captured from the inner surface of the sock (Fig. 6). All of the sock samples demonstrated substantial attached bacterial biomass, as well as the presence of fungal hyphae on some samples; however the damp samples exhibited more extensive biofilm formation than the dry socks. In some cases, preferential colonization was observed in the crevices present in many of the fibers. Many of the CSEM images did not clearly reveal the presence of EPS in these dry samples, however staining with Alcian blue did confirm the presence of EPS, albeit in lower amounts on the dry samples than on the damp samples. The images from the damp and dried towels recorded colonization by a diverse microflora, present in a variety of biofilm microcolony morphologies.

3.7. Damp and dry towels

In total, eight used cotton towels were examined, four dry and four damp. Observations of the damp towels via CSEM generally showed greater densities of microorganisms when compared to the dry towels.

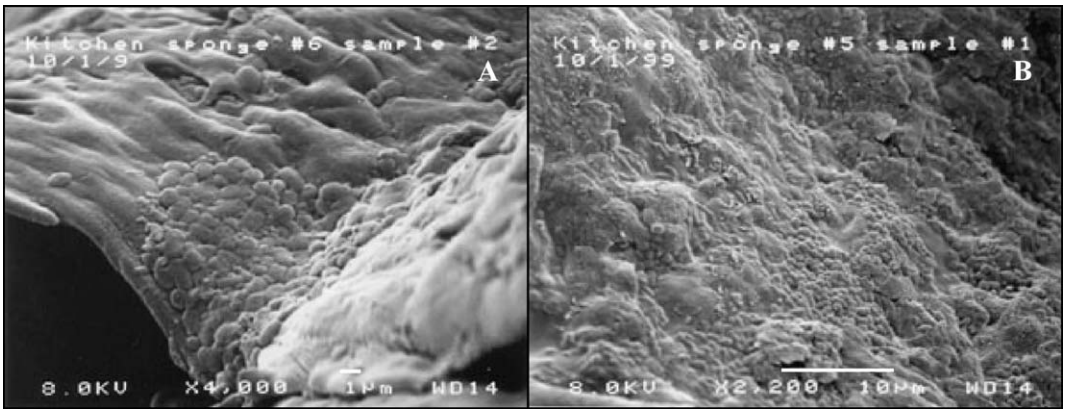

Fig. 5. CSEM images of biofilm present on used kitchen sponges.

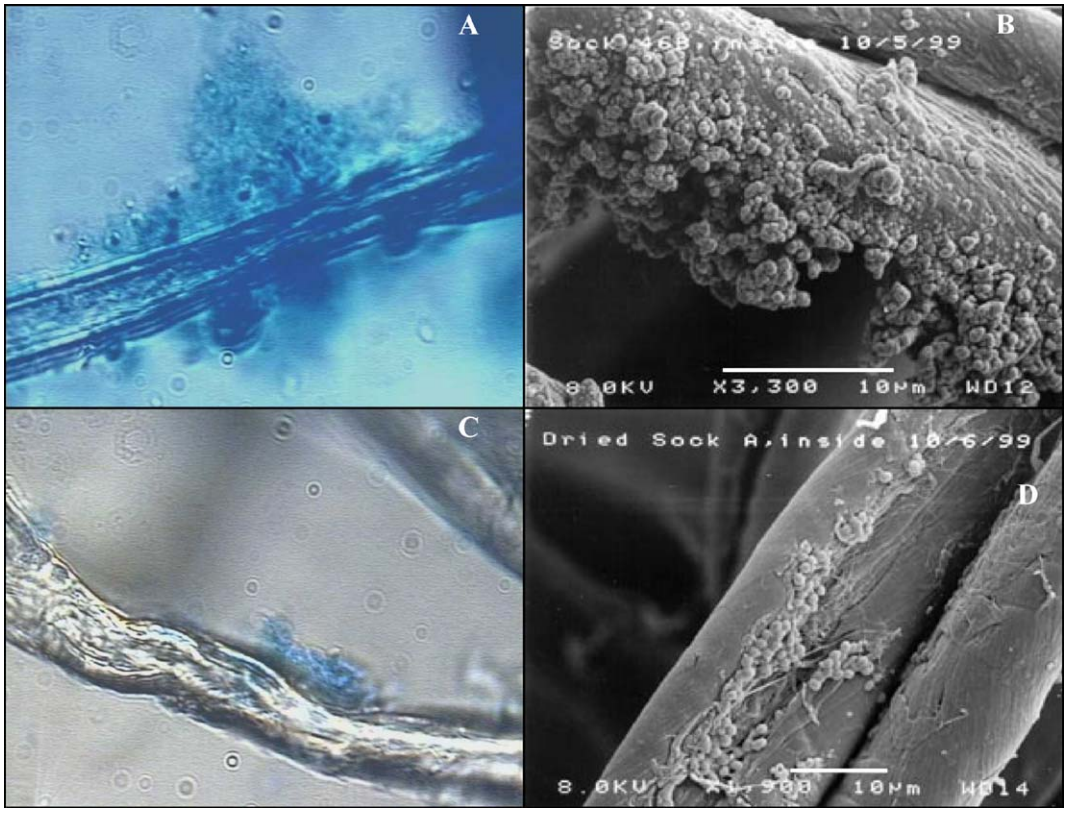

Fig. 6. Biofilm on sock fibers. (A) Damp sock (Alcian blue): 60 × magnification; (B) damp sock (CSEM); (C) dry sock (Alcian Blue): 60 × magnification; (D) dry sock (CSEM).

Considerable bacterial colonization was also observed on dry towels. Damp items were heavily colonized with microbial growth (macroscopic) after 1–2 weeks of storage. Naked eye observations prior to sampling the damp towels showed that they were visibly fouled, with spots of discoloration and apparent fungal contamination. The dry towels also had spots of discoloration, but they were fewer, and to a lesser degree than the damp-stored towels. Phase-contrast microscopy of the damp towels revealed bacterial colonization of the fibers and the presence of extensive fungal hyphae (Fig. 7). In some cases, the hyphae were surrounding fibers and appeared to penetrate into them. Cells tentatively identified as being yeast cells were also visible in a number of samples. On the dry towels, bacterial and fungal colonization of the fibers was also observed (Fig. 7), although to a lesser extent than the damp towels. In general, the dry towels exhibited less extensive biofilm growth.

4. Discussion

The majority of research papers concerning domestic environments involve laboratory-grown biofilms of microorganisms isolated from a particular environment (Anon, 1993; Ak et al., 1994; Park and Cliver, 1996; Seo and Frank, 1999), rather than observations of the in situ microbial flora. In one such typical study on fresh produce sanitization, a suspended culture of *E. coli* O157:H7 was sprayed onto lettuce leaves, then incubated for 24 h at 4 °C prior to treatment with anti-microbial product (Takeuchi and Frank, 2001). Since little or no microbial growth of these cultured organisms would have occurred on the lettuce due to the low nutrients and reduced temperature, this experimental approach focuses on the efficacy of the products against bacteria that are likely unprotected by an EPS matrix. Similarly, the proposed standard method of Harris et al.

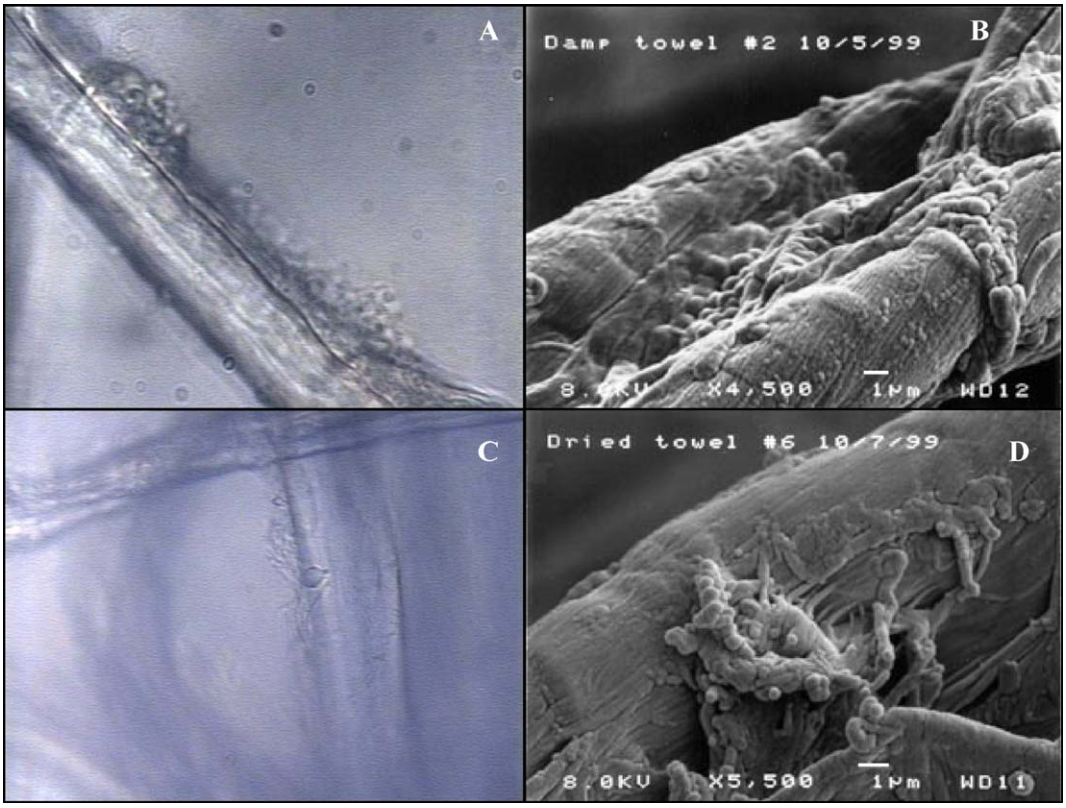

Fig. 7. Biofilm on towel fibers. (A) Damp towel: unstained, light microscopy image, 100× magnification; (B) damp towel (CSEM); (C) dry towel: unstained, light microscopy image, 100× magnification; (D) dry towel (CSEM).

(2001) provides a realistic test for mimicking recent cross-contamination events superimposed over endemic microbiota, however such a test is yet to be endorsed by regulatory agencies.

Our observations of the prevalence of biofilm on four common salad vegetables (tomatoes, carrots, lettuce and mushrooms) and on domestic household surfaces (cutting boards, sponges, socks and towels) have significant implications regarding the accurate assessment of antibacterial and cleansing efficacy of commercial sanitizing and cleaning products. This is especially relevant when the proposed product is to be used on fresh produce that will be incorporated into foods with minimal or no further processing or cooking, such as salads. Previous culturing analyses of lettuce, tomatoes, broccoli, and cauliflower purchased from grocery stores have detected coliform bacteria at densities of 10^5–10^7 CFU g^{-1} of produce (Albrecht et al., 1995). It is likely that a very large proportion of the endemic biofilm communities we observed were composed of non-pathogenic bacteria, and indeed a study of the microbial communities present on ready to eat lettuce found a predominance of Pseudomonads following storage at 10 °C (Rudi et al., 2002). It is however possible that potentially pathogenic microorganisms may sometimes become sequestered within the endemic biofilms, and be afforded some protection by these biofilms. Such sequestered pathogens may vary in their vulnerability to disinfection, and due to trans-location of biofilm clumps during food preparation, could result in other domestic surfaces, foods or utensils also becoming contaminated with biofilm-protected pathogens.

The sanitizers and detergents currently in use to address surface contamination are not generally tested against microbes within biofilms, and may be insufficient to remove or disinfect such microorganisms. The contamination of finished food products may occur

following contact with potentially detrimental bacterial sequestered within an equipment-associated biofilm (Zottola and Sashara, 1994). A study by Gibson et al. (1999) examined the efficacy of cleaning and disinfection protocols for removal of biofilms from industrial food factory surfaces. They found that the use of alkaline, acidic or neutral detergent prior to spraying a surface with water did not increase biofilm removal; however, the viability of *Pseudomonas aeruginosa* and *Staphylococcus aureus* (assessed using viable cell counts) was reduced when assayed immediately after treatment. In another study, adherent cells of *L. monocytogenes* were shown to be resistant to a variety of sanitizing products and their resistance was also surface-dependent, with polyester and polyurethane surfaces being the most difficult to disinfect (Krysinski et al., 1992). It has been demonstrated in several studies that rinsing with water is ineffective in removal of resident microflora. The inoculation of sterile bean sprouts with 10^8 CFU g^{-1} (*E. coli* or *Salmonella*), followed by triplicate washes in clean water, resulted in a reduction in viable cell numbers to 10^6 CFU g^{-1} (Raloff, 1998). The authors did not examine the bean sprouts after incubation to determine whether the cells were present as individual cells or whether biofilm formation had occurred. Even the inclusion of chlorination in a disinfection protocol may be ineffective in terms of completely removing or inactivating the microorganisms present. Beuchat et al. (1998) found that the use of chlorinated water for the washing of whole or cut produce had a mildly sanitizing effect, although the reductions in microbial numbers were generally less than 10^3 CFU following treatment with chlorine at 2000 ppm. Seo and Frank (1999) artificially inoculated lettuce leaves with *E. coli* O157:H7, and the leaves were treated with 20 mg l^{-1} chlorine for 5 min. After this time, viable cells could be visualized inside the leaf stomata, veins and cell wall junctions using fluorescein isothiocyanate and propidium iodide. These microorganisms were hypothesized to be protected from desiccation and environmental injury by the production of polysaccharide or other matrix material such as biofilm exopolysaccharides. This further reinforces the need for standardized, approved biofilm test methods that deal with the inherent difficulties associated with biofilm heterogeneity.

The formation and persistence of bacterial/fungal biofilms on fabrics is of particular importance with regard to laundry management in both hospital and food-processing environments. When washed inappropriately, fabrics can accumulate tenacious and difficult to remove encrustations of organic and inorganic substances. The amount of adhesion by *Bacillus megaterium* spores to encrusted fabrics has been shown to demonstrate a direct correlation with the amount of encrustation present (Ghione et al., 1989). In a study of soiled hospital linen, terry cloth towels were found to be colonized with 10^5–10^7 organisms 100 cm^{-2} (Smith et al., 1987), and the human pathogens *Klebsiella*, *Enterobacter*, *Serratia* and *Staphylococcus* spp. were identified as being present. Linen and clothing therefore represent potential sources of cross-infection (e.g., patient to patient or doctor to patient transfer). The observations and images obtained in this study clearly demonstrate the presence of biofilms on unlaundered used socks and towels, both damp and dry. Inadequate washing may leave behind both culturable and non-culturable biofilm microorganisms on the fabrics, from which subsequent bacterial regrowth can occur. Microorganisms that have the demonstrated potential to be transmitted via contaminated laundry include a number of important pathogenic species such as *Clostridium difficile*, *Cryptosporidium*, *P. aeruginosa*, Rotavirus, *Brucella* spp., Hepatitis C, *Streptococcus* spp., *Staphylococcus* spp., and *Mycobacterium fortuitum* (Bonifazi, 1999).

The carrot-, lettuce-, tomato- and mushroom-associated biofilms observed and described in this study suggest that there may be a need for fresh produce and surface sanitizing products that specifically target microbes present within this specialized mode. The observation of biofilms on a range of domestic fabrics also demonstrates a need to determine the efficacy of cleaning products such as anti-microbial laundry detergents against microorganisms present in this mode of growth. Consequently, standardized, biofilm test methods, such as the ASTM E2196-02 (http://www.astm.org), or as proposed by Harris et al. (2001), need to be evaluated for endorsement by regulatory agencies.

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References

Ak, N.O., Cliver, D.O., Kaspar, C.W., 1994. Decontamination of plastic and wooden cutting boards for kitchen use. Journal of Food Protection 57, 23–30.

Albrecht, J.A., Hamouz, F.L., Sumner, S.S., Melch, V., 1995. Microbial evaluation of vegetable ingredients in salad bars. Journal of Food Protection 58, 683–685.

Altekruse, S.F., Cohen, M.L., Swerdlow, D.L., 1997. Emerging foodborne diseases. Emerging Infectious Diseases 3 (3), 285–293.

Anon, 1993. On the cutting edge. Tufts University Diet and Nutrition Letter, 2–3.

Beuchat, L.R., Nail, B.V., Adler, B.B., Clavero, M.R., 1998. Effect of spray application of chlorinated water in killing pathogenic bacteria on raw apples, tomatoes and lettuce. Journal of Food Protection 61, 1305–1311.

Beumer, R.R., TeGiffe, M.C., Spoorenbert, E., Rombouts, F.M., 1997. *Listeria* species in domestic environments. Epidemiology and Infection 117, 437–442.

Bonifazi, W.L., 1999. Dirty laundry. Contemporary Long-Term Care 22 (9), 34–36.

Bruhn, C.M., 1997. Consumer concerns: motivating to action. Emerging Infectious Diseases 3 (4), 511–515.

Collins, J.E., 1997. Impact of changing consumer lifestyles on the emergence/reemergence of foodborne pathogens. Emerging Infectious Diseases 3 (4), 471–479.

Costerton, J.W., Lewandowski, Z., deBeer, D., Korber, D., James, G., 1994. Biofilms: the customized microniche. Journal of Bacteriology 176 (8), 2137–2142.

Fassel, T.A., Edmiston, C.E., 1999. Bacterial biofilms: strategies for preparing glycocalyx for electron microscopy. In: Doyle, R.J. (Ed.), Methods in Enzymology. Academic Press, San Diego, pp. 194–203.

Ghione, M., Parrello, D., Granucci, C., 1989. Adherence of bacterial spores to encrusted fabrics. Journal of Applied Bacteriology 67, 371–376.

Gibson, H., Taylor, J.H., Hall, K.E., Holah, J.T., 1999. Effectiveness of cleaning techniques used in the food industry in terms of the removal of bacterial biofilms. Journal of Applied Microbiology 87, 41–48.

Harris, L.J., Beuchat, L.R., Kajis, T.M., Ward, T.E., Taylor, C.H., 2001. Efficacy and reproducibility of a produce wash in killing *Salmonella* on the surface of tomatoes assessed with a proposed standard method for produce sanitizers. Journal of Food Protection 64 (10), 1477–1482.

Herwaldt, B.L., Ackers, M.L., and the Cyclospora Working Group, 1997. An outbreak in 1996 of cyclosporiasis associated with imported raspberries. The New England Journal of Medicine 336 (22), 1548–1556.

Hood, S.K., Zottola, E.A., 1997. Isolation and identification of adherent Gram-negative microorganisms from four meat-processing facilities. Journal of Food Protection 60, 1135–1138.

Krysinski, E.P., Brown, L.J., Marchisello, T.J., 1992. Effect of cleaners and sanitizers on *Listeria monocytogenes* attached to product contact surfaces. Journal of Food Protection 55, 246–251.

Kurtzweil, P., 1995. Can your kitchen pass the food safety test? FDA Consumer, 14–19.

Le Chevallier, M.W., Cawthon, C.D., Lee, R.G., 1988. Inactivation of biofilm bacteria. Applied and Environmental Microbiology 54, 2492–2499.

McDonough, L.C., 2000. Ways to germ-proof your kitchen. Reader's Digest, 100–102.

Park, P.K., Cliver, D.O., 1996. Disinfection of household cutting boards with a microwave oven. Journal of Food Protection 59, 1049–1054.

Raloff, J., 1996. Sponges and sinks and rags, oh my! Where microbes lurk and how to rout them. Science News, 172–174.

Raloff, J., 1998. Wash-resistant bacteria taint foods. Science News, 340–341.

Rudi, K., Flateland, S.L., Hanssen, J.F., Bengtsson, G., Nissen, H., 2002. Development and evaluation of a 16S Ribosomal DNA array-based approached for describing complex communities. Applied and Environmental Microbiology 68 (3), 1146–1156.

Seo, K.H., Frank, J.F., 1999. Attachment of *Escherichia coli* O157:H7 to lettuce leaf surface and bacterial viability in response to chlorine treatment as demonstrated by using CSLM. Journal of Food Protection 62, 3–9.

Smith, J.A., Neil, K.R., Davidson, C.G., Davidson, R.W., 1987. Effect of water temperature on bacterial killing in laundry. Infection Control 8, 204–209.

Takeuchi, K., Frank, J.F., 2001. Direct microscopic observation of lettuce leaf contamination with a prototype fruit and vegetable washing solution and 1% NaCl-$NaHCO_3$. Journal of Food Protection 64 (8), 1235–1239.

Tauxe, R.V., 1997. Emerging foodborne disease: an evolving public health challenge. Emerging Infectious Diseases 3 (4), 425–434.

Torok, T.J., Tauxe, R.V., Wise, R.P., Livengood, J.R., Sokolow, R., Mauvais, S., Birkness, K.A., Skeels, M.R., Horan, J.M., 1997. A large community outbreak of salmonellosis caused by intentional contamination of restaurant salad bars. JAMA 278 (5), 389–395.

Wirtanen, G., Husmark, U., Mattila-Sandholm, T., 1996. Microbial evaluation of the biotransfer potential from surfaces with *Bacillus* biofilms after rinsing and cleaning procedures in closed food-processing systems. Journal of Food Protection 59, 727–733.

Zottola, E.A., Sashara, K.C., 1994. Microbial biofilms in the food processing industry: should they be a concern. International Journal of Food Microbiology 23, 125–148.