Microscopy and Hygiene

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Most people know that it is important to adhere to hygienic rules - to wash their hands, to brush their teeth and to avoid unsanitary conditions in order to prevent infectious diseases. Electron microscopy shows that micro-organisms, particularly bacteria and fungi (moulds) are ubiquitous - on our skin, in our mouth and intestines, in foods, in the streets, on money, in animal and bird excrement, in the air - and they rapidly multiply in favourable conditions. This presentation shows bacteria at high magnification in various environments and in false colours.

Introduction

Until the invention of the electron microscope, people could not imagine how single-cell micro-organisms such as bacteria looked as three-dimensional objects. Yet, we all live with them in harmony irrespective of where they have colonised us - on the inside or outside. Most bacteria are beneficial to us, but a relatively small number of them are pathogenic and cause diseases. Our desire to stay healthy compels us to obey "hygienic rules" to keep the environment and our bodies clean and to avoid contact with infectious agents in order to prevent infection and illness.

There are many ways in which pathogenic bacteria invade animals and humans - through the air (e.g., *Mycobacterium tuberculosis*), through water (e.g., *Vibrio cholerae*), through food (e.g., various strains of *Salmonella, Campylobacter jejuni, Listeria monocytogenes*, toxigenic *Escherichia coli* O157:H7, etc.), or through direct body contact, including medical personnel not adhering to hygienic regulations. Scanning electron microscopy (SEM) shows a variety of bacteria in our environment. The micrographs may appear more appealing to viewers if they are shown in false colours as this form makes the message easier to remember. This article is focussed on bacteria with which most of us are in contact almost every day.

Bacteria in the human digestive system

Single-cell micro-organisms (microbes) were discovered by dedicated researchers such as Robert Koch, Louis Pasteur, Paul de Kruif and others in the 18th century. The primary objective was to explain the cause of diseases. The optical microscope revealed the causative agents as minuscule rods (bacilli), spirals (spirochetes) and dots (cocci).

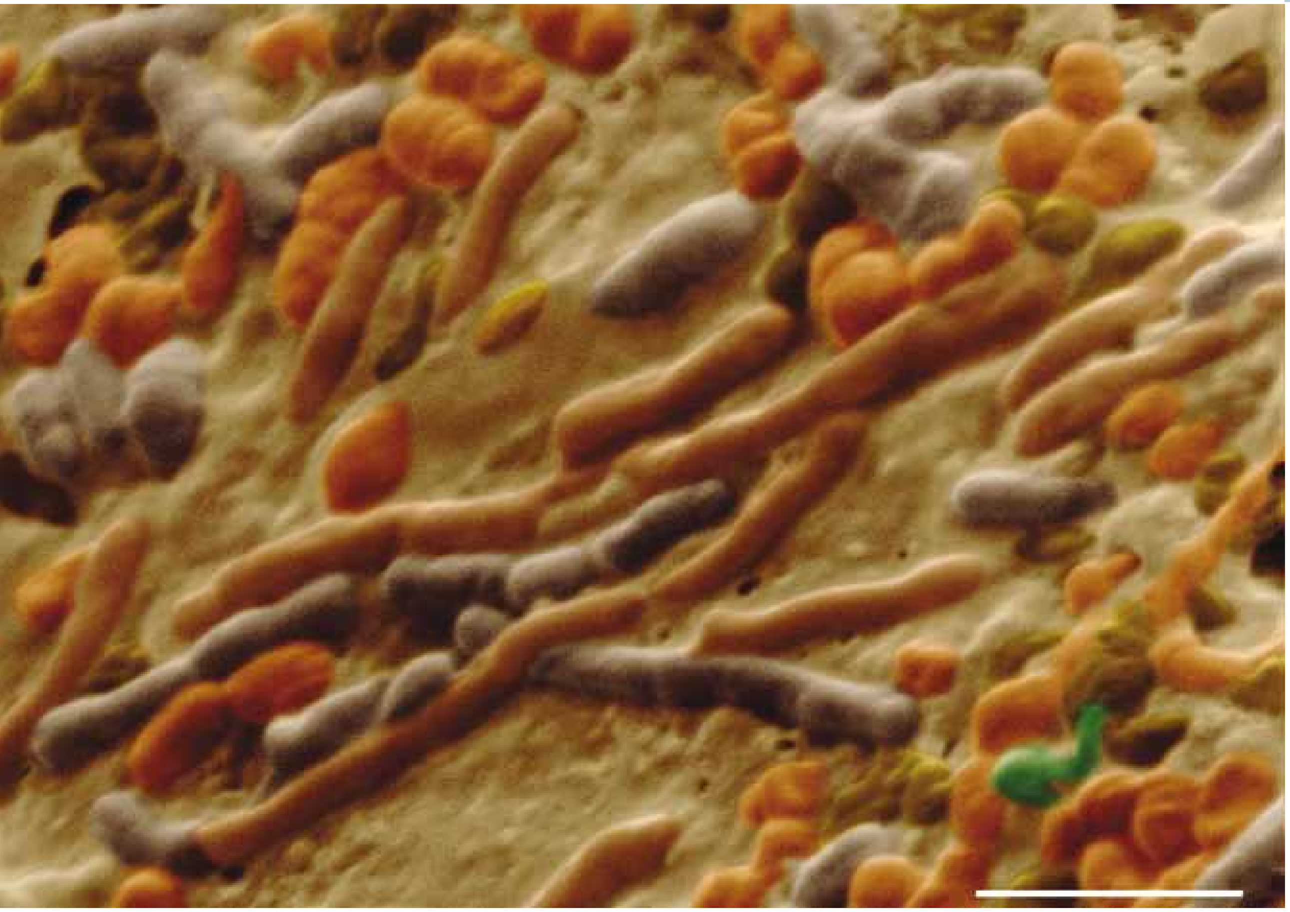

Fig. 1: Bacteria form a dental plaque. An extracted tooth was obtained in dry form from a dentist. It was coated with gold and photographed by SEM. Bar: 2 µm.

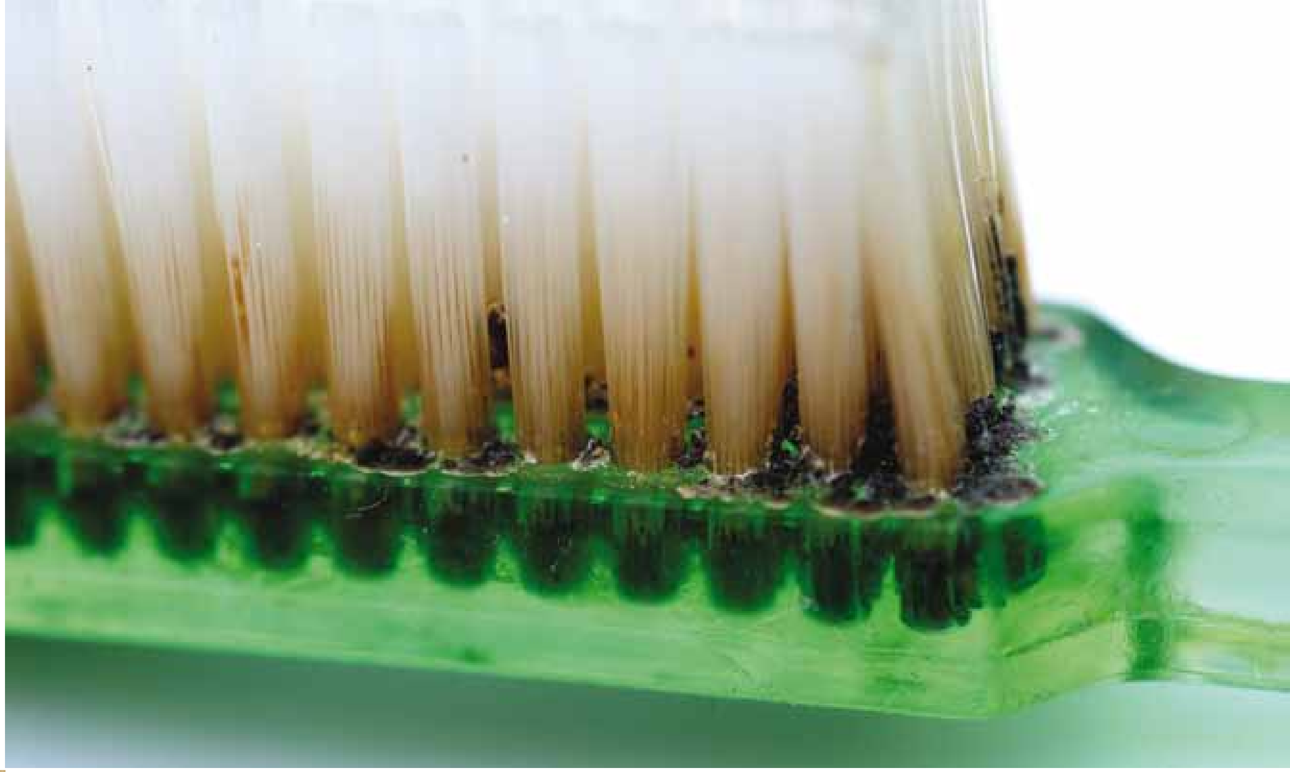

Fig. 2: An old toothbrush harbouring large quantities of dirt and bacteria.

Most pathogens hazardous to humans are associated with foods which bacteria may invade from various sources. After they are ingested, they can cause food poisoning depending on the immunity of the dining individual, the nature of the pathogen and the degree of food contamination. As soon as food is placed in the mouth, irrespective of whether it contains any micro-organisms, it encounters commensal bacteria, i.e. normally harmless bacteria which live in symbiosis with us and actually protect us, to some extent, from the pathogens. There are about 100 trillion commensal bacterial cells in our body - 10-times as many as our own body cells (Internet 1). Bacteria on the teeth are the first to meet the food. They use minute amounts of food to grow in the form of a plaque (Figure 1).

Dental plaque is an example of a biofilm or a community of micro-organisms attached to a wet or moist surface (Internet 2). In general, bacteria, fungi (yeasts) and algae may all participate together to form biofilms consisting, eventually, of complex structures designed to protect their members. It is more difficult to remove biofilms than individual bacteria.

Metabolites of dental plaque bacteria contain organic acids which are corrosive and contribute to the development of dental cavities. The proper hygienic procedure to prevent this from happening is to brush the teeth after every meal and to use dental floss to remove both the food residues and the bacteria. In spite of the use of toothpaste on the toothbrush and washing the brush after use, bacteria gradually develop on it (Figure 2) and even contaminate surfaces with which they may be in contact in the bathroom (Figure 3). Dental floss is used to clean areas between teeth (Figure 4) and is discarded after use.

In a healthy mouth, saliva protects the teeth and gums from harmful micro-organisms through histatins and cystatins. The former proteins punch holes

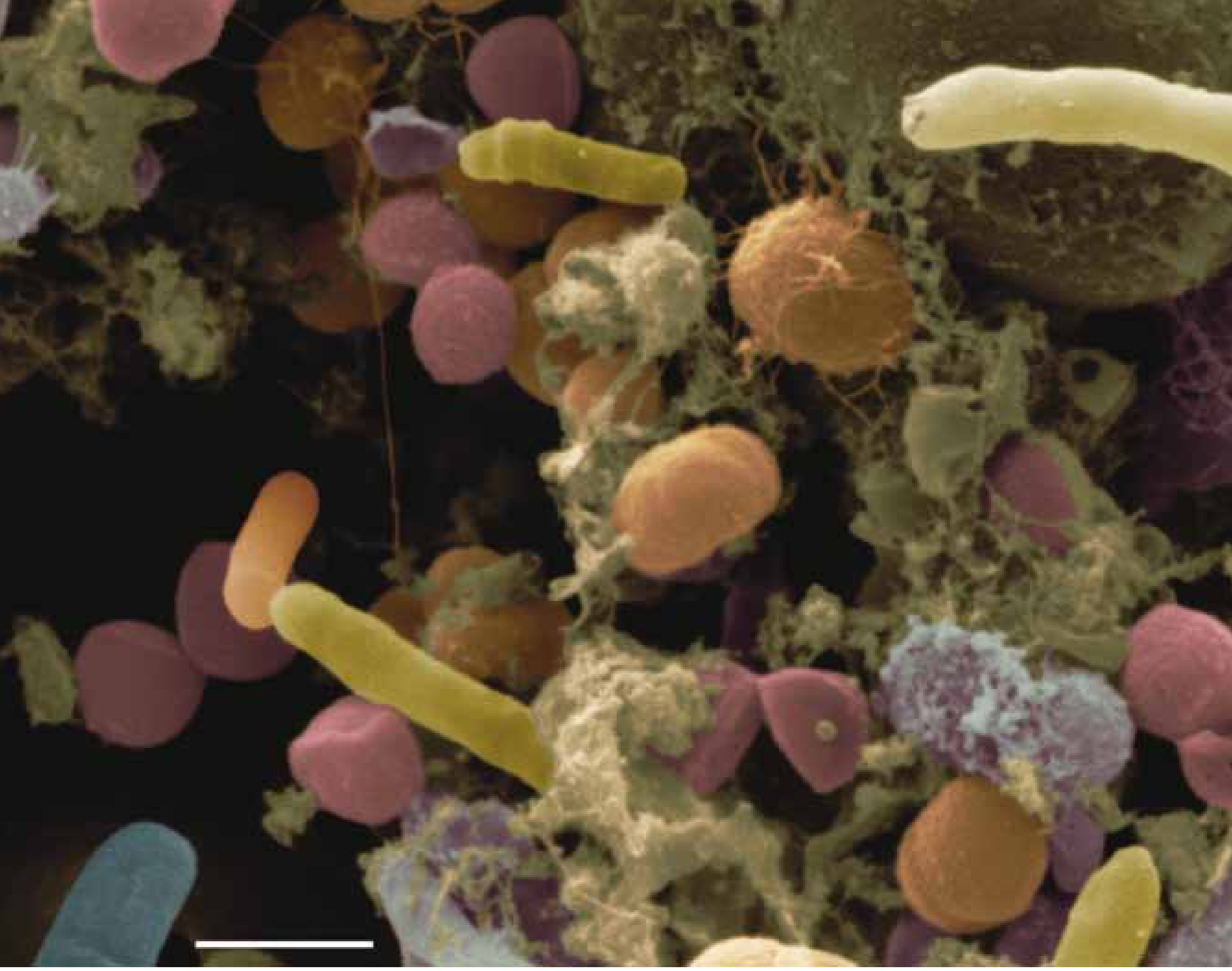

Fig. 3: Bacteria on the bristles of an old toothbrush. Bar: 2 µm.

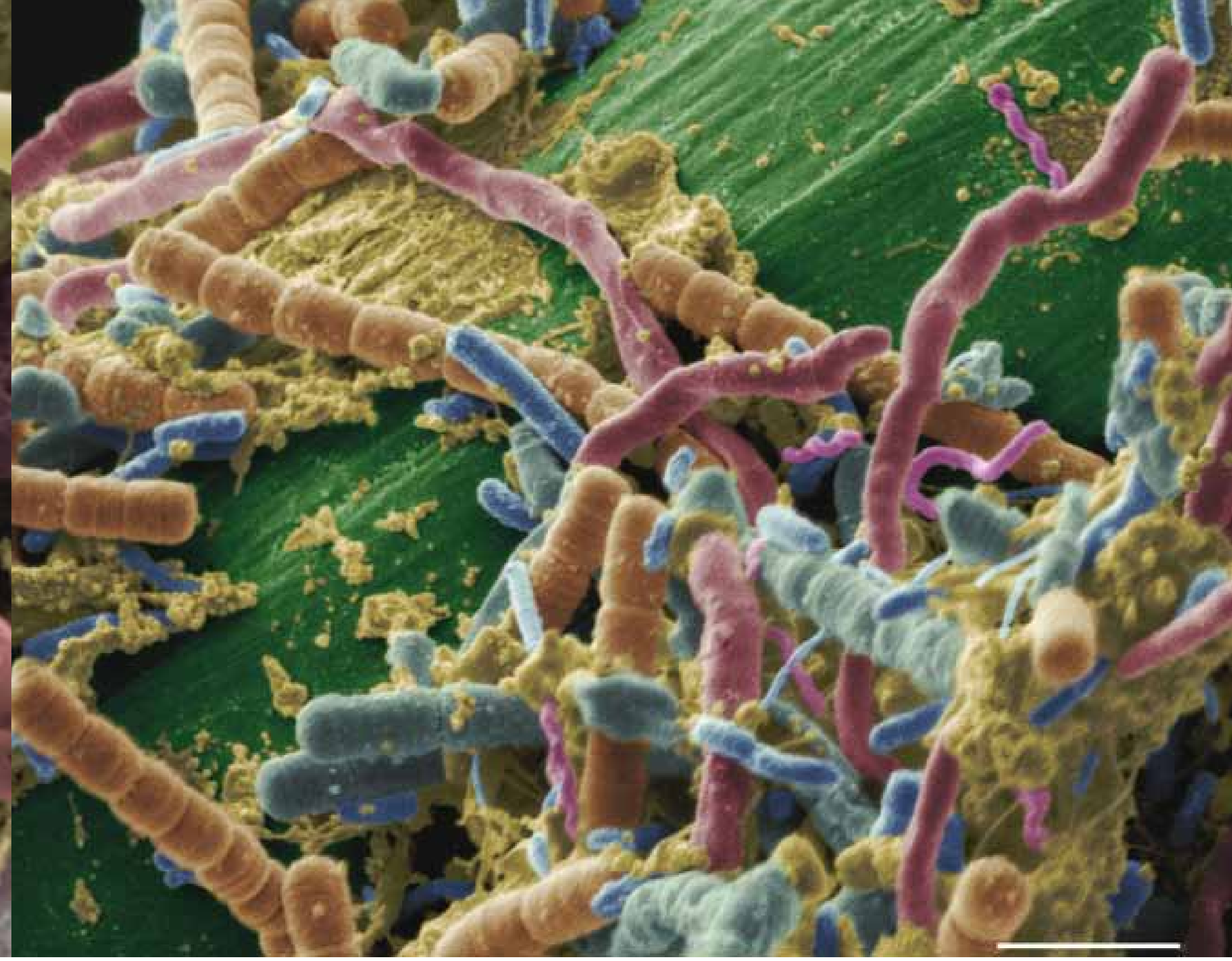

Fig. 4: Bacteria removed by dental floss from teeth. Bar: 2.5 µm.

in *Candida albicans* cell membranes and the latter proteins interfere with thiol proteases, which are important in the replication of viruses and bacteria (Levine, 1993). Yet, every person has many genera such as *Actinomyces*, *Enterobacter*, *Fusobacterium*, *Granulicatella*, *Haemophilus*, *Leptotrichia*, *Neisseria*, *Porphyromonas*, *Prevotella*, *Rothia*, *Serratia*, *Streptococcus* and *Veillionella* in their mouth (Internet 3). Images of many individual strains may be found on the websites of photo banks specialising in medical or scientific images (e.g., www.cmsp.com, www.mediscan.co.uk, www.sciencephoto.com, www.sciencesource.com, www.visualsunlimited.com, etc.).

It is natural to have a healthy population of micro-organisms living in the mouth, including on the tongue. They may range from a few (Figure 5) to a thick coating (Internet 4). Bacteria get into our mouths with foods, toys, hands, water, even the air - as early as the time of birth. If the tongue is healthy, it should be slightly moist, smooth, and pinkish in color. Some people, however, are finding that their tongue has a white coating, that may be the breeding ground for anaerobic bacteria which break down proteins in the absence of oxygen and produce malodorous sulfhydryl substances that cause bad breath (halitosis). Simple devices are commercially available to remove the coating from the tongue.

The role of tongue bacteria was found using laboratory rats. They harbour bacteria which reduce nitrates to nitrites. The most commonly found nitrite-producing organism is *Staphylococcus sciuri,* followed by *Staphylococcus intermedius*, *Pasteurella spp*., and finally *Streptococcus spp*. Nitrites produced on the tongue form nitric oxide in the acidic environment of the stomach. Since nitric oxide has antimicrobial properties, nitrate-reducing bacteria on the tongue may function as a defense against food-borne pathogens (Li *et al*., 1997).

Most bacteria are killed in the stomach, where a combination of hydrochloric acid and pepsin destroys them. *Helicobacter pylori* is an exception that is adapted to gastric conditions and in some people causes gastric ulcers. The subject is so important that a scientific journal, "Helicobacter", is being published bimonthly by Wiley-Blackwell.

As the foods that are partially digested in the stomach proceed into the small intestine, a rich nutritional mixture is created also for a large number (about 10,000/mL) of bacteria. Their number increases up to 1 x 10^9/mL in the colon. The dry matter of human stool contains about 60% of bacteria by mass (Guarner & Malagelada, 2003). The number of individual species is estimated at 300 - 1000 (Sears, 2005), with some estimates over 1000 (Internet 5); however, most are at 500 (Steinhoff, 2005). Many of these species are being identified by microbiologists using new molecular methods.

One particular bacterium, *Escherichia coli*, is characteristic of the intestinal contents. Its common name is "the intestinal rod". Wherever it is found, it indicates the presence of faecal material. *E. coli* did not harm us until several years ago when some of the bacteria mutated into an enterohaemorrhagic bacterium *E. coli* O157:H7 (Karch *et al*., 2005) and other serotypes, which may be deadly to humans through their Shiga toxin. This bacterium appeared in the year 2000 as a contaminant in drinking water in the small Canadian town of Walkerton (Prudham, 2004). As a result, 7 people died and over 20 became severely ill. Personal hygiene such as washing one's hands with soap and water would not protect the citizens in these cases, where communal hygiene plays vital role by taking care of the environment and providing the citizens with clean water and also ensuring that the air, the land and the water are not being contaminated.

Faeces are not homogeneous or uniform either macroscopically or when viewed using the microscope. Bacteria are prevalent but indigestible food components such as cellulose and lignin may also be found as an indication that fruits and vegetables had been consumed (Figure 6).

Bacteria may not be identified by microscopy

Electron microscopy alone is not sufficient to identify different strains of bacteria although it shows details of their anatomical features such as appendages (flagella and pili or fimbriae), the cell envelope (capsule, cell wall, and plasma membrane) and the cytoplasmic region (Internet 6). Microbiological procedures are thus used to identify them. Bacteria collected using a cotton swab must be first isolated as a single colony, cultured on specific nutrient media, and tested for their dependence on individual essential factors and for their metabolites. Recently, the nucleotide base sequence of the gene which codes for 16S ribosomal RNA is becoming an important standard for the definition of bacterial species (Internet 7). How is *E. coli* identified? During growth on a special medium such as MacConkey agar, deep-red colonies develop (Internet 8) when *E. coli* bacteria metabolise lactose present in the medium and the resulting lactic acid decreases its pH value turning the Neutral Red indicator red. Unlike most *E. coli* strains, *E. coli* O157:H7 does not metabolise sorbitol. Substituting it for lactose in the medium (abbreviated SMAC) makes it possible to detect *E. coli* O157:H7 in a bloody stool by differential analysis at 100% sensitivity, specificity of 85% and accuracy of 86% (March & Ratnam, 1986).

Although SEM cannot be used to distinguish which bacteria are harmless and which are pathological,

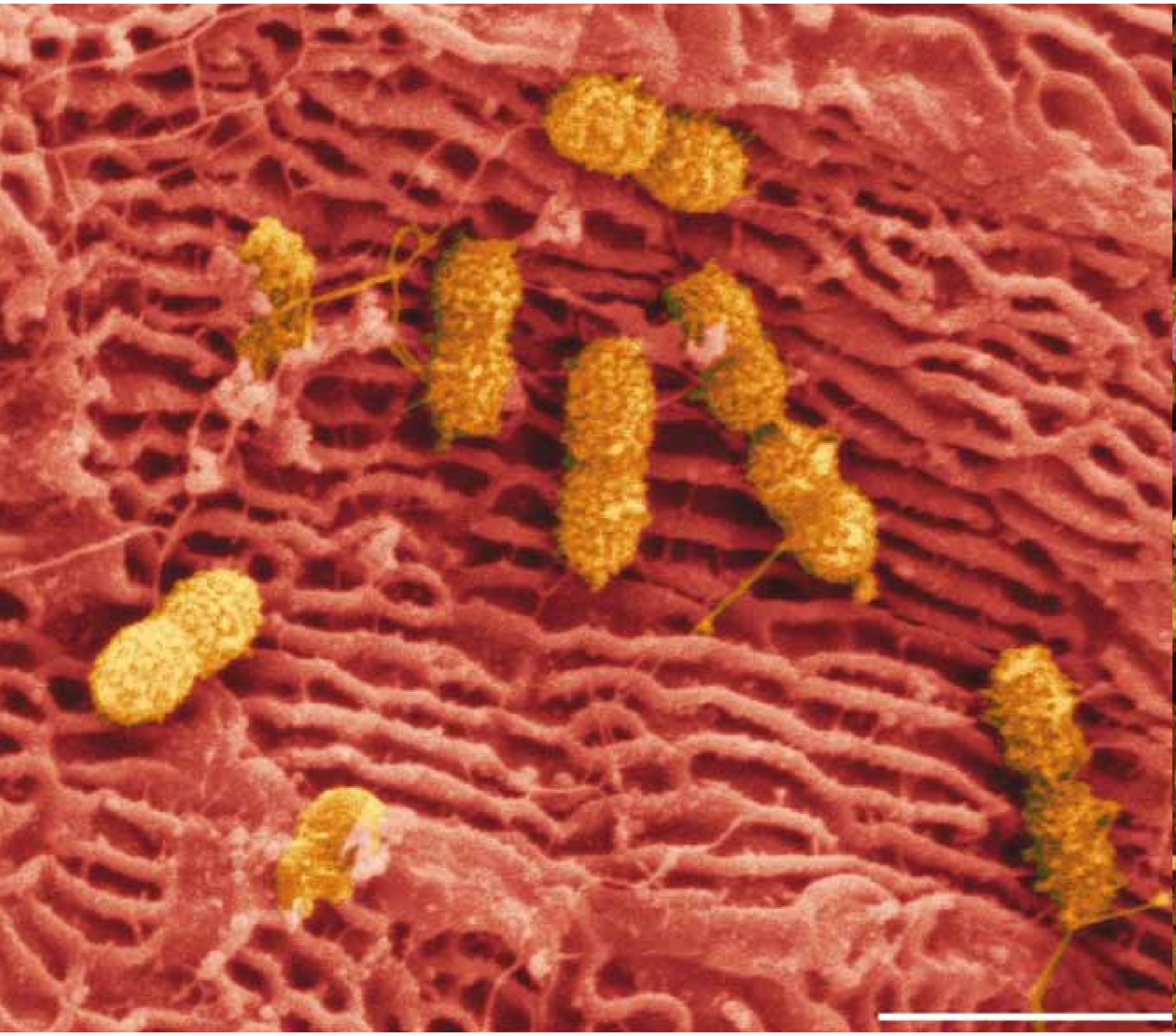

Fig. 5: Human tongue bacteria on a loose epithelial cell. Bar: 2 µm.

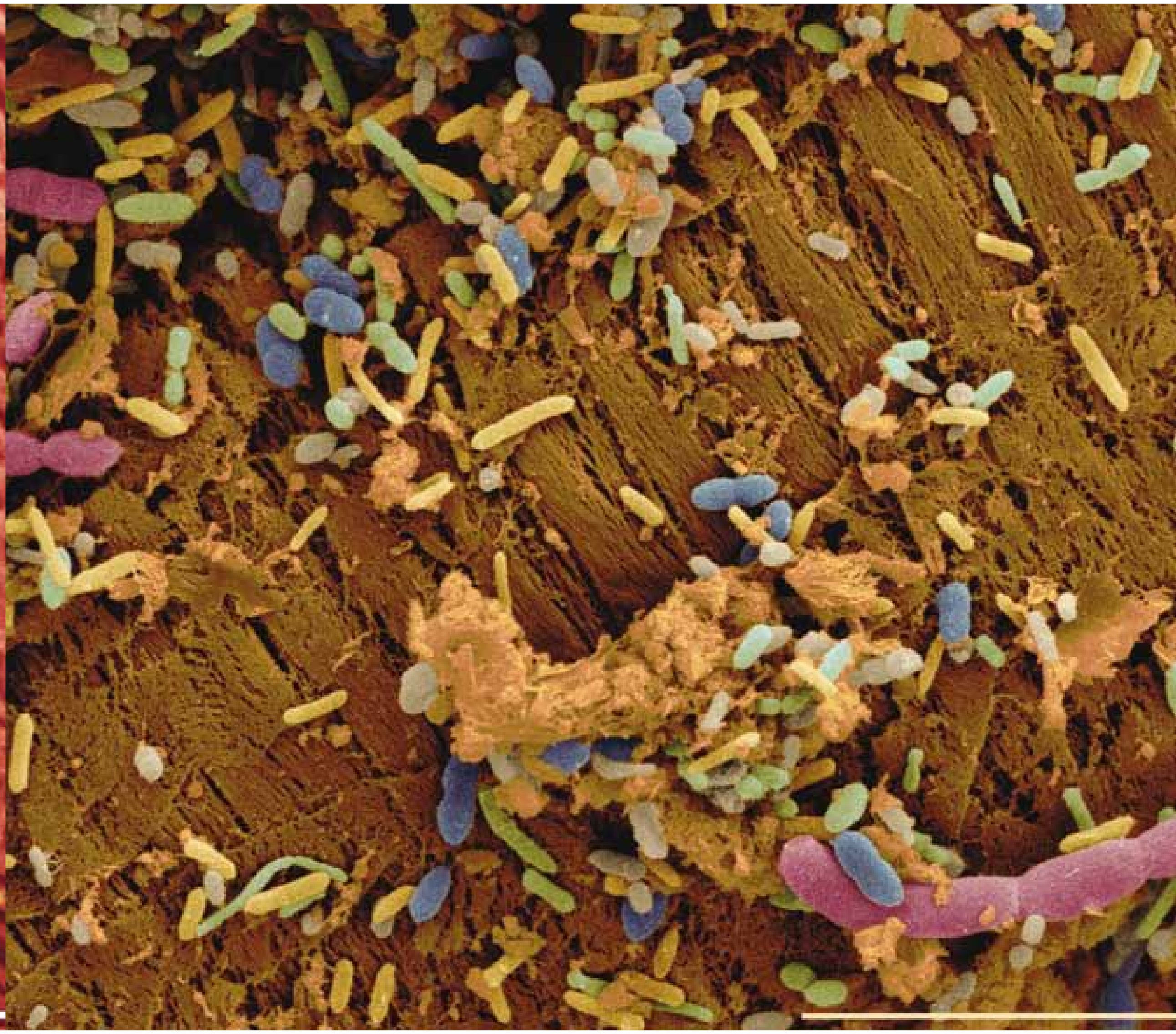

Fig. 6: Bacteria colonising plant tracheids from ingested raw vegetables in human faeces. Bar: 2 µm.

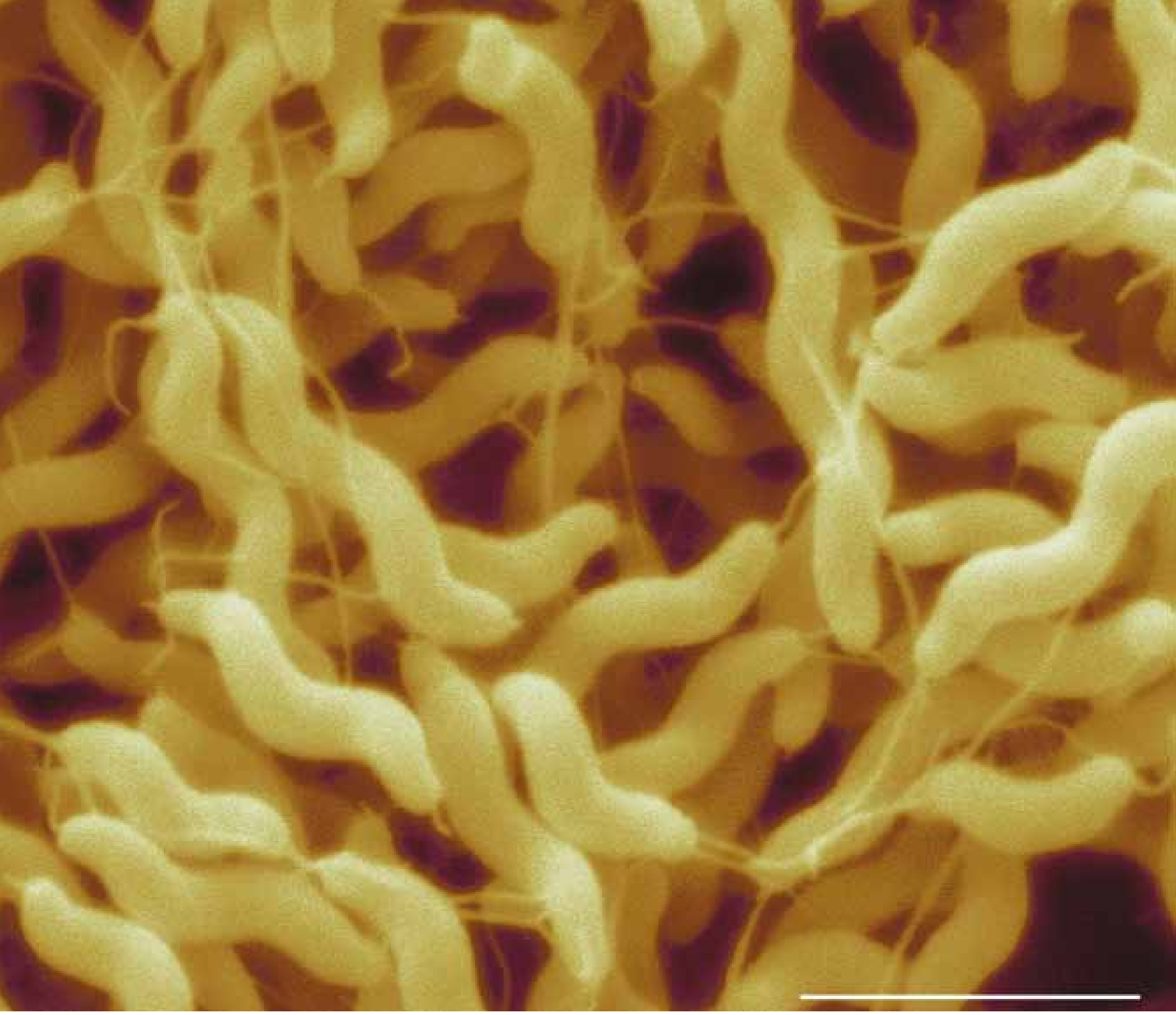

Fig. 7: Campylobacter jejuni bacteria have characteristic shapes. Bar: 2 µm.

there are a few exceptions such as *Campylobacter jejuni* which have characteristic features (Figure 7). This bacterium was at one time responsible for over 70% of food poisoning in the UK and is the most common cause of bacterial foodborne illness in the United States (Internet 9). Food poisoning caused by it can be severely debilitating. Pathogens such as *E. coli* O157:H7, *Salmonella spp*., *Campylobacter spp*., and *Yersinia enterocolitica* frequently cause outbreaks due to contamination of water or foods. As the epidemiology of food-borne disease is changing, new pathogens are emerging and some have spread worldwide. Many, including those mentioned above, have reservoirs in healthy food animals, from which they may spread to a variety of foods including raw milk and raw eggs (Internet 10). These pathogens cause millions of cases of sporadic illness and chronic complications, as well as large and challenging outbreaks over many geographic areas (Tauxe, 1997).

My first encounter with faeces was with babies' stools

In my professional career, I encountered faeces relatively early, when I developed an apparatus for the fermentation of faeces of newborn babies. Why did I do it? In the late 1950's, medical researchers learned that the new gas-liquid chromatographic analysis made it possible to analyse various gases including those developing in human intestines. Newborn babies are known to suffer from abdominal pain but they are unable to communicate their feelings

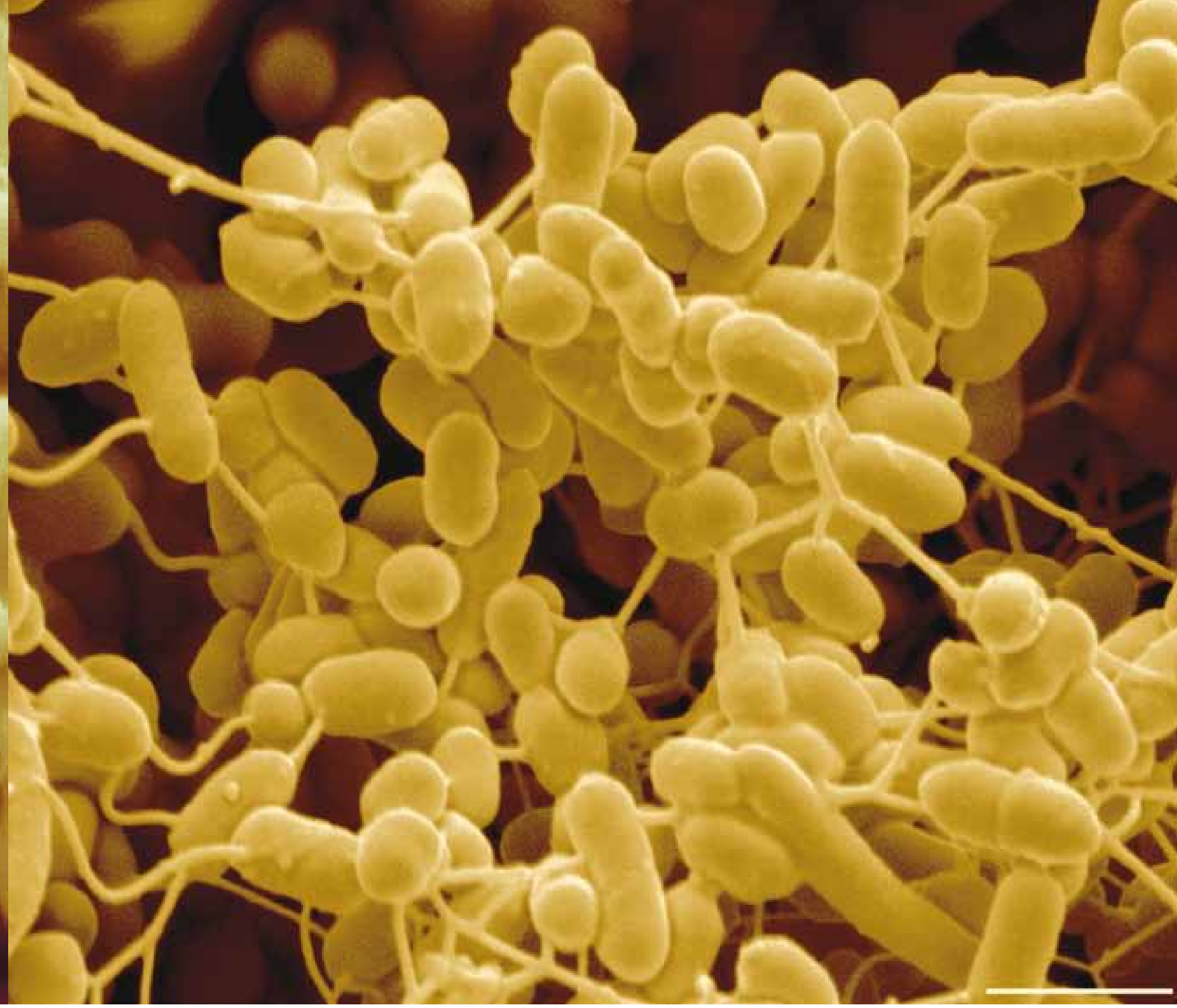

Fig. 8: Bacteria rapidly propagated on chicken skin kept at 22°C for 2 days. Bar: 2 µm.

in a way other than crying. It was assumed that the bad odour of such gases may be associated with digestive and intestinal disorders. It would not be easy to obtain intestinal gases from a newborn baby, so fermenting the faeces in the absence of air was hoped to result in gases similar in their composition to the gases produced by micro-organisms in the intestine. The glass apparatus available did not do the job for which it had been designed at the end of the 19th century. It exploded with very unpleasant consequences. As an engineer, I designed a better apparatus and soon I was producing enough samples to keep a medical chemistry technician busy running a gas-liquid chromatograph. The medical professors did not take me as a co-author of their findings (Němečková *et al*., 1961) but advised me to separately publish the description of my invention (Kaláb, 1961).

Should we put this into our mouths?

People prevent pathogenic bacteria from taking hold in their foods by using a variety of culinary procedures of which heat processing has the longest history. On the other hand, there are so-called probiotic bacteria, for example lactic acid bacteria, which convert some raw foods into new products (e.g. cabbage into sauerkraut, milk into yoghurt or cheeses etc.) and, thus, are useful as they also ward off pathogens from our bodies. In addition, the intestinal contents of all animals and birds also contain large quantities of commensal bacteria which are then excreted as faeces.

It seems difficult to prevent bacterial contamination of our foods because bacteria may not be seen by a naked eye and the effects of a good hygienic behaviour may not be immediately assessed. Any negligence, however, may have grave consequences in the form of painful food poisoning. Retail meats may be contaminated by (invisible) faecal micro-organisms. If, for example, a piece of retail chicken meat is left at room temperature, it will be covered soon with a large number of bacteria (Figure 8), mostly *Salmonella spp*. There have been trends to colonise the digestive tract of broilers with probiotic bacteria (Haghighi *et al*., 2006) to reduce the risk of food poisoning if the faeces contaminate the meat. I had the opportunity to view the distribution of bacteria in chicken intestines as part of similar experiments (Wheatcroft, 2005). Their highest numbers were found in the caecum (Figure 9). Is the caecum a redundant organ only ready to cause health problems in humans? Bollinger *et al*. (2007) do not think so. They have found that the caecum in humans may serve as a reservoir of the intestinal microflora and replenish the intestines if the regular microflora is depleted by the use of oral antibiotics.

An environment contaminated with faeces is probably one of the reasons that *Salmonella*

Fig. 9: Bacteria are stored in the caeca in chickens. Many are encased in polysaccharide capsules (fixed using Ruthenium Red). Some capsules have been opened by freeze-fracturing to reveal the bacteria inside. Bar: 2 µm.

bacteria may be found in egg yolks. In the USA, over 500 million eggs were recalled in the summer of 2010. More than 2000 people suffered from food poisoning when raw eggs were consumed in the form of mayonnaise, custard etc. Vaccination of hens against *Salmonella* in the United Kingdom ensured the production of eggs free of bacteria (Internet 11).

Inspiration for microscopy can come from various sources. Observing once Mr. J. F. Ceprano, a well-known rock artist, as he built rock sculptures directly in the shallow water of the Ottawa River in Canada (Internet 12), I noted that he had many "helpers" nearby - ducks, gulls and, particularly, Canada geese. Mr. Ceprano had no kind words for these large birds as he accused them of causing him severe diarrhoea. Although he used rubber gloves to handle the river rocks, he remembered that when he got river water into his mouth, he then became severely ill. I took samples of the water and goose faeces (Figure 10) and then examined them by SEM. At a low magnification, they show only partially digested grass (Figure 11). van der Wal and Loonen (1998) reported that Svalbard reindeer on Spitsbergen consumed goose droppings because other suitable food is scarce. At a higher magnification, goose faeces reveal a variety of bacteria (Figure 12). Spirochetes common to poultry droppings that cause gastrointestinal illness have also been reported in Canada goose droppings - they should be thoroughly cleaned from shoes, and the hands should carefully be washed if people come in contact with them (Internet 13).

Droppings of free-range hens, which searched for food in a forested area, differed from goose droppings. Macroscopically, the goose droppings were well formed - some people call them "cigars"- but hen droppings were thin with no specific shape. Using the microscope, slim pointed bacteria resembling *Borrelia burgdorferi* were found on them on several occasions (Figure 13). The situation is very different in the litter at commercial poultry farms. Antibiotics used in chicken feed to prevent

Fig. 10: Canada goose droppings in a city park.

disease lead to antibiotic-resistant bacteria which exchange resistance genes and mobile elements called integrons with other bacteria (Nandi *et al*., 2004). Resistance thus spreads in the litter and into other ecosystems.

Do we have to be concerned also with the faeces of wild animals? As recalls of field lettuce and spinach in the USA showed several years ago, wild animals frequenting the fields, defecate on the produce (Matthews, 2009) and contaminate them mostly with *Salmonella spp*., *Shigella spp*., and *Listeria spp*., *Escherichia coli* O157:H7, parasites such as Cryptosporidium and viruses, particularly Norovirus (Keller 2009), earlier known as "Norwalk-like virus". Manure from domestic animals must now be composted to kill human pathogens before it may be used as a source of nutrients for plants destined for human consumption (Jiang & Shepherd, 2009). Many food-poisoning outbreaks are associated with dining in restaurants. However, salad greens cannot be washed clean of the bacteria because part of them remain hidden in the leaf stomata (Figure 14).

In the spring this year, an *E. coli* outbreak in Germany resulted in more than 3000 people falling

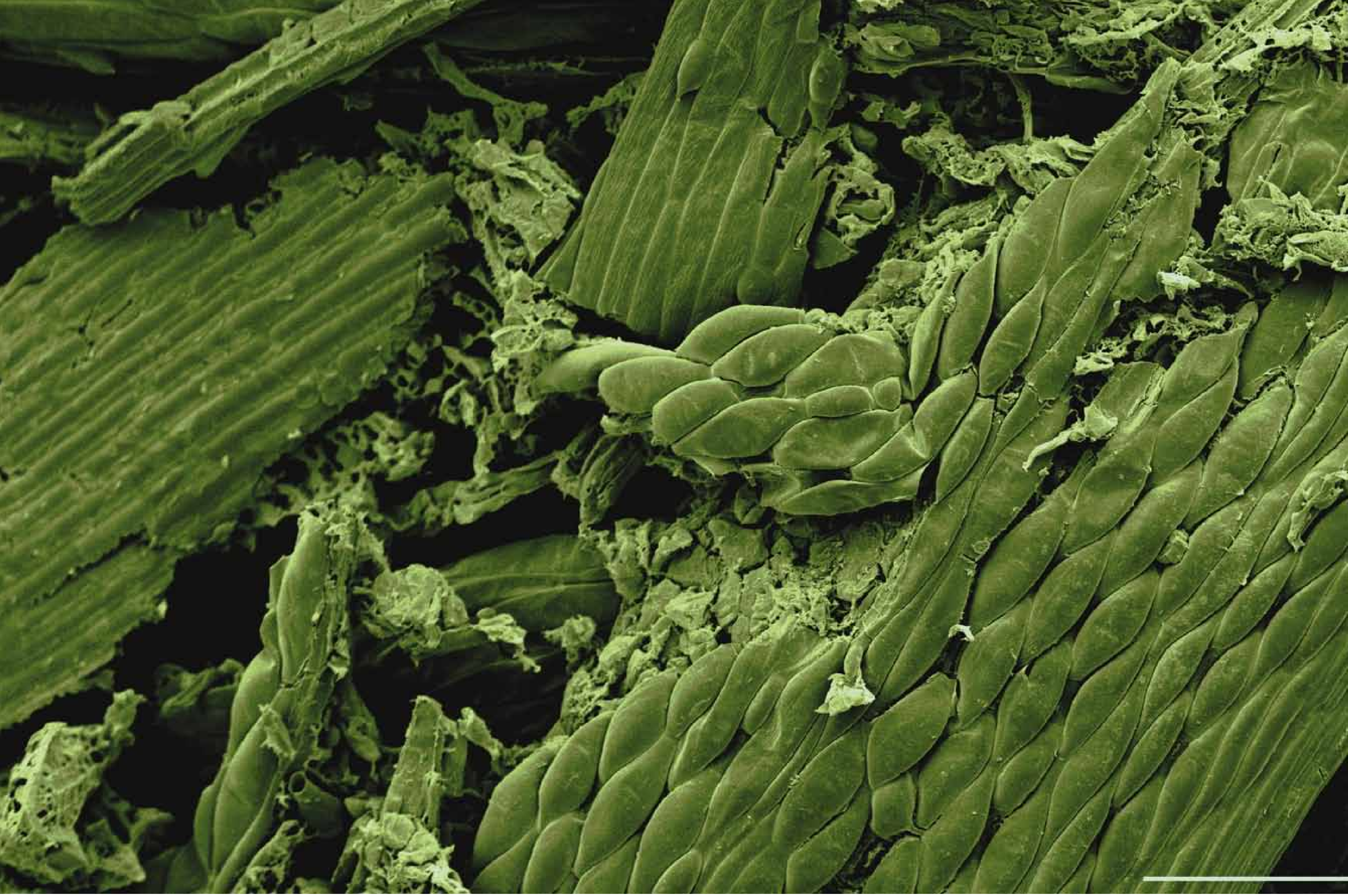

Fig. 11: Partially digested grass fragments are noticeable in goose droppings at a low magnification. Bar: 20 µm.

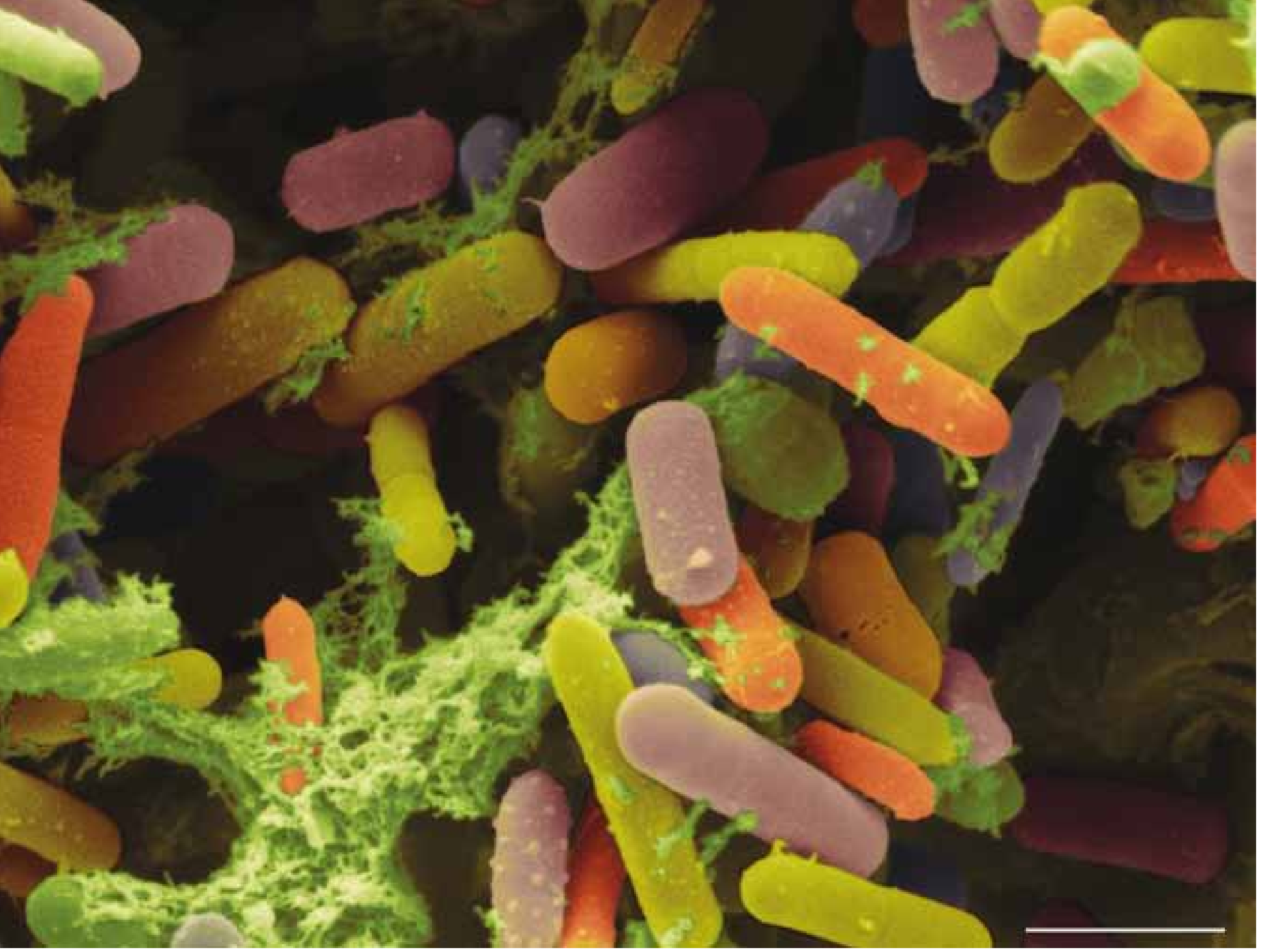

Fig. 12: The microflora in Canada goose droppings consists mostly of bacilli (rods). Bar: 2 µm.

ill, of which about 100 needed kidney transplants or life-long blood dialysis. Over 35 people died. The new *E. coli* strain of German origin, which contaminated bean sprouts, produces a potent toxin that causes neurological disorders in addition to the typical epidemiology of diarrhoea. This may include paralysis.

The real danger of the bacterium contaminating beans and other seeds from which sprouts are grown highlights that lapses in hygiene may have fatal consequences on a large scale wherever mass food production is concerned. For consumers it means that all potentially risky raw foods must be thoroughly cooked.

Where else may food pathogens be found?

Food pathogens may be contracted even in an environment free of land animals. Many people like to watch birds and provide feeders and bird baths for them. If bird bath water is not changed very often, particularly in warm weather, it may be a rich source of a variety of bacteria (Figure 15). The birds have a peculiar behaviour: they land on the rim of the bath, drink water, jump into the bath and bathe, then they jump up on the rim and defecate into the water and then they leave. Another bird repeats this sequence so that soon a microbiologist has a dense faecal suspension in the bird bath to sample.

Whereas Canada goose droppings are a seasonal hazard in North America and bacterial hazards of poultry droppings and bird bath water are limited, there are considerably more common dog (Figure 16) and, less frequently, cat faeces (Figure 17) occurring fresh daily year around in the streets and playgrounds. Municipal by-laws ordering the removal of such faeces from public places are good only if they are enforced. Rain disintegrates the faeces and a subsequent sunny period turns them into windborne particles which may be inhaled by unsuspecting humans.

There is, however, a risk of making airborne particles of human stool even in the comfort of a well-kept home. The culprit is the flushing toilet (Barker & Jones, 2005). The rapid flow of water produces an aerosol and minute droplets have been found to travel from the toilet bowl. A higher frequency of flushing toilets in public places such as restaurants, sport events, supermarkets etc. of course increases the amount of the aerosols formed.

To test the theory, I have attached plastic cover slips through a double-sided sticky tape to the rims of several toilet bowls in a private home for a week and then examined the cover slips by SEM (Figure 18). Bacteria are in our mind associated with toilets. They are, however, also associated with the kitchen sink where the dishes are being washed by hand with a rag or a sponge and hot water. Food residues provide nutrients for the bacteria whose counts double approximately every 20 minutes. Wet sponges and rags left in the sink or other moist places provide ideal conditions for the bacteria to multiply. Health experts advise (Internet 14) the use of rags rather than sponges, thoroughly wash either of them and let them dry before the next use. Would bacteria grow in a stainless steel sink, if it is only rinsed with hot water but is not cleaned with bleach and a rag after every dish washing? A stainless steel disk was attached to the bottom of a sink and was not touched for 2 weeks whereas the rest of the sink was cleaned properly. After that time, the disk was found to be covered with bacterial colonies (Figure 19).

In contrast with toilet aerosol particles, bacteria should not be associated with drinking (tap) water. It is common knowledge, however, that unless drinking glasses and cups are washed in a dishwasher or with a cloth, and then dried, an unsightly slimy coating develops on the glass walls. To test what develops in the glass, a plastic cover slip was attached to the bottom of a glass which was used twice a day

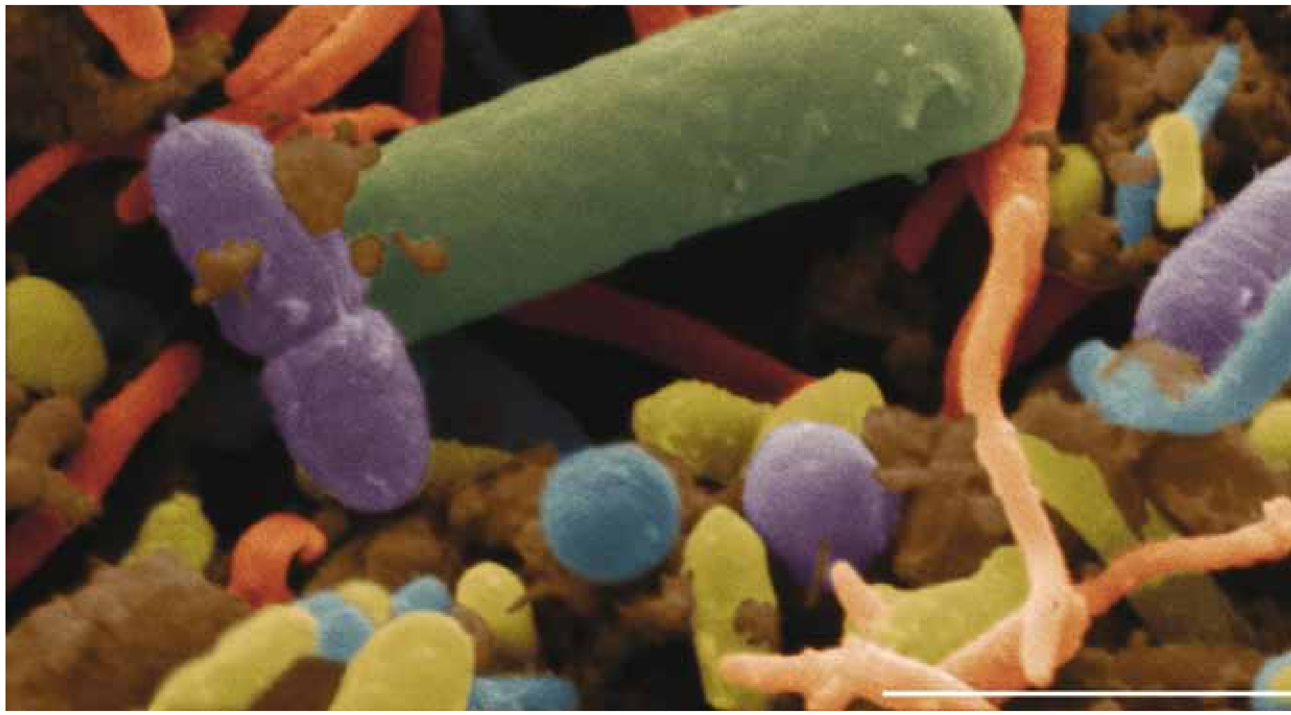

Fig. 13: Slim bacteria with pointed ends were found in free-range hens' droppings. Bar: 2 µm.

Fig. 14: Bacteria from the faeces of wild animals may contaminate spinach leaves by invading the leaf stomata. Bar: 5 µm.

(in the morning and in the evening) and was only rinsed with water between uses. Four weeks later, tightly coiled bacteria were found as the predominant kind of micro-organism on the cover slip (Figure 20). A similar kind of bacteria was found in distilled water obtained from a contaminated still. These bacteria are very similar to *Helicobacter heilmannii* (Une, 2003) except that the direction of their coiling runs in the opposite direction. *H. heilmannii* is found in the gastrointestinal tract of humans, dogs, cats and monkeys but not in water. Another possibility is spirochetes but their coiling is not as tight as shown in Figure 20. Thus, these bacteria remain unidentified. Over the past 30 years, bacteria that were not known to cause disease, have transformed into pathogens as they pass through wastewater treatment plants into surface water. Bacteria have become resistant to antibiotics (Radetsky, 1998) and several disinfecting agents including chlorine. Murray *et al.* (1984) isolated a total of 1,900 lactose-fermenting bacteria from raw sewage influent and chlorinated sewage effluent from a sewage treatment plant and found that 84% of them were resistant to one or more antibiotics. Whereas chlorination decreased the total number of bacteria in the sewage, there was a higher incidence of antibiotic resistance among the surviving bacterial strains.

There are many more hazards caused by poor hygienic conditions than those mentioned in this review. One of the worst, of course, are floods when water rises and raw sewage flows down the streets and floods human dwellings. These are, however, extraordinary conditions normally out of the control of humans.

Bacteria on money

An internet search for the incidence of micro-organisms on money (banknotes, coins) brings many links from various geographical areas. On their way

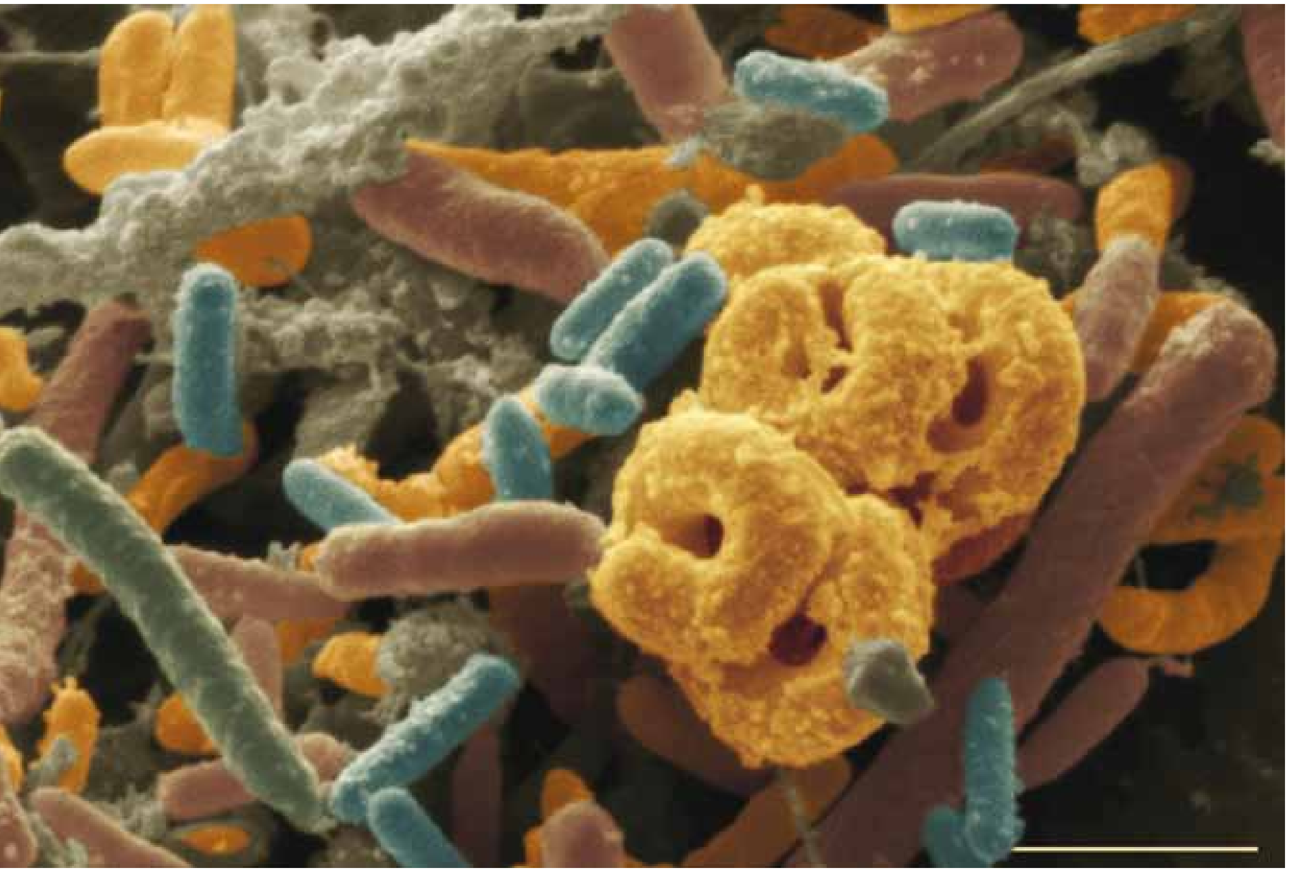

Fig. 15: Bacteria in the water of a garden bird bath frequented by small songbirds. Bar: 2 μm.

Fig. 16: Bacteria in the faeces of an urban dog. Bar: 2 μm.

from hand to hand, banknotes collect whatever is on our hands, be it dead skin cells, skin oils or food residues which we have not completely washed off - and, of course, we also transport bacteria and fungal spores on our hands, so they, too, may find the next banknote as their new destination. The surface of banknotes provides space for both the nutrients and the micro-organisms (Figure 21). Thus, the contamination of banknotes is a reflection of what is commonly present on human hands (Figure 22). Pope *et al.* (2002) incubated individual bills in nutrient broth and identified the bacteria growing in the culture dishes. The authors collected $1 bills in Ohio (USA) and found bacterial pathogens or potential pathogens in 94% of them. More than half hosted bacteria that commonly infect people in hospitals or those who have depressed immune systems. A total of 93 different types of bacteria were found on the bills, and two-thirds of the bills had at least one type. Several banknotes contained *Klebsiella pneumonia* or *Staphylococcus aureus* which can sicken healthy people. The fact that paper money harbours bacteria was not surprising but the wide variety was not expected. One of the factors affecting the incidence of bacteria on coins is the metal from which the coins are made. In contrast to paper money, library books contained negligible numbers of bacteria. Brook & Brook (1994) found only 1 to 4 cells of *Staphylococcus epidermidis* per page.

Bacteria on human skin

Commensal bacteria, such as *Staphylococcus epidermidis*, and *Micrococcus* and *Corynebacterium* species are also present on human skin, particularly in the armpits, on the perineum, palms, toes and soles, where they find moisture and nutrients. They are mostly beneficial but they may carry genes making them resistant to antibiotics. Worse yet, they may impart such genes in the form of DNA plasmids on pathogenic bacteria with which they come into contact (Internet 1). It is becoming increasingly difficult to treat infections caused by resistant pathogens. Our bodies produce an antimicrobial

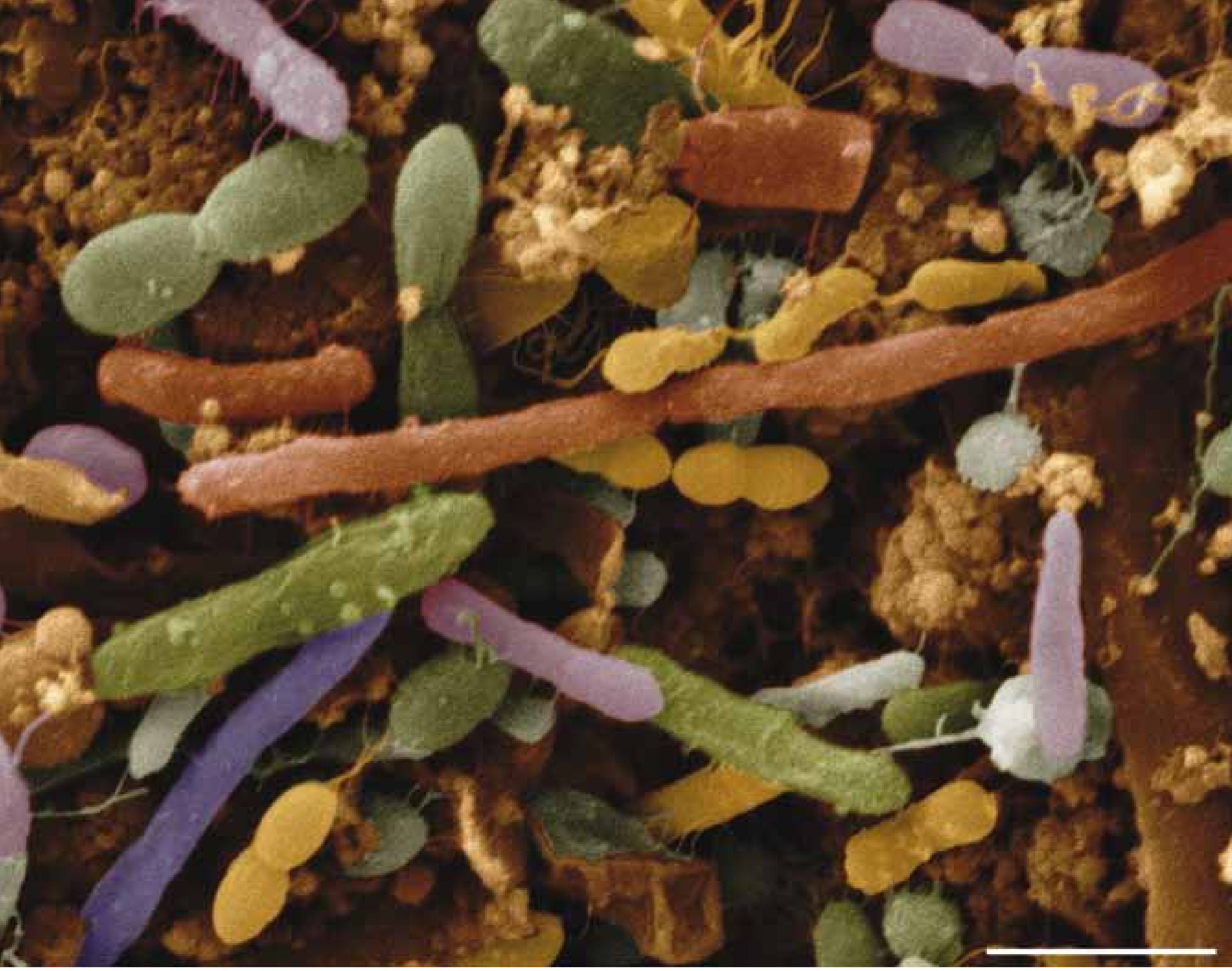

Fig. 17: Bacteria in rural cat faeces. Bar: 2 µm.

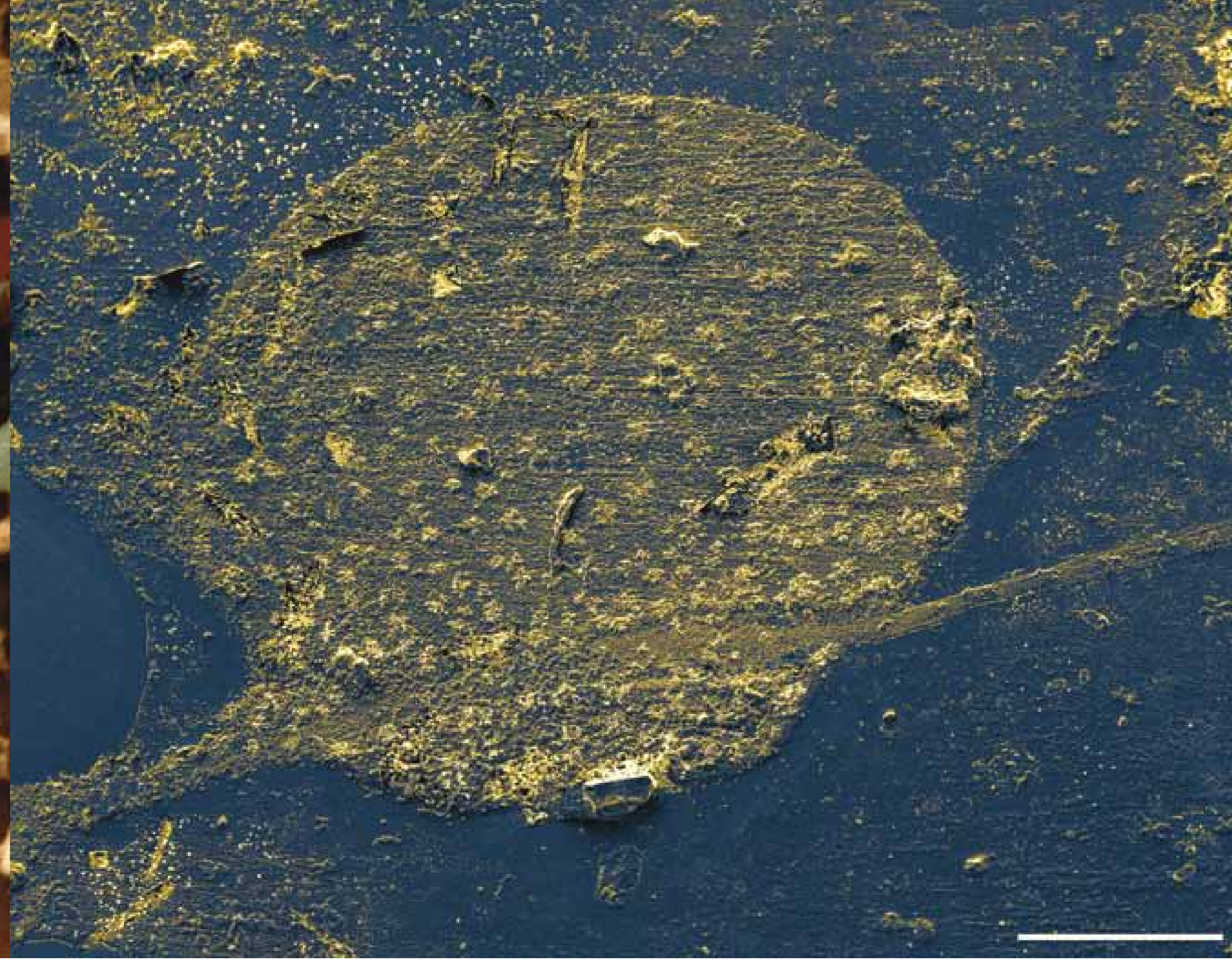

Fig. 18: Aerosol particles dried on the rim of a toilet bowl. Bar: 250 µm.

peptide called dermcidin in the sweat glands which limits infection by potential pathogens in the first few hours following bacterial colonisation (Schittek *et al.*, 2001).

The presence of bacteria on the palms may easily be demonstrated by making their imprints on nutrient agar gel (Figure 22). This experiment may be done at home or at school using ingredients available in food stores such as agar (Asian groceries), low-fat beef soup in powder, and soy sauce. A 2% agar with 1% beef soup powder and a teaspoon of a soy sauce ("hydrolysed soy protein") per half litre are boiled until the agar is fully dissolved. The mixture is poured onto a freshly washed and dried plate and allowed to solidify. A hand is then lightly pressed for 5 seconds on the agar plate and the agar gel is covered with a lid not touching the surface. Within two to four days, bacterial and fungal colonies start appearing on the agar gel (Figure 23).

It was not always recognised that bacterial pathogens can spread by hand contact. Ignaz Semmelweis, a Hungarian physician working in the 19th century in Vienna General Hospital's First Obstetrical Clinic, was the first (Hanninen *et al.*, 1983) to recognise that there are "some cadaverial particles" which may be transferred by the hands of students and doctors participating first in autopsies and then doing manual gynecological examinations on the maternity wards without having washed their hands in between these duties. His discovery preceded the germ theory of Louis Pasteur, published in 1861. Semmelweis introduced hand washing with chlorinated lime solutions which immediately reduced the incidence of fatal puerperal fever by 90%.

The term "nosocomial infections" is used for infections contracted by patients in hospitals or other healthcare centres. They are caused by insufficient personal hygiene, reduced immunity in some patients, the use of antibiotics (which eliminate useful commensal bacteria), etc. Bacteria most frequently involved in this kind of infection are *Acinetobacter baumannii*, Methicillin-resistant *Staphylococcus aureus* (MRSA), *Klebsiella pneumoniae*, and *Clostridium difficile*. The latter commensal spore-forming bacterium present in the intestine rapidly propagates in patients treated with antibiotics as they suppress the other members of the microflora. One of the best disinfectants for hospitals is hydrogen peroxide (Otter & French, 2009).

Body hygiene also includes the regular washing of the genitals and the removal of smegma (Krueger & Osborn 1986). Both females and males produce it. In females, it collects around the clitoris and in men under the foreskin. In healthy animals, smegma helps clean and lubricate the genitals. Smegma can protect both the penis and vagina from dirt and infection because it has antibacterial and antiviral properties, but it develops a strong odour if allowed to build up unchecked. Then it provides nutrients to other bacteria (Figure 24). *Mycobacterium smegmatis* is the characteristic bacterium present.

Thorough hand washing with soap and water is essentially important in preventing the transmission of pathogens. However, Aiello *et al.* (2007) have found that washing hands with an antibacterial soap is no more effective in preventing infectious illness than plain soap. The main active ingredient, triclosan, may cause some bacteria to become resistant to

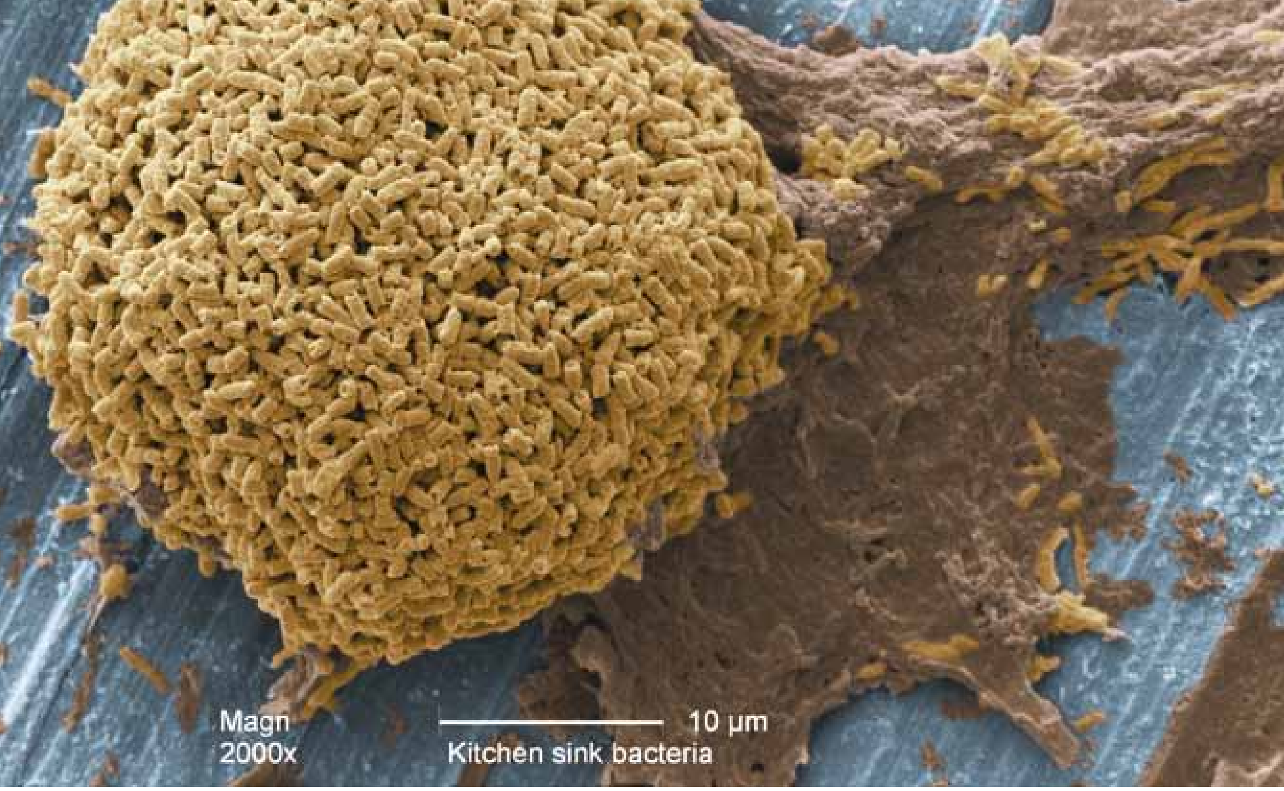

Fig. 19: Bacterial colony as a sign of microbial growth in a kitchen sink. Bar: 10 µm.

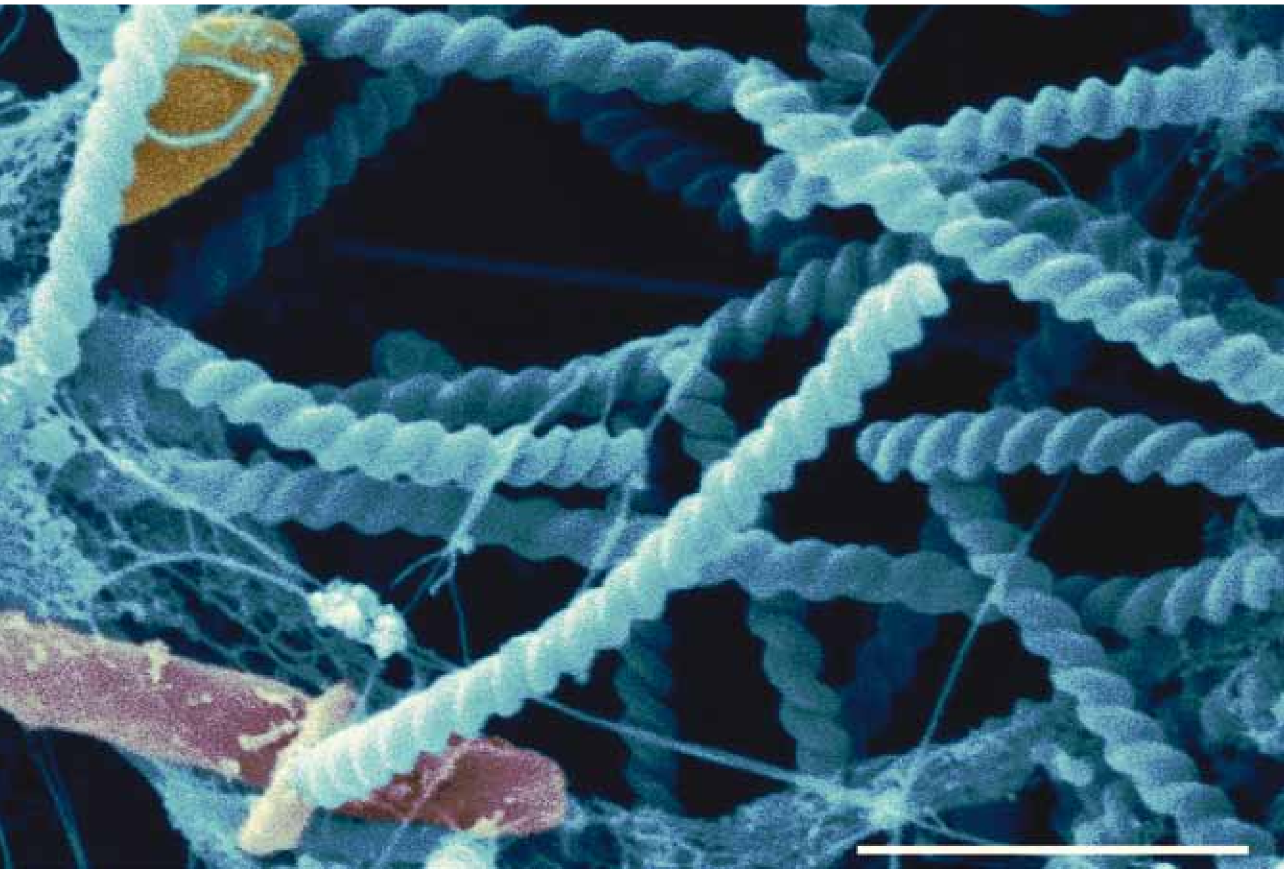

Fig. 20: Bacteria at the bottom of a drinking cup used for 4 weeks and only rinsed between uses. Bar: 2 µm.

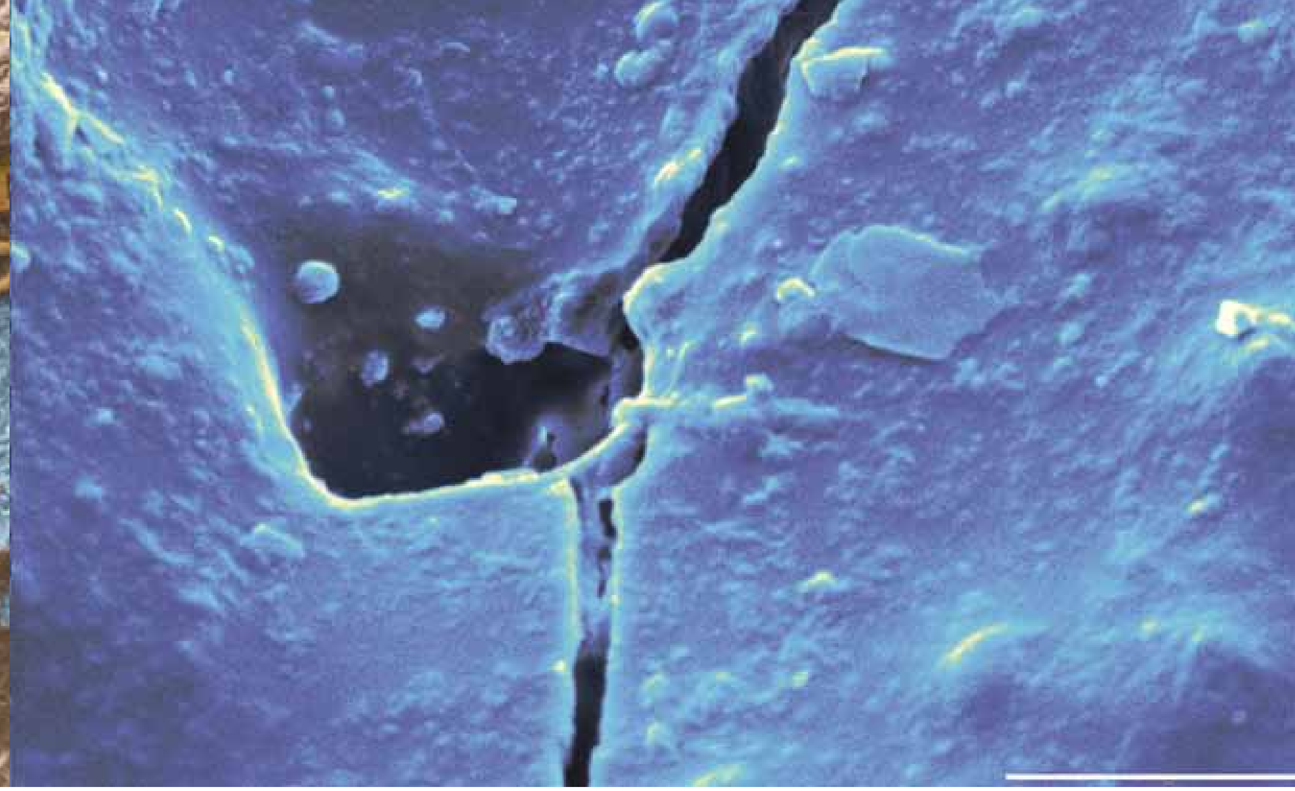

Fig. 21: The surface of a Canadian $5 banknote has depressions and cracks to accommodate bacteria as well as their nutrients. Bar: 10 µm.

Fig. 22: Fungal and bacterial colonies developed on a nutrient agar plate in a rectangle where a Canadian $5 banknote (6" x 2.75") was imprinted for 20 s and the plate was maintained at 22°C for 3 days. The margins around the banknote are free of microorganisms.

Fig. 23: A young girl imprints her hand on a nutrient agar gel plate.

Fig. 24: Bacterial and fungal colonies developed on an agar plate 36 h after a girl imprinted her left hand. Before the experiment, she played in a garden and only wiped her hand with a paper towel.

commonly used drugs such as amoxicillin. *E. coli* bacteria adapted in laboratory experiments to soaps containing as much as 0.1% w/v triclosan. Alcohol-containing (minimum 62% ethanol) sanitisers have been introduced for hand washing during the alert for the swine flu and they are being further developed by replacing alcohol with other ingredients, particularly benzalkonium chloride (the main ingredient of Lysol).

Closely associated with the presence of bacteria on human skin is the incidence of pathogenic bacteria on new swimsuits and underwear in clothing store departments as has been reported on the Internet.

Conclusions

The fact that we are colonised and surrounded with micro-organisms should not cause a panic. A foetus develops its immune system *in utero* and the development proceeds after birth as the infant is exposed to bacteria, viruses and allergens. Excessive emphasis on killing all micro-organisms in our surroundings may actually weaken our immune system and cause severe health problems. Vaccines are available only for a limited number of pathogens.

It is not possible to mention all aspects of our interactions with micro-organisms in a single article, but the micrographs and related bibliography presented may explain why it is important to avoid contact with bacterial pathogens. The most recent discovery of the bacterial gene for the NDM-1 enzyme (Internet 15), which confers resistance to β-lactam antibiotics onto any bacteria that have incorporated it into their genome, is another important reason to take hygiene seriously. The lack of sanitary conditions in areas afflicted by natural disasters such as floods, landslides or earthquakes or by large military conflicts is one of the major causes of epidemics of waterborne diseases such as cholera, gastroenteritis, diarrhoea, typhoid fever and other diseases.

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REFERENCES

The sources for this article were obtained from scientific papers (Part 1) and also from the Internet (Part 2) which is readily available to most readers and where new information appears quickly.

Part 1.

Aiello, A. E., Larson, E. L., Levy, S. B. (2007) Consumer antibacterial soaps: Effective or just risky? Clinical Infectious Diseases 45, 137–147.

Barker, J. & Jones, M.V. (2005) The potential spread of infection caused by aerosol contamination of surfaces after flushing a domestic toilet. Journal of Applied Microbiology 99(2), 339-47.

Bollinger, R. R., Barbasa, A. S., Busha, E. L., Lina, S. S., Parker, W. (2007) Biofilms in the large bowel suggest an apparent function of the human vermiform appendix. Journal of Theoretical Biology 249(4), 826-831.

Brook, S. J. & and Brook, I. (1994) Are public library books contaminated by bacteria? Journal of Clinical Epidemiology, 47(10), 1173-1174.

Guarner, F. & Malagelada, J. R. (2003). Gut flora in health and disease. Lancet 361(9356), 512–519.

Haghighi, H. R., Gong, J., Gyles, C. L., Hayes, M.A., Zhou, H., Sanei, B., Chambers, J. R., Sharif, S. (2006) Probiotics stimulate production of natural antibodies in chicken. Clinical and Vaccine Immunology, 13(9), 975–980.

Hanninen, O., Farago, M., Monos, E. (1983) Ignaz Philipp Semmelweis, the prophet of bacteriology, Infection Control 4(5), 367–370.

Jiang, X. & Shepherd, M. (2009) The role of manure and compost in produce safety. In: Microbial Safety

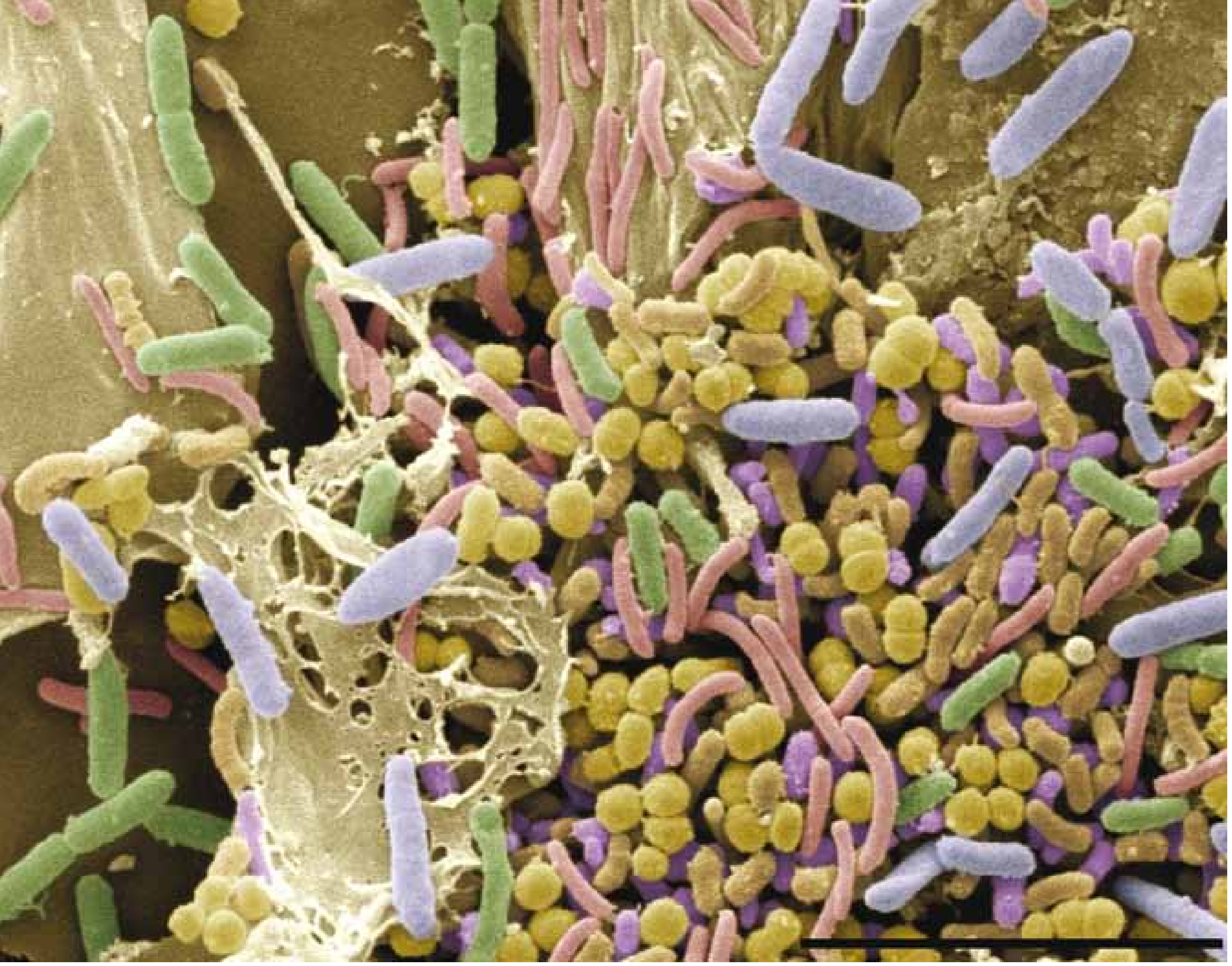

Fig. 25: Bacteria find nutrients in smegma; they propagate quickly and are the cause of foul odour. They are removed by washing with soap and water. Bar: 2 µm.

of Fresh Produce. Fan, X., Niemira, B.A., Doona, C.J., Feeherry, F.E., Gravani, R.B., eds., Wiley-Blackwell, 143-166.

Kaláb, M. (1961) Apparatus to ferment feces. Československá Fysiologie 10(6), 480.

Kaláb, M., Yang, A.-F., Chabot, D. (2008) Conventional scanning electron microscopy of bacteria. infocus, Issue 10, 42-61.

Karch, H., Tarr, P., Bielaszewska, M. (2005). Enterohaemorrhagic Escherichia coli in human medicine. International Journal of Medical Microbiology 295(6-7), 405–18.

Kazor, C. E., Mitchell, P. M., Lee, A. M., Stokes, L. N. Loesche, W. J., Dewhirst, F. E., Paster, B. J. (2003) Diversity of bacterial populations on the tongue dorsa of patients with halitosis and healthy patients. Journal of Clinical Microbiology 41(2), 558–563.

Keller, S.E. (2009) Microbial contamination of fresh produce. In: Intentional and Unintentional Contaminants in Food and Feed. ACS Symposium Series, Vol. 1020, 25-45. American Chemical Society.

Krueger H. & Osborn L. (1986) Effects of hygiene among the uncircumcised. Journal of Family Practice 22(4), 353-355.

Levine, M. J. (1993) Critical Reviews in Oral Biology and Medicine, 4(3/4), 279-286.

Li, H., Duncan, C., Townend, J., Killham, K., Smith, L. M., Johnston, P., Dykhuizen, R., Kelly, D., Golden, M., Benjamin, N., and Leifert, C. (1997). Nitrate-reducing bacteria on rat tongues. Applied Environmental Microbiology 63(3), 924–930.

March, S. B. & Ratnam, S. (1986) Sorbitol-MacConkey medium for detection of Escherichia coli O157:H7 associated with hemorrhagic colitis. Journal of Clinical Microbiology 23(5), 869-872.

Matthews K. R. (2009): Leafy vegetables. In: The Produce Contamination Problem. Causes and Solutions. G. M. Sapers, E. B. Solomon, K. R. Matthews (eds.), Academic Press - Elsevier, USA, 165-187.

Murray, G. E., Tobin, R. S., Junkins, B., Kushner, D. J. (1984) Effect of chlorination on antibiotic resistance profiles of sewage-related bacteria. Applied Environmental Microbiology 48(1), 73-77.

Nandi, S., Maurer, J. J., Hofacre, C., Summers, A. O. (2004) Gram-positive bacteria are a major reservoir of Class 1 antibiotic resistance integrons in poultry litter. Proceedings of the National Academy of Sciences of the United States 101(18), 7118-7122.

Němečková, A., Janák, J, Pelikán, V., Šantavý, F., Šmíd, F. (1961) Analysis of intestinal gases with gas chromatography (in Czech). Československá Fysiologie 10, 461-3.

Otter, J.A. & L. French, G. L. (2009) Survival of nosocomial bacteria and spores on surfaces and inactivation by hydrogen peroxide vapor. Journal of Clinical Microbiology 47(1), 205–207.

Pope, T.W., Ender, P.T., Woelk, W. K., Koroscil, M. A., Koroscil, T. M. (2002) Bacterial contamination of paper currency. Southern Medical Journal 95(12), 1408-1410.

Prudham, S. (2004) Poisoning the well: Neoliberalism and the contamination of municipal water in Walkerton, Ontario. Geoforum 35(3), 343-359.

Radetsky, P. (1998) Last days of the wonder drugs. Discover 19(11) 76-85.

Reynolds, K. A., Watt, P. M., Boone, S. A., Gerba, C. P. (2005) Occurrence of bacteria and biochemical markers on public surfaces. International Journal of Environmental Health Research 15, 225-234.

Sears, C. L. (2005). A dynamic partnership: celebrating our gut flora. Anaerobe 11(5), 247–51.

Schittek, B., Hipfel R., Sauer, B., Bauer, J., Kalbacher, H., Stevanovic, S., Schirle, M., Schroeder, K., Blin, N., Meier, F., Rassner, G., Garbe1, C. (2001) Dermcidin: a novel human antibiotic peptide secreted by sweat glands. Nature Immunology 2, 1133-1137.

Steinhoff, U. (2005) Who controls the crowd? New findings and old questions about the intestinal microflora. Immunol. Letters 99(1), 12–6.

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

Une, Y. (2003) Helicobacter species and helicobacter infection in animals. 28th World Congress of the World Small Animal Veterinary Association, October 24-27, Bangkok, Thailand.

Vaahtovuo, J., Korkeamäki, M., Munukka, E., Viljanen, M. K., Toivanen, P. (2005) Quantification of bacteria in human feces using 16S rRNA-hybridization, DNA-staining and flow cytometry. Journal of Microbiological Methods, 63(3) 276-286.

van der Wal, R. & Loonen, M. J. J. E. (1998) Goose droppings as food for reindeer. Can. J. Zool. 76, 1117-1122.

Wheatcroft, R. (2006) Personal communication.

Xu, J., Moore, J. E., Millar, B. C. (2005) Ribosomal DNA (rDNA) identification of the culturable bacterial flora on monetary coinage from 17 currencies. Journal of Environmental Health, 67(7), 51-55.

Part 2. Links to the Internet:

Internet 1: http://www.roarproject.org/ROAR/html/commensal.htm

Internet 2: http://www.entkent.com/biofilms.html

Internet 3: http://sandwalk.blogspot.com/2009/03/bacteria-in-your-mouth.html

Internet 4: http://db2.photoresearchers.com/search?function=query&key=SC3627

Internet 5: http://en.wikipedia.org/wiki/Gut_flora

Internet 6: http://www.textbookofbacteriology.net/themicrobialworld/Structure.html

Internet 7: http://www.jlindquist.net/generalmicro/102bactid.html

Internet 8: http://www.nmconline.org/ecoli.htm

Internet 9: http://www.foodborneillness.com/campylobacter_food_poisoning/

Internet 10: http://www.fda.gov/Food/ResourcesForYou/Consumers/ucm079516.htm

Internet 11: http://www.denverpost.com/business/ci_15885059
Internet 12: http://web.ncf.ca/ek867/rmw.working.remic.jpg
Internet 13: http://nogeese.com/
Internet 14: http://www.aces.edu/dept/extcomm/newspaper/feb5a04.html
Internet 15: http://en.wikipedia.org/wiki/New_Delhi_metallo-beta-lactamase

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As a chemical engineer (M.Eng., 1952) and scientist (Ph.D. 1957) in Czechoslovakia, Miloš arrived in 1966 as a post-doctoral fellow at the National Research Council of Canada in Ottawa to study blood serum lipoproteins. In 1968 he was hired by then Agriculture Canada for the Dairy Research Team. To better understand the gelation of milk, Miloš learned electron microscopy in the laboratory of Dr. G. H. Haggis.

Since 1979, Miloš helped Dr. Om Johari (Scanning Microscopy International, USA) organise international meetings of food microscopists and in 1982 to establish a new scientific journal "Food Microstructure" (later renamed "Food Structure"). He served as the Editor-in-Chief for 12 years. The tables of contents are available at www.foodsci.uoguelph.ca/dairyedu/journal2.htm.

The American Dairy Science Association conferred the Pfizer Award on Miloš in 1982 for his microstructural research of cultured milk products. He served as a United Nations FAO consultant at the National Dairy Research Institute in Karnal (India) and shared his expertise with food scientists in Japan thanks to a grant from the Government of Japan. Miloš has published over 150 scientific and technical papers. After his retirement in 1995, Miloš has volunteered as an Honorary Research Associate doing part-time electron microscopy of micro-organisms, foods and blood cells. This is his third contribution to infocus.