CHAPTER 1
A primer on microbiology

David O. Carter[1], Emily N. Junkins[1,2] and Whitney A. Kodama[1]

[1] *Laboratory of Forensic Taphonomy, Forensic Sciences Unit, Division of Natural Sciences and Mathematics, Chaminade University of Honolulu, Honolulu, HI, USA*

[2] *Department of Microbiology and Plant Biology, University of Oklahoma, Norman, OK, USA*

1.1 Introduction

In many ways microorganisms are an ideal form of physical evidence. They can be found virtually everywhere and are certainly present in every habitat occupied by humans. Thus, microbes could be collected from every scene under a forensic investigation, yet not all microorganisms are everywhere; like many forms of trace evidence, some microbes are found only in certain locations due to having a preferred habitat, much like how insects, birds, and reptiles have a preferred habitat range. Another valuable characteristic of microorganisms is that many of them can transform themselves into a highly durable structure that is designed to survive harsh conditions, which increases the likelihood of their survival and discovery. Considering all of these attributes, it is probably not surprising that microorganisms have been used as physical evidence since the early days of forensic science, particularly to establish the cause of death (e.g., MacCallum and Hastings, 1899). Forensic microbiology has since grown into an exciting discipline relevant to several areas of forensic science including medicolegal death investigation (Caplan and Koontz, 2001; Forbes *et al.*, 2016), bioterrorism (Budowle *et al.*, 2011), and product authenticity (Brzezinski and Craft, 2012). It will be absolutely fascinating to learn of the new discoveries in forensic microbiology over the next few decades.

Historically microbes have been used almost exclusively as spatial evidence—physical evidence that is used to associate people with diseases, objects, and/or locations (Locard, 1930a, b, c; Caplan and Koontz, 2001; Tridico *et al.*, 2014; Wiltshire *et al.*, 2014; Young *et al.*, 2015). This application is similar to the use of any other form of trace evidence, such as soil (Bisbing, 2016), paint (Kirkbride, 2016), glass (Almirall and Trejos, 2016), and fibers (Houck, 2016). However, recent research has shown that microorganisms

Forensic Microbiology, First Edition. Edited by David O. Carter, Jeffery K. Tomberlin, M. Eric Benbow and Jessica L. Metcalf.

represent a relatively unique form of physical evidence that can also serve as temporal evidence, evidence that is used to establish a timeline. This application uses the ability of microorganisms to respond rapidly to changes in their environment (e.g., Carter and Tibbett, 2006), and these changes are temporally predictable (Metcalf *et al.*, 2013; Pechal *et al.*, 2014; Guo *et al.*, 2016; Metcalf *et al.*, 2016), with an apparent ability to serve as an estimate of the postmortem interval (Chapter 2) and human habitation (Chapter 13) interval.

We are currently in an exciting time when multiple research groups around the world are leading advances in postmortem microbiology and trace microbiology (Fierer *et al.*, 2010; Benbow *et al.*, 2015; Lax *et al.*, 2015; Metcalf *et al.*, 2016). These advances are occurring rapidly and have great potential to significantly change how microorganisms are used as physical evidence. Microorganisms will likely play a greater role as physical evidence in the future, so the purpose of the current chapter is to provide an introduction to some fundamental aspects of microbiology and microbial ecology to help the reader develop an appreciation for the vast diversity of microorganisms and how they can be used to identify a location or time period of investigative interest. It is not possible for this chapter to review all known microorganisms, so the contents hereinafter will place an emphasis on bacteria that are of interest to the most recent research relevant to the scope of this book, postmortem microbiology and trace microbiology (e.g., Benbow *et al.*, 2015; Iancu *et al.*, 2015; Finley *et al.*, 2016; Metcalf *et al.*, 2016). However, domains Archaea and Eukarya are also highly relevant, and the current chapter will reference relevant work, when possible, that focuses on these very important taxa within a forensic context.

1.2 Microbial characteristics

Microorganisms can differ in many ways including their morphology, method of movement (motility), metabolic strategy, environmental requirements, and several other characteristics (Brown, 2015). The current chapter will address this issue with relative simplicity by describing how microorganisms survive with a brief description of some relevant taxa.

1.2.1 Microbial taxonomy and function

Classification of life has proven to be a challenge. Presently, there are a number of opinions on how we should organize organisms in terms of their relationship to one another. Although not the focus of this chapter, this topic is of great importance as it impacts our ability to assess microbial communities in general. Thus, we suggest individuals with an interest in forensic microbiology remain cognizant of the ever-shifting landscape of microbial taxonomy.

For this text, we focus our discussion on three major groups of microorganisms organized as domains: Archaea, Bacteria, and Eukarya (Woese *et al.*, 1990) although a new

Table 1.1 Phyla of domain Bacteria included in the list of prokaryotic names with standing in nomenclature, http://www.bacterio.net

Acidobacteria	Cyanobacteria	Nitrospira
Actinobacteria	Deferribacteres	Planctomycetes
Aquificae	Deinococcus–Thermus	Proteobacteria
Armatimonadetes	Dictyoglomi	Spirochaetes
Bacteroidetes	Elusimicrobia	Synergistetes
Caldiserica	Fibrobacteres	Tenericutes
Chlamydiae	Firmicutes	Thermodesulfobacteria
Chlorobi	Fusobacteria	Thermomicrobia
Chloroflexi	Gemmatimonadetes	Thermotogae
Chrysiogenetes	Lentisphaerae	Verrucomicrobia

perspective on this classification was presented recently (Hug *et al.*, 2016). The List of Prokaryotic Names with Standing in Nomenclature (http://www.bacterio.net) currently divides Bacteria into 30 phyla (Table 1.1) and Archaea into five phyla (Crenarchaeota, Euryarchaeota, Korarchaeota, Nanoarchaeota, Thaumarchaeota). These microbes can vary morphologically, with spherical (cocci; Figure 1.1a) and rod-shaped (historically termed bacilli; Figure 1.1b) being the most common. Variations on these general morphologies exist (Figure 1.1c), as do other morphologies such as club-shaped cells (coryneform; Figure 1.1d) and curved rods (e.g., vibrio). Thus, referring to a bacterium as a rod or a coccus is a helpful way to begin the identification process.

As the term microbiology indicates, microbes are small. A bacterial cell will likely have a diameter of 1–5 μm (see Figure 1.1), which means that microscopy is necessary to view individual microbial cells. Thus, the shape and size of microbial cells can be used for a general identification, usually to exclude possible identities. Other commonly used characteristics to identify microbes include the reaction to the Gram stain and the ratio of nucleotides in a cell, which is presented as guanine–cytosine (GC) content.

Stains play a significant role in the identification of microorganisms. For example, the Gram stain was developed in the nineteenth century to help visualize microbial cells. Without staining, many microbial cells are transparent and difficult to see. The Gram-positive and Gram-negative designation also provides some insight into the structure of the microbial cell wall. The cell wall of a Gram-positive bacterium is approximately 90% peptidoglycan, whereas the cell wall of a Gram-negative bacterium is approximately 10% peptidoglycan (Madigan *et al.*, 2012). Interestingly, the Gram designation also provides information about the taxonomy of bacteria. Gram-positive bacteria are generally found in phyla Actinobacteria and Firmicutes. Some relatively well-known Gram-positive bacteria include genera *Bacillus* (rod), *Clostridium* (rod), and *Streptococcus* (coccus), all of which are in phylum Firmicutes. The GC content of a cell can be used to generally distinguish between Actinobacteria and Firmicutes. The GC content represents

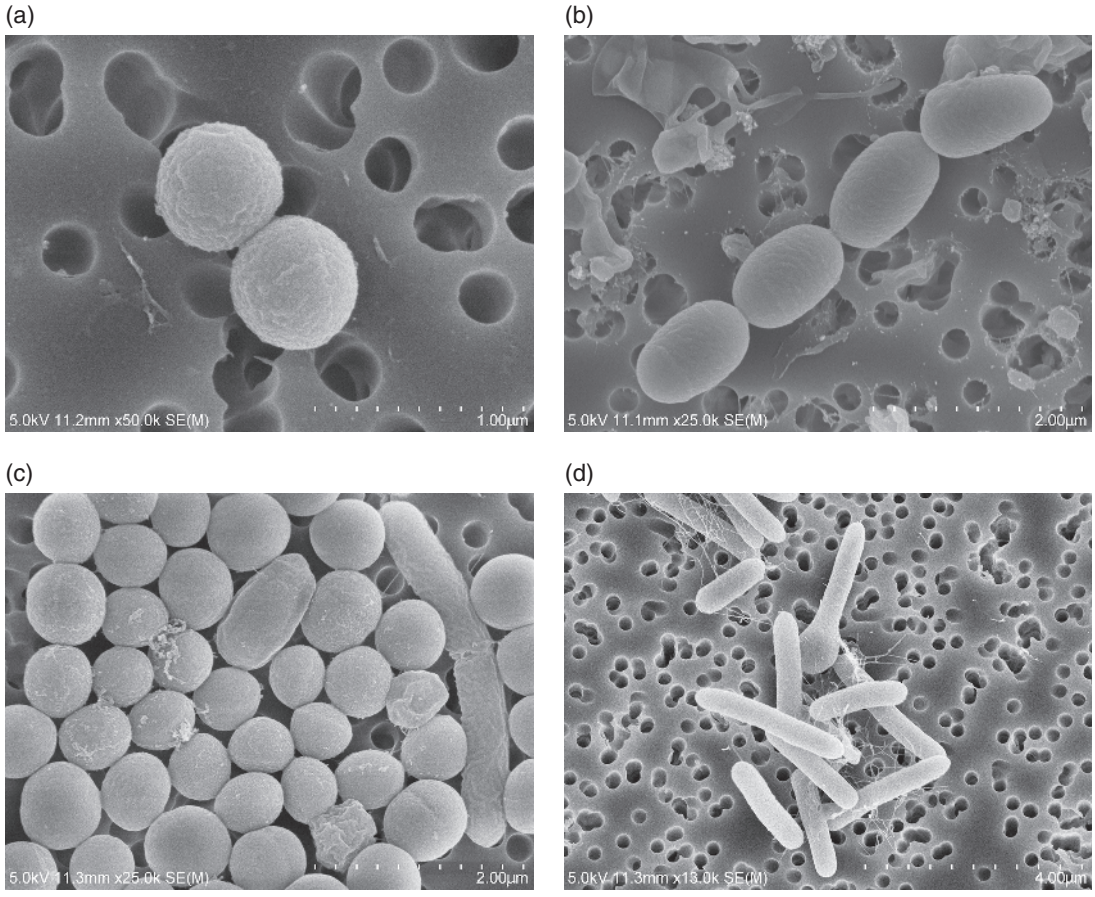

Figure 1.1 Scanning electron micrograph showing some of the contrasting morphologies observed in microbial cells including the widely observed coccus (a: *Staphylococcus cohnii*) and rod (b: *Bacillus subtilis*) shapes, which can vary and occur in association with other morphologies (c). Microbial cells can be observed as several other shapes including spiral (spirilla), curved rods (vibrio), and club shaped (coryneform), the latter of which is observed in *Sporosarcina contaminans* (d)

the proportion of the bacterial genome that comprises GC base pairs, rather than adenine–thymine base pairs, and is presented as high GC (>50% GC content) or low GC (<50% GC content). Phylum Actinobacteria includes high GC bacteria, while phylum Firmicutes includes low GC bacteria.

Microbes can also be identified based on their function. In fact, one of the reasons that microbes are so important to life on Earth is the vast diversity of functions that they carry out. Microbes decompose organic material, utilize carbon dioxide, fix nitrogen, and help plants and animals to acquire nutrients, and they contribute many other vital functions that keep habitats stable. These functions are the result of microorganisms

competing for that which is essential to microbial life, water, energy, and nutrients, where the energy source is often organic (contains carbon) and the nutrients include several essential elements such as nitrogen, phosphorus, potassium, sulfur, and calcium. As seen with other organisms, the ability of microbes to function is greatly influenced by their environment: oxygen availability, temperature, chemistry (particularly pH and Eh), and light (Ball, 1997). Thus, humans live in habitats that are the result of several processes, many of which are carried out by microorganisms. To effectively use microorganisms as physical evidence, we must therefore understand where microbes live, what their function is within that habitat, and how their environment affects their function and survival.

Microbes use several strategies to acquire energy and nutrients, referred to here as metabolic strategy. Some microbes even have the ability to change their metabolic strategy depending on environmental conditions. A microbe that is restricted to a single metabolic strategy is known as obligate. An obligate aerobe is a microbe that requires oxygen, for example. A microbe that can change metabolic strategies is termed facultative. A facultative anaerobe is a microbe that can maintain metabolic function regardless of oxygen availability. Understanding these metabolic strategies is important for forensic microbiology because it forms the foundation for interpreting microbial evidence or identifying a particular microorganism. For example, one would expect to see decomposing remains associated with chemoorganotrophic microbes, like the Enterobacteriaceae (e.g., Benbow *et al.*, 2015), because a dead body is a high quality source of water, energy, and nutrients (Carter *et al.*, 2007b).

The metabolic terms defined in Table 1.2 are presented to help understand the diversity of metabolic strategies used by microbes and show that they are regularly described by their metabolic strategy. Phototrophs, such as those in the phylum Cyanobacteria, use light to conduct photosynthesis. Chemotrophs, such as those in class Gammaproteobacteria, consume organic compounds (e.g., human remains, plant detritus) to generate energy. Because of this they are often referred to as decomposers. These decomposers represent the bulk of microorganisms associated with decomposing remains and trace evidence. It is important to accept that some taxa have physiologies that are not easily described using the terminology in Table 1.2 because of the vast array of metabolic strategies employed by microorganisms (e.g., Slonczewski and Foster, 2011; Madigan *et al.*, 2012)

1.2.2 Enzyme activity

Enzymes are important in microbial ecology. Microbial activity and metabolism can be measured in many different ways (e.g., carbon mineralization, calorimetry), but these metrics are often the result of enzyme-substrate reactions, if not a direct measure of potential enzyme activity (e.g., Carter *et al.*, 2008). Microbial enzymes can be classified in many ways, for example, as hydrolase, lyase, oxidoreductase, and transferase enzymes (Table 1.3). They can also be classified as intracellular and extracellular, which are important distinctions to consider; intracellular enzymes react with substrates within

Table 1.2 Glossary of terms commonly used to describe the habitat preferences and metabolic strategies of microorganisms

Aerobe	An organism that lives in the presence of oxygen
Aerotolerant	An organism that can live in aerobic and anaerobic conditions
Anaerobic	An organism that lives in the absence of oxygen
Autotrophy	Using carbon dioxide as the only source of carbon
Chemoautotrophy	Oxidizing chemical compounds to obtain energy while acquiring carbon only from carbon dioxide
Chemolithotrophy	Oxidizing inorganic compounds to obtain energy
Chemoorganotrophy	Oxidizing organic compounds to obtain energy
Chemotrophy	Oxidizing chemical compounds to obtain energy
Extremotroph, -phile	An organism that lives in extreme environmental conditions such as temperature and pH
Facultative	Not required, optional
Fermentation	Obtaining energy by using an organic compound as both an electron donor and an electron acceptor
Halotroph, -phile	An organism that lives in high salt concentrations
Heterotrophy	Using organic compounds as sources of carbon
Hyperthermotroph, -phile	An organism with an optimal growth temperature of ≥80°C
Mesotroph, -phile	An organism that lives at moderate temperature with an optimal growth temperature from 15 to 40°C
Metabolism	All reactions, anabolic and catabolic, in a cell
Microaerotroph, -phile	An organism that requires low levels of oxygen
Obligate	Required
Photoautotrophy	Using light as an energy source while acquiring carbon only from carbon dioxide
Photoheterotrophy	Using light as an energy source while using organic compounds as sources of carbon
Phototroph	An organism that obtains energy from light
Psychrotroph, -phile	An organism that lives at low temperature with an optimal growth temperature of <15°C
Respiration	Obtaining energy by oxidizing chemical compounds through a series of reactions to a terminal electron acceptor
Thermotroph, -phile	An organism that lives at high temperature with an optimal growth temperature from 45 to 80°C

See also Madigan *et al.* (2012) and Slonczewski and Foster (2011).

the microbial cell, while extracellular enzymes are released from microbial cells so that the enzyme–substrate product can then be transported across the cell membrane into the cell. Microorganisms commonly release extracellular enzymes into their habitat so that they can use the products to acquire the resources necessary for survival. This degradative and overall enzyme profile can be used to determine the range of substrates

Table 1.3 General classification of enzymes commonly associated with microorganisms

Enzyme	Examples	Function
Hydrolase	Amylase, chitinase, lipase, peptidase, phosphatase, phosphodiesterase, protease, sulfatase, urease	Catalyzes the hydrolysis of chemical bonds
Lyase	Aldehyde lyase, amino acid decarboxylase, cyclase, dehydratase	Catalyzes an elimination reaction and oxidation to break chemical bonds
Oxidoreductase	Alcohol oxidoreductase, amino acid oxidoreductase, ammonia monooxygenase, glucose oxidase, nitrite oxidoreductase, methane monooxygenase, monoamine oxidase, peroxidase	Catalyzes the transfer of electrons from the electron donor (reductant) to the electron acceptor (oxidant)
Transferase	Amino transferase, CoA transferase, methyltransferase, polymerase, kinase	Catalyzes the transfer of a functional group from one molecule (donor) to another (acceptor)

that can be used by a microbe (e.g., Pechal *et al.*, 2013), known as community level physiological profiling (Degens and Harris, 1997). This potential substrate use is then modified by resource availability/quality, decomposer community, and physicochemical environment (Swift *et al.*, 1979; Killham and Prosser, 2007).

The release of extracellular enzymes into a habitat does introduce some complexity into interpreting the ecology of a microbe. Once extracellular enzymes are released from a microbial cell, they can function independently of the microbial cell. This ability means that microbial habitats can contain free enzymes capable of reacting with substrates. As a result, a microbial cell might take up the product of a reaction that used an enzyme it did not release. To further complicate matters, these free enzymes do not necessarily react with substrates soon after release from the microbial cell. They can be bound to organic material and inorganic surfaces, such as soil minerals, and even accumulate in habitats so that the measurement of enzyme activity might provide a misleading metric of microbial activity (Carter *et al.*, 2007a). This phenomenon must be researched in greater detail before we truly understand postmortem microbial ecology.

1.3 Microorganisms and their habitats

Microorganisms and their activity are greatly influenced by their environment. Temperature, relative humidity, pH, oxidation–reduction potential (Eh), and oxygen availability are all environmental parameters that play a role in defining the microbial

community. For example, the *Psychrobacter* sp. observed by Carter *et al.* (2015) in gravesoil during the winter in Nebraska, United States, is a taxon that prefers cold temperature. Many of these environmental parameters, such as ambient temperature and precipitation, can be measured with relative ease (see Chun *et al.*, 2015), and these quantitative measures can be used to understand the ecology of microbes associated with a given sample or habitat. In addition, some of these chemical measures can be used as physical evidence in their own right. For example, these measures have allowed us to observe that fly (Diptera) larval masses in decomposing remains are slightly acidic-reducing environments of high temperature (Chun *et al.*, 2015). As a result we would expect to see larval masses select for bacteria and archaea that can grow in a habitat of neutral pH with little/no oxygen at approximately 35°C.

1.3.1 Oxygen and moisture

Microorganisms are regularly classified as aerobes or anaerobes. Thus, the presence or absence of oxygen is a useful index for classifying microorganisms. Obligate aerobes like *Pseudomonas aeruginosa* and *Mycobacterium tuberculosis* require oxygen as it serves as the terminal electron acceptor in the electron transport chain. In contrast, obligate anaerobes like *Clostridium* spp. cannot tolerate oxygen, and they must employ another metabolic strategy for survival; *Clostridium* spp. are well-known fermenters of carbohydrates, amino acids, and ethanol. Fermentation is a metabolic strategy where an organic compound is used as the terminal electron acceptor. Fermentation is an important process to understand as it drives putrefaction, which is essentially the fermentation of human remains.

Facultative anaerobes, such as *Staphylococcus aureus* can grow with or without oxygen. Many microbes can switch between oxidative phosphorylation (aerobic respiration) and anaerobic fermentation. Others may respire in the absence of oxygen using alternative inorganic electron acceptors such as nitrate, sulfate, or oxidized metals. For example, a denitrifier from the order Rhizobiales would reduce nitrate, instead of oxygen, to either nitrous oxide (N_2O) or free nitrogen (N_2) (Metcalf *et al.*, 2016). The switch between aerobic and anaerobic metabolism by a microorganism can occur within a few hours (Killham and Prosser, 2007), easily within the early postmortem period. However, the energy yield of anaerobic metabolism is low because it results in the accumulation of incompletely oxidized products. As a result, anaerobic and microaerobic habitats tend to result in a slower decomposition process, such as the formation of adipocere (Forbes, 2008).

The influence of oxygen is also related to the microbial need for moisture as moisture and oxygen availability are coupled in many habitats. Moisture concentration is negatively correlated to the concentration of oxygen. Moisture is necessary for microbial life, but many microbes have contrasting moisture preferences. Some microorganisms, like the Actinobacteria, Firmicutes, and Proteobacteria detected on dry human skin (Grice and Segre, 2011), are able to thrive in habitats of low moisture content, whereas some microorganisms, like those observed on vertebrate remains in postmortem submersion interval studies (Benbow *et al.*, 2015), require an aquatic habitat to maintain

growth. Because of this, many microorganisms have potential as microbial trace evidence because they can survive on human skin as well as the surfaces on which they come into contact (Lax *et al.*, 2014).

An excellent example of the relationship between oxygen and moisture was presented by Carter *et al.* (2010): the addition of moisture to a sufficiently aerated habitat can lead to anaerobic conditions as air diffuses more slowly through water. This effect is enhanced by the introduction of organic material available for decomposition, such as human remains. In this scenario, the presence of human remains would stimulate microbial activity because it is a highly attractive food source. This microbial activity would consume air more rapidly than it can be replenished, which is significantly slower in a wet environment (Carter *et al.*, 2010). This effect is particularly pronounced when decomposing remains are buried in soil, but the relationship also holds true for remains decomposing on the surface. The remains will remain an anaerobic hotspot (because air cannot be replenished rapidly enough to meet demand) until moisture levels drop sufficiently to retard enzyme activity. At this point, often referred to as advanced decay (Carter *et al.*, 2007b), it becomes difficult for bacteria to compete and the fungi can proliferate (Carter and Tibbett, 2003; Sagara *et al.*, 2008).

1.3.2 Temperature

Microorganisms and their activity are significantly influenced by temperature. Microbial activity increases as temperature increases and slows with decreasing temperatures. Doubling of microbial activity with a 10°C increase in temperature is not uncommon (e.g., Carter and Tibbett, 2006) up to approximately 40°C. As temperature increases from 40°C, it becomes too hot for many microorganisms. Similarly, a lot of microbial activity ceases at 0°C. Several studies (Putman, 1978; Carter *et al.*, 2008; Pechal *et al.*, 2013) have reported an increase in postmortem microbial activity with an increase in temperature, which is one of the reasons why decomposition is more rapid during warmer months. Although insects and scavengers often consume the majority of remains, microorganisms make a significant contribution to taphonomy (Lauber *et al.*, 2014).

Microbes have preferred temperature ranges. Microbes are typically characterized as one of the three temperature regimes: psychrotrophic, mesotrophic, or thermotrophic. Psychrotrophs live in low temperature habitats, such as glaciers, and have an optimal growth temperature <15°C. Mesotrophs live at moderate temperature and grow optimally between 15 and 40°C. Thermotrophs live at high temperature and grow optimally between 50 and 80°C in environments such as thermal vents and hot springs. Some microbes, particularly archaea, are recognized as hyperthermotrophs. These microbes have an optimal growth temperature ≥80°C.

One way to analyze the influence of temperature on biological activity is by using the accumulated degree day (ADD), which is essentially the sum of median daily temperature and represents physiological time (e.g., Pechal *et al.*, 2014). This metric is commonly used, and it has been incorporated into recent postmortem microbiome studies

(Metcalf *et al.*, 2013; Pechal *et al.*, 2014; Hauther *et al.*, 2015; Metcalf *et al.*, 2016), yet one important issue requires resolution: the use of a minimum developmental threshold. The minimum developmental threshold is a variable used to compensate for the cessation of biological activity when calculating ADD. For example, if an organism is known to cease function at 6°C, then these six degrees are subtracted from each degree day. One problem that forensic microbiology currently faces is the establishment of a minimum developmental threshold for microorganisms. Vass *et al.* (1992) used 0°C based on the rationale that most microbial activity ceases at 0°C. However, this is not always the case, as microsites might remain slightly above 0°C or free extracellular enzymes might remain active below 0°C. Establishing a minimum developmental threshold for forensic microbiology might be straightforward for studies using a single taxon or simple microbial communities comprising only a few taxa. However, the complex microbial communities that are currently the focus of postmortem microbiology and trace microbiology could make the issue quite difficult to resolve.

1.4 Competition for resources

At this point we hope that you can appreciate the usefulness of identifying a microbe, for example, as a psychrotrophic obligate aerobe. This brief description refers to a microorganism that would be found in a cold, well-aerated habitat. However, this categorization does not provide insight into the ability of a microbe to compete for nutrients. This competition is an issue of growth kinetics where the growth rate of a microbe is a function of substrate concentration and availability (Figure 1.2). Figure 1.2 shows that

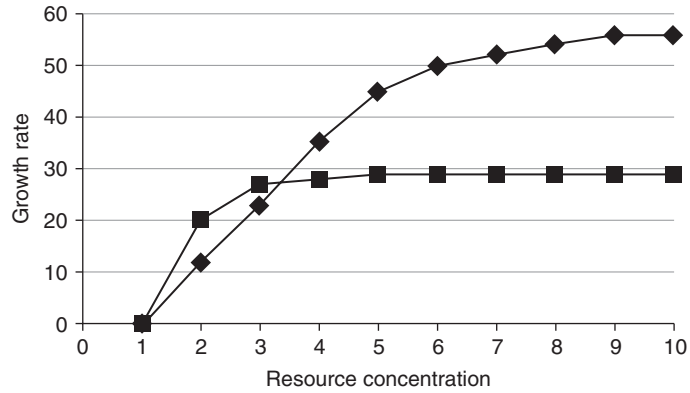

Figure 1.2 The growth rate of microorganisms is regulated, in part, by the availability of resources. Zymogenous (◆) microbes are able to use resources and multiply rapidly so they can dominate a habitat in which resources are abundant. In contrast, autochthonous (■) microbes tend to represent the basal, resting community that uses resources and multiplies slowly and forms the foundation of a microbial community

the microbial population represented by a diamond symbol will outcompete the microbial population represented by the square symbol at high resource concentration. This organism is termed zymogenous. Zymogenous organisms are associated with a high growth rate and a high substrate affinity. Zymogenous microbes will dominate habitats following a disturbance, such as digging in the soil, or when habitats contain a high quality source of energy. In contrast, the square population will outcompete the other populations at low substrate concentrations. This organism is termed autochthonous. Autochthonous organisms are associated with a low growth rate and substrate affinity. Autochthonous microbes will dominate environments with low resource availability or low resource quality and likely represent the foundational, basal microbial community for a given habitat. These populations might occur in the less accessible microenvironments where C availability is low so that, in reality, a scale of autochthony and zymogeny exists. Furthermore the scale changes over time so that microbial succession will occur as a resource is being decomposed.

The autochthony–zymogeny concept can be viewed as analogous to the r-K continuum used in plant and animal ecology. K-strategists and oligotrophic microbes are adapted to growth under conditions of low energy and nutrient availability. Oligotrophy also refers to low nutrient content and the use of unusual C resources and volatile organic acids. In contrast, copiotrophs are adapted to an excess of nutrients. Recall that microorganisms are physiologically versatile and flexible; facultative anaerobes metabolize most efficiently as aerobes, but they can survive when oxygen is insufficient for aerobic metabolism. Another aspect of flexibility is the ability to metabolize more than one substrate, often simultaneously. This is reflected in the diversity of enzymes associated with microbes. Also, microorganisms can adapt to the repeated introduction of compounds such as pesticides (Jayachandran *et al.*, 1998) and muscle tissue (Carter and Tibbett, 2008), which results in more rapid decomposition.

1.5 The ecology of some forensically relevant bacteria

In recent years unprecedented insight into the ecology of microorganisms relevant to medicolegal death investigation and trace evidence analysis has been gained (Fierer *et al.*, 2010; Nagasawa *et al.*, 2013; Song *et al.*, 2013; Lax *et al.*, 2014; Tridico *et al.*, 2014; Bouslimani *et al.*, 2015; Lax *et al.*, 2015). This research has identified many bacteria from several phyla, but there are three phyla that have been observed regularly: Actinobacteria, Firmicutes, and Proteobacteria. The remainder of this chapter will provide an introduction to the characteristics of some microorganisms from these phyla.

1.5.1 Actinobacteria

The phylum Actinobacteria is both morphologically and metabolically diverse. The taxonomy of this phylum is somewhat confusing as it has only one class (Actinobacteria) but

several subclasses (www.bacterio.net). Actinobacteria comprises 10 orders and 58 families. These Gram-positive, high GC bacteria can be observed as cocci, rods, and filaments while conducting respiration or fermentation. The Actinobacteria are primarily aerobic and are common inhabitants of soil, skin, and aquatic environments. Many of the more familiar genera in this phylum are members of subclass Actinobacteriales, including *Corynebacterium*, *Micrococcus*, and *Mycobacterium* (Brown, 2015). As with most phyla, there are several ways to categorize the Actinobacteria. For this chapter, phylum Actinobacteria is organized into three groups: coryneform bacteria, propionic acid bacteria, and filamentous bacteria.

1.5.1.1 Coryneform bacteria

Although the term coryneform indicates club-shaped cells (Figure 1.1d), the coryneform bacteria are also rod-shaped. The coryneform bacteria are commonly associated not only with human skin and mucosae but are also observed in soils and foods (Table 1.4). Common genera in this group include *Arthrobacter*, C*orynebacterium*, and *Kurthia* (Madigan *et al.*, 2012). *Corynebacterium* is a diverse group of greater than 100 species that includes decomposers as well as pathogenic bacteria (e.g., *Corynebacterium diphtheriae*). *Corynebacterium glutamicum* is of great economic interest because it is used to generate monosodium glutamate. Coryneform bacteria have been observed in association with decomposing remains and present an interesting trend in this context; their abundance can remain relatively consistent over time (Carter *et al.*, 2014). This persistence during decomposition will likely lead to great forensic interest because it might indicate their ability to survive in dynamic habitats, which is an important characteristic for trace evidence.

Table 1.4 General characteristics of two genera recognized as coryneform bacteria

	Corynebacterium	***Arthrobacter***
Morphology	Gram-positive rods, club shaped	Gram-variable, rod–coccus–rod developmental cycle (e.g., *Arthrobacter globiformis*)
Oxygen	Aerobic or facultative anaerobe	Mostly aerobic, but capable of anaerobic growth with nitrate (e.g., *Arthrobacter globiformis*)
Metabolism	Lactic acid fermentation	Mostly respiratory; can decompose herbicides, pesticides, nicotine, caffeine, and phenol
Habitat	Soil, food, human skin	Primarily upper layers of soil
Other	Of significant interest to industry (*Corynebacterium glutamicum*) and public health (*Corynebacterium diphtheriae*)	Highly resistant to desiccation and starvation (no spore formation)

From Brown (2015), Madigan *et al.* (2012), and Slonczewski and Foster (2011).

1.5.1.2 Propionic acid bacteria

The propionic acid bacteria are anaerobic or aerotolerant, and their morphology can be rod or filamentous. Common genera in this group include *Propionibacterium* and *Eubacterium*. The ecology of these bacteria is interesting because of their ability to ferment lactic acid. Typically the lactic acid bacteria, such as *Lactobacillus* (see Section 1.5.2.2), are very effective competitors that are able to inhibit the proliferation of other bacteria due to their ability to decrease the pH of a habitat. This is not the case with the propionic acid bacteria. In fact, the propionic bacteria decompose lactic acid to generate propionic acid, acetic acid, and carbon dioxide. A succession then occurs whereby propionic acid bacteria succeed lactic acid bacteria. This metabolic strategy, where propionic bacteria use a fermentation product released by other bacteria, is known as secondary fermentation (see Madigan *et al.*, 2012). The propionic acid bacteria are also among the many Gram-positive taxa present in the human oral cavity. The propionic bacteria thrive on human skin and in the ducts of sebaceous glands and are associated with acne, although they do not necessarily cause it. They also contribute to some components of body odor. One species in particular, *Propionibacterium acnes*, is ubiquitous on the human skin and is generally harmless.

1.5.1.3 Filamentous bacteria

The filamentous bacteria are a large group of phylogenetically related aerobic Gram-positive bacteria that form filamentous hyphae that are similar to fungal hyphae. This ability makes these bacteria particularly interesting because they essentially function as multicellular bacteria. Filamentous bacteria have cells to generate and release vast amounts of spores that are sometimes called arthrospores (Brown, 2015). These spores are not as durable as endospores released by *Bacillus* spp. and *Clostridium* spp. (see following text); however, they are resistant to desiccation (Killham and Prosser, 2007). Commonly observed filamentous bacteria include genera *Streptomyces* and *Actinomyces*.

Filamentous bacteria are primarily soil dwellers and are important decomposers because they can generate extracellular enzymes to decompose polysaccharides, proteins, and lipids. They prefer alkaline and neutral soils that are well drained and aerobic. They are able to proliferate in well-drained soils because, like fungi, their filaments can bridge gaps in water and nutrients, an ability that single-celled bacteria do not possess. The hallmark of these bacteria, however, is their prolific production of antibiotics, such as streptomycin, spectinomycin, tetracycline, erythromycin, clindamycin, and chloramphenicol. It remains to be seen if these antibiotics play a relevant role in postmortem or trace microbiology.

1.5.2 Firmicutes

The phylum Firmicutes are a large and diverse group of bacteria comprising 5 classes, 9 orders, and 39 families (www.bacterio.net). Firmicutes are usually decomposers (heterotrophic) found in soils, sediments, and in association with skin, mucosae, and

gastrointestinal tract. Members of the class Bacilli are primarily aerobic, while many of the other taxa in this phylum are usually anaerobic but often aerotolerant. Firmicutes are able to use a wide range of carbon sources, and therefore their fermentation products are similarly diverse, which might explain the vast diversity of volatile organic compounds that are released during decomposition (e.g., Statheropoulos *et al.*, 2005; Vass, 2012; Forbes *et al.*, 2016). Here we have divided Firmicutes into three main groups: sporulating firmicutes, lactic acid bacteria, and non-sporulating, non-lactic acid Firmicutes.

1.5.2.1 Sporulating Firmicutes

These bacteria are characterized by their ability to form endospores. These structures are highly heat-, chemical-, and radiation-resistant, thick-walled, and metabolically inactive. Endospores allow these organisms to endure unfavorable conditions such as extreme temperature, desiccation, or nutrient depletion. For example, endospore-forming Firmicutes can be selectively isolated from an environment by heating the sample to 80°C for 10 minutes. This exposure will kill active cells but will not harm the endospore. The endospore-forming Firmicutes are a large group of bacteria that are commonly found in association with soil, food, dust, and animals. Key genera in this group include *Bacillus*, *Clostridium*, *Heliobacterium*, and *Sporosarcina* (Figure 1.3). The common endospore formers can be grouped into two categories: aerobic (*Bacillus* and relatives) and anaerobic (*Clostridium* and relatives).

The aerobic endospore formers are found in the class Bacilli. Many Bacilli generate extracellular hydrolytic enzymes that can break down complex polymers, including polysaccharides, nucleic acids, and lipids, which explains their association with decomposing remains (Chun *et al.*, 2015). One of the most well-documented bacteria in this

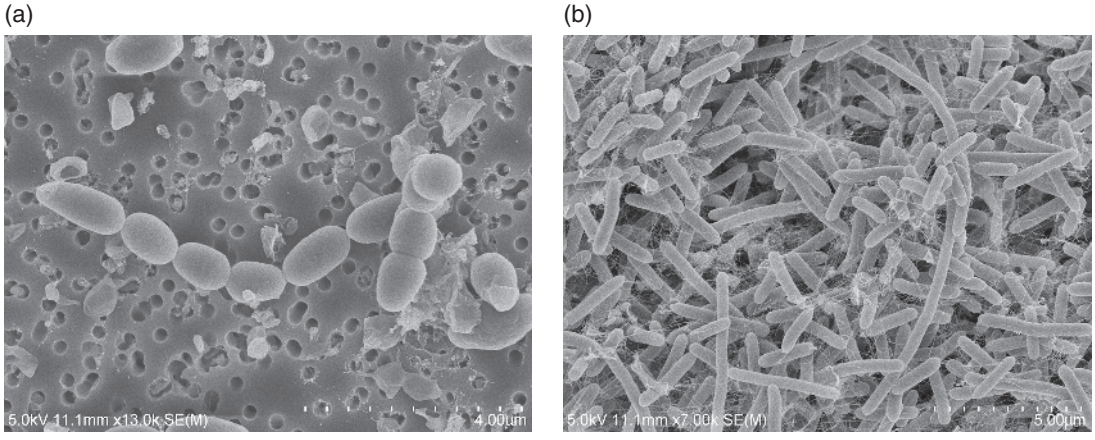

Figure 1.3 Scanning electron micrograph of endospore-forming Firmicutes *Bacillus subtilis* (a) and *Sporosarcina contaminans* (b) collected from the skin of a decomposing swine carcass on Oahu, Hawaii

group is *Bacillus anthracis*, the causative agent of anthrax. This species has the ability to produce endospores that can withstand harsh environments and have the ability to be industrialized. These characteristics have allowed terrorists to mass-produce and distribute the endospores as a biological weapon via simple methods, such as the postal service, as seen in 2001 (Government Accountability Office (GAO), 2005). Like many pathogens, this bacterium is an opportunist and will remain as an endospore until an ideal host or environment presents itself, such as a *B. anthracis* spore that is introduced to the human body through inhalation, ingestion, or through a break in the skin (cutaneous). The endospore then germinates and becomes an active microbial cell when it is introduced to these favorable environments. A number of aerobic endospore formers were formerly members of genus *Bacillus* but have since been reassigned to new genera such as *Paenibacillus*, *Lysinibacillus*, and *Geobacillus*. These genera have also been observed with decomposing remains (Chun *et al.*, 2015).

The anaerobic spore formers, class Clostridia, commonly reside in the soil and the animal gut where they primarily live in anaerobic microsites. Clostridia are fermenters since they are unable to use an electrochemical gradient and inorganic final electron acceptor. This trait has allowed further classification since there is variation in the fermentation substrates of *Clostridium* spp. For example, members of the class Clostridia are well-known fermenters of amino acids and fatty acids, which are in high abundance in decomposing vertebrate remains. Consequently, these microbes are regularly observed in postmortem microbial communities (Pechal *et al.*, 2014; Damann *et al.*, 2015; Metcalf *et al.*, 2016). Other Clostridia with medicolegal relevance include *Clostridium botulinum*, *Clostridium tetani*, and *Clostridium difficile* (Caplan and Koontz, 2001).

1.5.2.2 Lactic acid Firmicutes

The lactic acid bacteria are fermenters that produce lactic acid. This group of bacteria (e.g., *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Streptococcus*) are usually Gram-positive rods or cocci. One way to further classify the lactic acid bacteria is based on the pattern of products formed from their fermentation. The lactic acid bacteria are either homofermentative (only produce lactic acid) or heterofermentative (produce lactic acid and other compounds like ethanol and carbon dioxide). All lactic acid bacteria grow anaerobically but many are aerotolerant. The genera *Streptococcus* and *Lactococcus* are homofermentative and play an important role in dairy production. *Enterococcus* is typically grouped among the fecal bacteria. *Lactobacillus* can be homo- or heterofermentative and is more tolerant of acidic habitats than most other lactic acid bacteria; lactobacilli can grow at pH 4. They are often responsible for the final stages of most lactic acid fermentations, when the pH is too acidic for other bacteria. Because of this, they are able to outcompete many other bacteria because their production of lactic acid inhibits the growth of their microbial competitors.

1.5.2.3 Non-sporulating, non-lactic acid Firmicutes

The non-sporulating Firmicutes, such as *Sarcina*, *Staphylococcus*, and *Erysipelothrix*, are catalase positive, which separates them from *Streptococcus* and other Gram-positive cocci and rods. Oftentimes, *Micrococcus* is included among the non-sporulating Firmicutes even though it belongs to phylum Actinobacteria. *Staphylococcus* and *Micrococcus* are both aerobic bacteria that use respiration to generate energy. Staphylococci are common in humans and other animals. *Micrococcus* can be isolated from skin, but they are also common in soil and dust. In general, these Gram-positive cocci are tolerant of low moisture content and high salt concentrations (e.g., 7.5% NaCl in growth medium will select for these taxa), which makes them ideal as trace evidence since the human skin is a relatively salty environment.

1.5.3 Proteobacteria

The phylum Proteobacteria is by far the largest and most diverse of the bacterial phyla. Many bacteria commonly cultured from soil, water, and animals are species of Proteobacteria. Proteobacteria show a high metabolic diversity with autotrophic, heterotrophic, chemolithotrophic, chemoorganotrophic, and phototrophic taxa. They are equally diverse in their relationship to oxygen; obligate anaerobes, obligate aerobes, microaerophilic organisms, and facultative anaerobes are known. 16S rRNA gene sequences divide the phylum Proteobacteria into five classes: Alpha-, Beta-, Gamma-, Delta-, and Epsilon-. However, all of the aforementioned characteristics appear to be widely dispersed within these classes, making Proteobacteria difficult to organize. Brown (2015) groups Proteobacteria according to class, in which there is value (Table 1.5), but here we have grouped these bacteria by considering function as done by Madigan *et al.* (2012).

1.5.3.1 Enteric Proteobacteria

The enteric Proteobacteria are a major group of highly related bacteria of major medical importance that are found in class Gammaproteobacteria, family Enterobacteriaceae. These bacteria are facultatively anaerobic, Gram-negative, non-sporulating rods that have relatively simple nutrient requirements, can ferment sugars, and are often grouped by their metabolic strategy (Table 1.6). These bacteria are regularly found in soil and water but are most known for their presence in the digestive tracts of humans and several other animals. For example, *Escherichia* spp. are nearly universal inhabitants of human and other animal intestinal tracts. *Proteus* spp. are typically a frequent cause of urinary tract infections and have been shown to play a significant role in attracting flies to decomposing remains (Zheng *et al.*, 2013). Many enteric bacteria can be pathogenic, such as *Escherichia coli*, and the enterics are sometimes called coliforms because of their relatively close relationship with *E. coli*. Enteric bacteria have been observed in several recent studies (e.g., Lax *et al.*, 2014; Carter *et al.*, 2015), and it will be interesting to observe their use as physical evidence because they are relevant to cause of death, postmortem interval, and trace evidence.

Three genera from this group have recently garnered great attention because of their consistent association with human remains. These bacteria are *Proteus*, *Ignatzschineria* (Figure 1.4), and *Wohlfahrtiimonas*. *Proteus mirabilis* plays an important role in attracting

Table 1.5 Some general characteristics of the classes that comprise phylum Proteobacteria classes

Class	Morphology	Metabolism	Habitat	Forensically significant divisions
Alpha-	Rod, coccus, spirilla, appendaged	Phototrophy, heterotrophy, methylotrophy, autotrophy	Aerobic and anaerobic aquatic environments	Nitrifiers, methanotrophs, methylotrophs, sulfur, and iron oxidizers
Beta-	Rod, coccus, spirilla, filamentous	Aerobic, facultative anaerobic, heterotrophy, chemolithotrophy	Organic-rich environments (soils, wastewater, sediments)	Sulfur-, iron-, manganese-, methane-oxidizers, nitrifiers
Gamma-	Rod, coccus, spirilla, vibrio, filamentous	Aerobic, facultative anaerobic, microaerotrophic, obligate anaerobic; heterotrophy, chemoautotrophy, autotrophy	Ubiquitous; psychro-, meso-, and thermophiles	Methanotrophs, methylotrophs, sulfur and iron oxidizers, pseudomonads, enterics
Delta-	Straight or curved rod	Anaerobic sulfate and sulfur reduction	Varies with phenotype (i.e., sulfate-reducers in sulfur-rich anaerobic environments)	Sulfate and sulfur reducers, sulfur oxidizers, nitrifiers
Epsilon-	Helically curved rods with flagella	Microaerotrophy, heterotrophy, chemoautotrophy	Intestinal symbionts, deep sea	Sulfate and sulfur reducers

From Brown (2015), Madigan *et al.* (2012), and Slonczewski and Foster (2011).

Table 1.6 Grouping of enteric bacteria by metabolic product

Group	Metabolism	Taxa
Mixed acid fermenters	Forms acetic, lactic, and succinic acids; ethanol, CO_2, and H_2; equal amounts of CO_2 and H_2 are produced	*Escherichia, Proteus, Salmonella, Yersinia*
2,3-butanediol producers	Less acid formed, main products are butanediol, ethanol, CO_2, and H_2; more CO_2 is produced than H_2	*Enterobacter, Klebsiella, Serratia*

From Brown (2015), Madigan *et al.* (2012), and Slonczewski and Foster (2011).

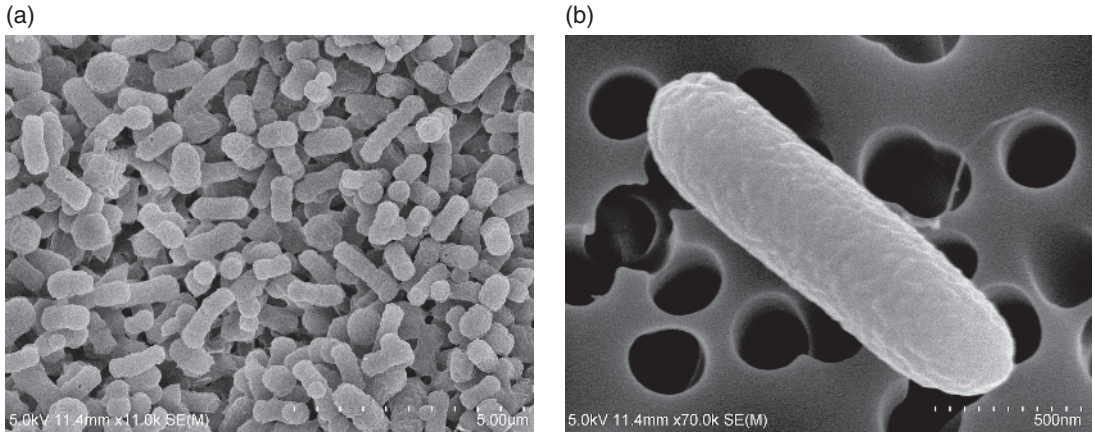

Figure 1.4 Scanning electron micrographs of enteric Proteobacteria *Ignatzschineria indica* collected from the skin of a decomposing swine carcass on Oahu, Hawaii presented as a colony (a) and as a single cell on a filter (b)

forensically important insects to resources (Ma *et al.*, 2012; Zheng *et al.*, 2013). This bacterium can colonize resources rapidly through a process called swarming where the cells grow and spread rapidly, ultimately forming biofilm. *Ignatzschineria* and *Wohlfahrtiimonas* also have a strong association with insects. Most likely as a consequence of this interaction, they are also regularly found in association with decomposing remains (e.g., Chun *et al.*, 2015). The forensic value of these microbes is not yet fully understood, but these bacteria are already an excellent example in the importance of understanding insect–microbe interactions (Chapter 11).

1.5.3.2 *Pseudomonas* and the pseudomonads

Pseudomonas is a genus within Gammaproteobacteria and the term pseudomonad refers to other Proteobacteria that share similar characteristics: Gram-negative chemoorganotrophic rods with polar flagella, such as *Burkholderia* and *Xanthomonas* (Madigan *et al.*, 2012). Common in most aerobic environments, the pseudomonads typically use aerobic respiration for energy. However, many pseudomonads can grow under anoxic conditions, often using nitrate as an alternative electron acceptor. The pseudomonads can thrive at relatively low nutrient levels because of their simple nutrient requirements and ability to use a vast array of different organic compounds. Since they tend to lack the hydrolytic enzymes necessary for catalysis of high molecular compounds, pseudomonads tend to use lower molecular weight organic compounds. Pseudomonads are also effective decomposers of xenobiotics, such as herbicides and pesticides. Because of their ability to capitalize on low nutrient environments, grow anoxically, and vary their electron acceptor to what is available, these organisms are well equipped to thrive in a decomposition environment.

1.5.3.3 Nitrifying Proteobacteria

Nitrification is a process where ammonia (NH_3) is oxidized to nitrite (NO_2^-), which is then oxidized to nitrate (NO_3^-). This process is critical for microbial function in all habitats and probably explains some of the pH changes observed in gravesoil (Vass *et al.*, 1992; Carter and Tibbett, 2008). These bacteria are widespread in soil and water and proliferate where the concentration of ammonia is high, such as in gravesoil (e.g., Anderson *et al.*, 2013) and bodies of water that receive an input of sewage or wastewater.

All aerobic ammonia oxidizers are members of the class Betaproteobacteria except for *Nitrosococcus*, which is in Gammaproteobacteria. Most nitrifying bacteria are obligate chemolithotrophs and obligate aerobes that oxidize inorganic nitrogen. However, *Nitrobacter* spp. are able to grow via chemoorganotrophy on acetate or pyruvate as the carbon source. Phylogenetically, nitrifying bacteria are found in Alpha-, Beta-, Gamma-, and Deltaproteobacteria. However, the genus *Nitrospira* forms its own phylum and represents nitrifying bacteria in metabolism only, even though it has been suggested that it is the most abundant nitrifying bacterial taxon in nature (Madigan *et al.*, 2012).

1.5.3.4 Methanotrophic and methylotrophic Proteobacteria

Methylotrophs are bacteria that can grow using C_1 compounds such as methanol, formate, and formamide. Thus, all organic compounds in the cell must be synthesized from C_1 precursors. Methanotrophs are methylotrophs that can also oxidize methane (CH_4). Methane is found in anoxic environments throughout the planet, including the mammalian digestive tracts, decomposing remains, water-treatment areas, and landfills. A methane cycle almost certainly exists in any coupled anaerobic/aerobic system making it a likely inhabitant of a postmortem microbial community. The main consumers of methane are Gammaproteobacteria and Alphaproteobacteria. These consumers are aerobic and present in several soil, water, and digestive systems, often located at the interface of methane and oxygen-rich systems. They conduct chemoautotrophy using methane released from the anaerobic environment to generate energy in the aerobic environment. Because of this, methanotrophs play an important role in the carbon cycle as they convert methane into cellular material and carbon dioxide.

1.5.3.5 Sulfur- and iron-oxidizing Proteobacteria

Sulfide oxidizers are found among the Alpha-, Beta-, Gamma-, Delta-, and Epsilonproteobacteria (Plante, 2007). However, the Gammaproteobacteria are the most commonly observed. These bacteria are typically found in black hydrogen sulfide-rich soils and sediments, such as those associated with decomposing remains (Vass *et al.*, 1992). Ecosystems in which hydrogen sulfide is produced tend to be microaerobic or anaerobic. Because of this, sulfur oxidizers must position themselves at the interface of sulfur-rich and oxygenated habitats, in a similar way as the methanotrophs. These bacteria oxidize hydrogen sulfide and other reduced sulfur compounds for energy with oxygen or nitrate as

electron acceptors. They use either carbon dioxide or organic compounds as carbon sources. Sulfur oxidizers form the foundation of a food web, just like photosynthesizers in other ecosystems. Sulfur and hydrogen sulfide provide energy for various autotrophic sulfur or sulfide oxidizers, and the oxidation of these substrates generates sulfuric acid (H_2SO_4).

1.5.3.6 Sulfate- and sulfur-reducing Proteobacteria

Sulfate and sulfur reducers proliferate in sulfur-rich environments. The most obvious indicator of these organisms is the characteristic odor associated with decomposing vertebrate remains often encountered during forensic investigations. These bacteria use organic compounds or H_2 in anaerobic respiration. Sulfate and sulfur are electron acceptors, while hydrogen, lactate, and pyruvate are often the electron donors. Hydrogen sulfide is the end product of both reductions. The genera of dissimilative sulfate and sulfur reducers can be placed in one of the two groups: (i) those that can oxidize acetate and other fatty acids to carbon dioxide, such as *Desulfobacter*, *Desulfonema*, and *Desulfosarcina* and (ii) those that cannot, such as *Desulfovibrio*, *Desulfuromonas*, *Desulfotomaculum*, and *Desulfobulbus*. Dissimilative simply means that the sulfate or sulfur is used for energy, not for growth. Sulfate-reducing bacteria are widespread and often form communities with sulfur oxidizers. *Desulfosarcina* and *Desulfonema* are actually chemolithotrophs with carbon dioxide serving as the sole C source. Several other taxa are also able to reduce sulfur, including *Proteus*, *Campylobacter*, *Pseudomonas*, and *Salmonella*.

1.6 Archaea and microbial eukaryotes

The amount of space dedicated to the domains Archaea and Eukaryota does not accurately reflect their importance to forensic microbiology. Yet, there are major gaps in our understanding of these organisms. Here we will briefly discuss the fungi, other microbial eukaryotes, and domain Archaea.

Fungi have received the most research attention of the members of this section. Fungi are regularly associated with decomposing remains (Hitosugi *et al.*, 2006; Sagara *et al.*, 2008; Chimutsa *et al.*, 2015; Olakanye *et al.*, 2015; Metcalf *et al.*, 2016) and have value as trace evidence (Hawksworth and Wiltshire, 2011; Wiltshire *et al.*, 2014; Young *et al.*, 2015, 2016). The fungi associated with decomposing remains tend to proliferate after the flush of bacterial and insect activity or when they can colonize the skin (Hitosugi *et al.*, 2006; Ishii *et al.*, 2006). These fungi apparently proliferate in response to the availability of ammonia and nitrate, so they probably have a relationship with the nitrifying Proteobacteria.

The Archaea are virtually unstudied from a forensic perspective. They are currently organized into five phyla with the majority of Archaea representing phylum Crenarchaeota or phylum Euryarchaeota. These organisms, like bacteria, are small (0.5–5.0 μm) prokaryotes that are widely distributed around the planet. However, the Archaea are more ecologically diverse than the bacteria and eukaryotes because they inhabit several extreme environments. Some archaea are hyperthermophiles that can thrive at hot temperatures like those found in hot springs and hydrothermal vents. Other archaea are psychrophiles

that can live in association with sea ice and glacial lakes. Others are tolerant to extreme pressure, acidity, alkalinity, and salinity, and it will be valuable to determine if these microbes have forensic value due to their extremely diverse habitat ranges. For example, some archaea are methanogens, generating methane from carbon dioxide and hydrogen. These archaea might be of value to the investigation of decomposing remains.

1.7 Conclusions

Modern advances in microbiology and microbial ecology allow for the study of prokaryotes and eukaryotes at an unprecedented resolution. This knowledge is potentially helpful for forensic science because it allows us to better understand microorganisms and their environment. Some key environmental parameters that significantly affect microorganisms are moisture, oxygen, and temperature. These parameters must be considered when interpreting microbial evidence. Also critical is the understanding that all microbes are in competition for energy and nutrients. Understanding how microbes compete to survive is vital for a robust interpretation of microbial evidence. In terms of taxonomy, many recent studies have highlighted the forensic value of bacterial phyla Actinobacteria, Firmicutes, and Proteobacteria. We expect that these taxa will continue to be explored as physical evidence, and we are hopeful that significant attention will be paid to Archaea and microbial eukaryotes, as many of these organisms have already shown some potential to serve as bioindicators of past events.

Acknowledgments

We kindly thank R. W. Carter and C. Carter for the helpful comments on the manuscript. We also thank T. Carvalho and the University of Hawaii at Manoa Biological Electron Microscope Facility for assistance with scanning electron micrographs.

References

Almirall, J. and Trejos, T. (2016) Analysis of glass evidence, in *Forensic Chemistry: Fundamentals and Applications* (ed J.A. Siegel), John Wiley & Sons, Ltd., Chichester, UK, pp. 228–272.

Anderson, B., Meyer, J. and Carter, D.O. (2013) Dynamics of ninhydrin-reactive nitrogen and pH in gravesoil during the extended postmortem interval. *Journal of Forensic Sciences*, **58**, 1348–1352.

Ball, A.S. (1997) *Bacterial Cell Culture: Essential Data*, John Wiley & Sons, Ltd., Chichester, UK.

Benbow, M.E., Pechal, J.L., Lang, J.M. *et al.* (2015) The potential of high-throughput metagenomic sequencing of aquatic bacterial communities to estimate the postmortem submersion interval. *Journal of Forensic Sciences*, **60**, 1500–1510.

Bisbing, R.E. (2016) The forensic comparison of soil and geological microtraces, in *Forensic Chemistry: Fundamentals and Applications* (ed J.A. Siegel), John Wiley & Sons, Ltd., Chichester, UK, pp. 273–317.

Bouslimani, A., Porto, C., Rath, C.M. *et al.* (2015) Molecular cartography of the human skin surface in 3D. *Proceedings of the National Academy of Sciences of the United States of America*, **112**, E2120–E2129.

Brown, J.W. (2015) *Principles of Microbial Diversity*, American Society for Microbiology Press, Washington, DC.

Brzezinski, J.L. and Craft, D.L. (2012) Characterization of microorganisms isolated from counterfeit toothpaste. *Journal of Forensic Sciences*, **57**, 1365–1367.

Budowle, B., Schutzer, S.E., Breeze, R.G. *et al.* (eds) (2011) *Microbial Forensics*, 2nd edn, Academic Press, Burlington, MA.

Caplan, M.J. and Koontz, F.P. (2001) *Cumitech 35 Postmortem Microbiology*, American Society for Microbiology Press, Washington, DC.

Carter, D.O. and Tibbett, M. (2003) Taphonomic mycota: fungi with forensic potential. *Journal of Forensic Sciences*, **48**, 168–171.

Carter, D.O. and Tibbett, M. (2006) Microbial decomposition of skeletal muscle tissue (*Ovis aries*) in a sandy loam soil at different temperatures. *Soil Biology and Biochemistry*, **38**, 1139–1145.

Carter, D.O. and Tibbett, M. (2008) Does repeated burial of skeletal muscle tissue (*Ovis aries*) in soil affect subsequent decomposition? *Applied Soil Ecology*, **40**, 529–535.

Carter, D.O., Yellowlees, D. and Tibbett, M. (2007a) Autoclaving can kill soil microbes yet enzymes can remain active. *Pedobiologia*, **51**, 295–299.

Carter, D.O., Yellowlees, D. and Tibbett, M. (2007b) Cadaver decomposition in terrestrial ecosystems. *Naturwissenschaften*, **94**, 12–24.

Carter, D.O., Yellowlees, D. and Tibbett, M. (2008) Temperature affects microbial decomposition of cadavers (*Rattus rattus*) in contrasting soils. *Applied Soil Ecology*, **40**, 129–137.

Carter, D.O., Yellowlees, D. and Tibbett, M. (2010) Moisture can be the dominant environmental parameter governing cadaver decomposition in soil. *Forensic Science International*, **200**, 60–66.

Carter, D.O., Knight, R. and Metcalf, J.L. (2014) *A preliminary survey of a postmortem skin microbiome on Oahu*. Proceedings of 66th Annual Meeting of the American Academy of Forensic Sciences, vol. **20**, Seattle, WA, February 17–22, 2014, American Academy of Forensic Sciences, Colorado Springs, CO, pp. 361–362.

Carter, D.O., Metcalf, J.L., Bibat, A. and Knight, R. (2015) Seasonal variation of postmortem microbial communities. *Forensic Science, Medicine, and Pathology*, **11**, 202–207.

Chimutsa, M., Olakanye, A.O., Thompson, T.J.U. and Komang Ralebitso-Senior, T. (2015) Soil fungal community shift evaluation as potential cadaver decomposition indicator. *Forensic Science International*, **257**, 155–159.

Chun, L., Miguel, M., Junkins, E.N. *et al.* (2015) An initial investigation in the ecology of culturable postmortem bacteria. *Science and Justice*, **55**, 394–401.

Damann, F.E., Williams, D.E. and Layton, A.C. (2015) Potential use of bacterial community succession in decaying human bone for estimating postmortem interval. *Journal of Forensic Sciences*, **60**, 844–850.

Degens, B.P. and Harris, J.A. (1997) Development of a physiological approach to measuring the catabolic diversity of soil microbial communities. *Soil Biology and Biochemistry*, **9–10**, 1309–1320.

Fierer, N., Lauber, C.L., Zhou, N. *et al.* (2010) Forensic identification using skin bacterial communities. *Proceedings of the National Academy of Sciences*, **107**, 6477–6481.

Finley, S.J., Pechal, J.L., Benbow, M.E. *et al.* (2016) Microbial signatures of cadaver gravesoil during decomposition. *Microbial Ecology*, **71**, 524–529.

Forbes, S.L. (2008) Decomposition chemistry in a burial environment, in *Soil Analysis in Forensic Taphonomy: Chemical and Biological Effects of Buried Human Remains* (eds M. Tibbett and D.O. Carter), CRC Press, Boca Raton, FL, pp. 203–223.

Forbes, S.L., Troobnikoff, A.N., Ueland, M. *et al.* (2016) Profiling the decomposition odour at the grave surface before and after probing. *Forensic Science International*, **259**, 193–199.

Government Accountability Office (GAO) (2005) Anthrax Detection: Agencies Need to Validate Sampling Activities in Order to Increase Confidence in Negative Results. GAO, Washington, DC.
Grice, E.A. and Segre, J.A. (2011) The skin microbiome. *Nature Reviews Microbiology*, **9**, 244–253.
Guo, J., Fu, X., Liao, H. *et al.* (2016) Potential use of bacterial community succession for estimating postmortem interval as revealed by high throughput sequencing. *Scientific Reports*, **6**, 24197.
Hauther, K.A., Cobaugh, K.L., Meadows-Jantz, L. *et al.* (2015) Estimating time since death from postmortem human gut microbial communities. *Journal of Forensic Sciences*, **60**, 1234–1240.
Hawksworth, D.L. and Wiltshire, P.E.J. (2011) Forensic mycology: the use of fungi in criminal investigations. *Forensic Science International*, **206**, 1–11.
Hitosugi, M., Ishii, K., Yaguchi, T. *et al.* (2006) Fungi can be a useful forensic tool. *Legal Medicine*, **8**, 240–242.
Houck, M. (2016) Textiles, in *Forensic Chemistry: Fundamentals and Applications* (ed J.A. Siegel), John Wiley & Sons, Ltd., Chichester, UK, pp. 40–74.
Hug, L.A., Baker, B.J., Anantharaman, K. *et al.* (2016) A new view of the tree of life. *Nature Microbiology*, **1**, 1–6.
Iancu, L., Carter, D.O., Junkins, E.N. and Purcarea, C. (2015) Using bacterial and necrophagous insect dynamics for post-mortem interval estimation during cold season: novel case study in Romania. *Forensic Science International*, **254**, 106–117.
Ishii, K., Hitosugi, M., Kido, M. *et al.* (2006) Analysis of fungi detected in human cadavers. *Legal Medicine*, **8**, 188–190.
Jayachandran, K., Stolpe, N.B., Moorman, T.B. and Shea, P.J. (1998) Application of ^{14}C most probable number technique to enumerate atrazine-degrading microorganisms in soil. *Soil Biology and Biochemistry*, **30**, 523–529.
Killham, K. and Prosser, J.I. (2007) The prokaryotes, in *Soil Microbiology, Ecology, and Biochemistry*, 3rd edn (ed E.A. Paul), Academic Press, Burlington, MA, pp. 119–144.
Kirkbride, P. (2016) Paint and coatings examination, in *Forensic Chemistry: Fundamentals and Applications* (ed J.A. Siegel), John Wiley & Sons, Ltd., Chichester, UK, pp. 75–134.
Lauber, C.L., Metcalf, J.L., Keepers, K. *et al.* (2014) Vertebrate decomposition is accelerated by soil microbes. *Applied and Environmental Microbiology*, **80**, 4920–4929.
Lax, S., Smith, D.P., Hampton-Marcell, J. *et al.* (2014) Longitudinal analysis of microbial interaction between humans and the indoor environment. *Science*, **345**, 1048–1052.
Lax, S., Hampton-Marcell, J.T., Gibbons, S.M. *et al.* (2015) Forensic analysis of the microbiome of phones and shoes. *Microbiome*, **3**, 21.
Locard, E. (1930a) The analysis of dust traces. Part III. *American Journal of Political Science*, **1**, 496–514.
Locard, E. (1930b) The analysis of dust traces. Part II. *American Journal of Political Science*, **1**, 401–418.
Locard, E. (1930c) The analysis of dust traces. Part I. *American Journal of Political Science*, **1**, 276–298.
Ma, Q., Fonseca, A., Liu, W. *et al.* (2012) *Proteus mirabilis* interkingdom swarming signals attract blow flies. *The ISME Journal*, **6**, 1356–1366.
MacCallum, W.G. and Hastings, T.W. (1899) A case of acute endocarditis caused by *Micrococcus zymogenes* (nov. spec.), with a description of the microorganism. *Journal of Experimental Medicine*, **4**, 521–534.
Madigan, M., Martinko, J., Stahl, D. and Clark, D. (2012) *Brock Biology of Microorganisms*, 13th edn, Pearson, San Francisco, CA.
Metcalf, J.L., Wegener Parfrey, L., Gonzalez, A. *et al.* (2013) A microbial clock provides an accurate estimate of the postmortem interval in a mouse model system. *eLife*, **2**, e01104.

Metcalf, J.L., Xu, Z.Z., Weiss, S. *et al.* (2016) Microbial community assembly and metabolic function during mammalian corpse decomposition. *Science*, **351**, 158–162.

Nagasawa, S., Motani-Saitoh, H., Inoue, H. and Iwase, H. (2013) Geographic diversity of *Helicobacter pylori* in cadavers: forensic estimation of geographical origin. *Forensic Science International*, **229**, 7–12.

Olakanye, A.O., Thompson, T. and Komang Ralebitso-Senior, T. (2015) Soil fungal community shift evaluation as a potential cadaver decomposition indicator. *Forensic Science International*, **257**, 155–159.

Pechal, J.L., Crippen, T.L., Tarone, A.M. *et al.* (2013) Microbial community functional change during vertebrate carrion decomposition. *PLoS One*, **8**, e79035.

Pechal, J.L., Crippen, T.L., Benbow, M.E. *et al.* (2014) The potential use of bacterial community succession in forensics as described by high throughput metagenomic sequencing. *International Journal of Legal Medicine*, **128**, 193–205.

Plante, A.F. (2007) Soil biogeochemical cycling of inorganic nutrients and metals, in *Soil Microbiology, Ecology, and Biochemistry* (ed E.A. Paul), Academic Press, Burlington, MA, pp. 389–432.

Putman, R.J. (1978) Patterns of carbon dioxide evolution from decaying carrion. 1. Decomposition of small mammal carrion in temperate systems. *Oikos*, **31**, 47–57.

Sagara, N., Yamanaka, T. and Tibbett, M. (2008) Soil fungi associated with graves and latrines: toward a forensic mycology, in *Soil Analysis in Forensic Taphonomy: Chemical and Biological Effects of Buried Human Remains* (eds M. Tibbett and D.O. Carter), CRC Press, Boca Raton, FL, pp. 67–108.

Slonczewski, J.L. and Foster, J.W. (2011) *Microbiology: An Evolving Science*, 2nd edn, W. W. Norton and Company, New York, NY.

Song, S.J., Lauber, C., Costello, E.K. *et al.* (2013) Cohabiting family members share microbiota with one another and with their dogs. *eLife*, **2**, e00458.

Statheropoulos, M., Spiliopoulou, C. and Agapiou, A. (2005) A study of volatile organic compounds evolved from the decaying human body. *Forensic Science International*, **153**, 147–155.

Swift, M.J., Heal, O.W. and Anderson, J.M. (1979) *Decomposition in Terrestrial Ecosystems*, Blackwell Scientific, Oxford, UK.

Tridico, S.R., Murray, D.C., Addison, J. *et al.* (2014) Metagenomic analyses of bacteria on human hairs: a qualitative assessment for applications in forensic science. *Investigative Genetics*, **5**, 16.

Vass, A.A. (2012) Odor mortis. *Forensic Science International*, **222**, 234–241.

Vass, A.A., Bass, W.M., Wolt, J.D. *et al.* (1992) Time since death determinations of human cadavers using soil solution. *Journal of Forensic Sciences*, **37**, 1236–1253.

Wiltshire, P.E.J., Hawksworth, D.L., Webb, J.A. and Edwards, K.J. (2014) Palynology and mycology provide separate classes of probative evidence from the same forensic samples: a rape case from southern England. *Forensic Science International*, **244**, 186–195.

Woese, C.R., Kandler, O. and Wheelis, M.L. (1990) Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. *Proceedings of the National Academy of Sciences of the United States of America*, **87**, 4576–4579.

Young, J.M., Weyrich, L.S., Breen, J. *et al.* (2015) Predicting the origin of soil evidence: high throughput eukaryote sequencing and MIR spectroscopy applied to a crime scene scenario. *Forensic Science International*, **251**, 22–31.

Young, J.M., Weyrich, L.S. and Cooper, A. (2016) High throughput sequencing of trace quantities of soil provides reproducible and discriminative fungal DNA profiles. *Journal of Forensic Sciences*, **61**, 478–484.

Zheng, L., Yu, Z., Crippen, T.L. *et al.* (2013) Bacteria mediate oviposition by the black soldier fly, *Hermetia illucens* (L.), (Diptera: Stratiomyidae). *Scientific Reports*, **3**, 2563.