

Chapter 2

Handling and growing microorganisms



Sampling bacteria from a Petri dish

KEY FACTS

- The word 'sterile' means the complete absence of life, whereas 'aseptic' means a procedure designed to avoid unwanted transfer of organisms from one item or place to another.
- Aseptic procedures can protect the operator from hazardous organisms and the material being handled from contamination.
- Microorganisms are placed in one of four categories according to the infection risk they pose; these are designated hazard groups 1–4, with 4 being the most dangerous. Organisms of pharmaceutical interest are usually in groups 1 and 2.
- Culture media for bacteria usually contain hydrolysed protein as a source of amino acids and B-group vitamins from yeast extract. Media for fungi often have higher sugar concentrations and a lower pH than those for bacteria.
- Microorganisms are normally grown either in liquid media (called broths) or on 'solid' media in Petri dishes. Anaerobic organisms must be grown without oxygen.
- The growth of microorganisms may be affected by a variety of factors including temperature, pH, redox potential, osmotic pressure, nutrient availability and the gaseous environment.

2.1 Sterility and asepsis – what do they mean?

It is obvious that microbial cultures need to be handled in a safe manner in order to avoid the risk of infection, so the important aspects of microbiological safety will be considered in this chapter. But first, it is essential to fully understand the meaning of words used in descriptions of safety procedures. Two of these words, *sterile* and *aseptic*, are commonly used both in relation to safety and in the

context of manufacturing and dispensing of medicines. Unfortunately, the words are often misunderstood and used incorrectly as if they mean the same thing – they don't!

Sterile, in a pharmaceutical context, means the complete absence of life. So any medicine or surgical device, or, indeed, any object that is sterile, has no living organisms at all in it or on it. It is an absolute term, so an object is either sterile or it is not; there are no levels of microbial contamination that are so low as to be regarded as insignificant and therefore acceptable. If a medicine is

contaminated with a single organism it is not sterile, so phrases like 'almost sterile' or 'more sterile' should be avoided because they simply display a lack of understanding of the concept.

Several categories of medicines, notably injections and eye products, are required to be sterile, and there are two manufacturing strategies available: the preferred method, known as terminal sterilization, is where the medicine is made from nonsterile raw materials and subjected to a heat, radiation or other sterilization procedure at the end of the manufacturing process. The alternative, used when the product cannot withstand the high temperatures or radiation doses of a terminal sterilization process, is to start with raw materials that are individually sterilized – often by passing solutions of them through bacteria-proof filters – and then mixing them together under conditions that do not allow the entry of microorganisms, followed by filling into presterilized containers; this is termed 'aseptic manufacture'.

Aseptic, therefore, is a word used to describe a procedure that is intended to avoid the unwanted transfer of microorganisms from one object or location to another. It works both ways: it can be a procedure designed to avoid the introduction of organisms into a medicine whilst it is being made, dispensed or administered to a patient, but it also describes procedures for handling hazardous organisms which pose an infection risk to operators. In this case, therefore, the aseptic procedure is intended to keep the organism in its container and avoid it being dispersed into the atmosphere and inhaled, or transferred onto the body of the person handling it. Regardless of whether the intention is to protect the product or protect the operator, aseptic procedures would normally require gowns, gloves, facemasks, disinfectants and the use of safety cabinets or isolators supplied with filtered, decontaminated air.

2.2 Hazard categories of microorganisms

Clearly, microorganisms differ in terms of the infection risk they pose, and many are harmless; others, termed opportunist pathogens, usually only infect individuals with impaired immunity; and some cause incurable, possibly fatal infections. Hazardous organisms need to be contained – that is, handled in laboratories that are designed to protect operators and with facilities which are proportionate to the risk the organism represents. To ensure that laboratory staff are aware of the hazard involved and the degree of laboratory containment

required for any individual organism, the UK government's Advisory Committee on Dangerous Pathogens has assigned microorganisms into one of four categories (designated hazard groups 1–4 with 4 being the most dangerous) in an *Approved List of Biological Agents*, and described the laboratory facilities necessary for the containment of organisms in each group. It is important for persons working in microbiology laboratories in the pharmaceutical industry, hospital pathology laboratories or laboratories attached to hospital manufacturing units to be familiar with the classification scheme and, in particular, with the laboratory design features associated with each category of containment. An organism is classified on the following criteria:

- Is it pathogenic for humans?
- Is it a hazard to laboratory workers (does it survive in the dry state or outside the body for example)?
- Is it readily transmissible from person to person?
- Are effective prophylaxis (vaccines) and treatment (antibiotics) available?

Organisms in group 4 are exclusively viruses, and all of them are readily transmissible pathogens causing untreatable infections with high mortality rates for which there is usually no vaccine. Even some of the notorious bioterrorism pathogens like those responsible for anthrax and bubonic plague are only hazard group 3 because both are bacteria for which there are effective antibiotics. Table 2.1 summarizes the characteristics of each group.

The three classes of safety cabinets all provide operator protection and are designed to minimize the risk of aerosol generation and inhalation of microorganisms; classes II (Figure 2.1) and III additionally provide product protection. The working area in a class III cabinet is totally enclosed and the operator is separated from the materials being handled by a physical barrier, for example integral gloves attached to the front of the cabinet. The design features of the laboratories for each category of containment are too detailed to reproduce here, but are available in a UK Health and Safety Executive book titled *The Management, Design and Operation of Microbiological Containment Laboratories*. There are recommendations on ease of cleaning and absorbency of work surfaces (metal or impervious plastic rather than wood, for example), control of personnel access, operator working space, ventilation, washing facilities, disinfection, laboratory clothing, disposal of waste and accident reporting. Level 3 containment is not commonly encountered in a pharmaceutical setting, and level 4 not at all; containment level 2 would be appropriate for

Table 2.1 Hazard classification of microorganisms by the UK Advisory Committee on Dangerous Pathogens.

Hazard group	Description	Typical organisms	Containment
1	Organisms not normally considered harmful	Lactobacilli (found in milk) and baker's yeast	Open laboratory bench
2	Possible hazard for laboratory staff, but unlikely to spread in the community; antibiotics and vaccines usually available	<i>E. coli</i> , staphylococci and streptococci	Open bench, or class I or II safety cabinets
3	Definite hazard to laboratory staff and may spread in the community; antibiotics and vaccines may be available	The organisms responsible for typhoid, diphtheria, HIV and rabies	Class I or class III safety cabinets ^a
4	Serious hazard to laboratory staff and high risk to the community; antibiotics and vaccines not normally available. Exclusively viral infections	Marburg, Ebola and Lassa fever viruses	Class III safety cabinets

^aClass II cabinets are designed to protect the product being handled against microbial contamination, and the level of operator protection they afford is not considered adequate for some hazard group 3 pathogens.

organisms reasonably likely to be encountered as contaminants of pharmaceutical materials or organisms used in tests and assays.



Figure 2.1 A microbiologist working with influenza virus using a class II safety cabinet in which the air is passed through filters to remove microorganisms. Source: PHIL ID #7988; Photo Credit: James Gathany, Centers for Disease Control and Prevention.

2.3 Sources and preservation of microorganisms

There are several tests and assays described in international standards, pharmacopoeias and regulatory documents for which specified organisms must be used, for example antibiotic assays, preservative efficacy tests and tests for sterility. Usually a particular strain of the organism is required. A strain is a subdivision of a species; it conforms to the standard species description in all major respects but is distinguishable by the possession of a particular property, for example the production of a particular metabolite, enzyme or toxin, or resistance to an antibiotic. Authentic, pure cultures of the test strains are obtainable from national or international culture collections, and strains are identified by the culture collection initials and reference number. The American Type Culture Collection (ATCC) and the UK National Collection of Industrial and Marine Bacteria (NCIMB) are two whose strains are often specified in official methods, but there are others specializing in particular categories of organisms, such as pathogenic bacteria or fungi.

Bacteria and fungi can grow quickly and pass through many generations in a short time, so if a strain is grown in the lab for months or years there is a real possibility of mutants arising with properties that differ significantly from the original cells. If a strain that has changed in this way is used for a test or assay it may not give results that

are consistent with those obtained previously, so many test methods direct that the cells to be used must not be more than a specified number of subcultures removed from the original culture collection specimen (subculturing simply means regrowing the organism in fresh, sterile culture medium).

Organisms may be obtained from many other sources and preserved in the laboratory for research or reference purposes. This is common with antibiotic-resistant strains, and the research laboratories of companies developing new antibiotics may keep literally hundreds or thousands of strains with which to compare the effectiveness of a new antibiotic with established ones. It is also common for pharmaceutical companies to keep organisms isolated from their manufacturing areas (these are termed environmental isolates) and from contaminated products in which microorganisms have grown, particularly if they have done so despite the presence of chemical preservatives.

Strains from culture collections are usually supplied in sealed ampoules containing the organism freeze-dried in gelatin, which has to be reconstituted with growth medium when the ampoule is opened (Figure 2.2). Most of the bacteria and fungi of pharmaceutical and medical importance can be stored freeze-dried for many years without significant loss of viability. Alternatively, they can be stored in sealed containers (plastic screw-capped ampoules called cryotubes) either in liquid

nitrogen at -196°C , or in freezers at -80°C . In each case, glycerol (5–15% w/w) is often added to the culture beforehand to protect against freeze-thaw damage.

Many organisms will readily survive storage in a refrigerator at 4°C for several weeks or even months, so it is common for bacteria to be kept in this way in Petri dishes sealed with tape, or in screw-capped bottles in which the culture gel was allowed to set at an angle in order to increase the surface area available for growth; these are referred to as slopes or slants (Figure 2.2). If Petri dishes are left unsealed, water evaporates from the culture gel and this may accelerate the death of the organism.

When preserving cultures it is not only important to ensure that the cells are alive but also that they are not contaminated with other species – the purity of the culture is essential. This is confirmed by streaking a liquid culture onto a Petri dish using an inoculating loop (a thin wire attached to an insulated handle; the wire is twisted at the end into a loop of approximately 5 mm diameter so that it will retain a thin film of any liquid in which it is immersed – similar to a child's soap bubble tub!). Streaking is simply a means of spreading a small volume over the surface of the gel in order to dilute it and separate the cells or colony-forming units (often abbreviated to CFU and meaning a group of cells attached to each other so that they give rise to just a single colony after incubation); the operator can then see any contaminant colonies that may differ from the others in terms of shape, size, colour etc. There are several ways in which a Petri dish can be streaked, one of which is shown in Figure 2.3A. The inoculating loop is first heated to redness in a flame to kill any organisms that may already be on it (or a sterile, disposable plastic loop may be used). The Petri dish in Figure 2.3B has been streaked well and indicates that the culture is pure because all of the colonies look alike, but the streaking in Figure 2.3C is unsatisfactory because there is inadequate separation of the individual colonies so that the presence of contaminants may not be recognized.



Figure 2.2 A freeze-dried ampoule of *Bacillus subtilis* NCIMB 3610, as received from the culture collection, and an agar slope of the mould *Aspergillus niger*.

2.4 Growth media and methods

Some bacteria can grow on simple culture media containing sugar as a carbon and energy source, an ammonium salt as a source of nitrogen for making proteins, and a mixture of minerals like magnesium and iron sulfates. However, such bacteria are in the minority, at least amongst those of interest in pharmacy and medicine, so although simple glucose/salts media are sometimes used for research purposes it is much more common to use so-called 'complex' media (also known as general-purpose



Figure 2.3 Streaking a Petri dish to give isolated colonies and check culture purity (see text).

media). They are complex in the sense that they contain a large number of individual ingredients and the precise composition may vary slightly from batch to batch.

The major ingredients of a complex medium are (in addition to purified water):

- Protein (from meat, milk or soya) which has been hydrolyzed with trypsin or acid to provide the amino acids necessary for growth. Proteins that are only partially hydrolyzed are termed peptones, and peptones produced by the use of trypsin are often called tryptone. Thus tryptone soya medium is one of the most commonly used in routine microbiology and is often specified in pharmacopoeial methods although, confusingly, the pharmacopoeias refer to it as soya bean casein digest medium – a name that is otherwise rarely used.
- A source of B-group vitamins, which enhance microbial growth, so yeast extract is a common ingredient; fat-soluble vitamins are not required.
- Sodium chloride may be included to adjust the osmotic pressure.

A liquid medium containing such ingredients is often termed a 'broth' because of the nutritional similarities to broths used in cooking. In addition to growth in tubes of liquid broth, microorganisms are commonly grown in Petri dishes (often referred to by microbiologists as 'plates') on the surface of a medium solidified by the addition of a gelling agent. These so-called 'solid' media are liquid when hot (so that they may easily be poured into the plates) but the medium sets to a gel upon cooling to room temperature. The gelling agent universally used is agar (a carbohydrate extracted from seaweed) because it cannot easily be digested by bacterial and fungal enzymes and it remains as a gel at 37°C (i.e. body temperature, which is also the preferred growth temperature for most

pathogens) whereas gelatin, a possible alternative, is easily digested and is molten at 37°C. Agar is unusual in that the temperatures at which its gels liquefy and set are not the same: it liquefies at 85°C, but does not set to a gel again until it has cooled to below 40°C. This last property can be an inconvenience, because if an agar medium starts to set whilst Petri dishes are being poured, it is not simply a question of heating by a few degrees to make it more fluid again – it has to be reheated nearly to boiling point. Despite the term 'solid' being used to describe gelled media in Petri dishes, it is important to recognize that they still contain approximately 97% water.

2.4.1 Growing anaerobes

Some microorganisms, termed anaerobes, do not grow on Petri dishes incubated in air; they do not even grow well in an ordinary liquid medium because it contains dissolved oxygen from the air. Anaerobes, therefore, will only grow in reducing conditions – in media with a low redox potential (this term describes whether oxidizing or reducing conditions exist and is measured in mV on a scale having positive and negative values – the larger the negative value the more reducing are the conditions). Air may be removed from liquid media by boiling, but it slowly redissolves after the liquid has cooled so the more common option is to add a reducing agent to the medium; sodium thioglycollate is the usual choice, although sulfur-containing amino acids like cysteine are also used. Anaerobic media often contain redox indicators which change colour according to redox potential rather than pH, for example resazurin, which is pink in oxidizing conditions and colourless when reduced.

In order to check the purity of cultures of anaerobic organisms it is necessary to grow isolated colonies by incubating Petri dishes in an anaerobic jar from which

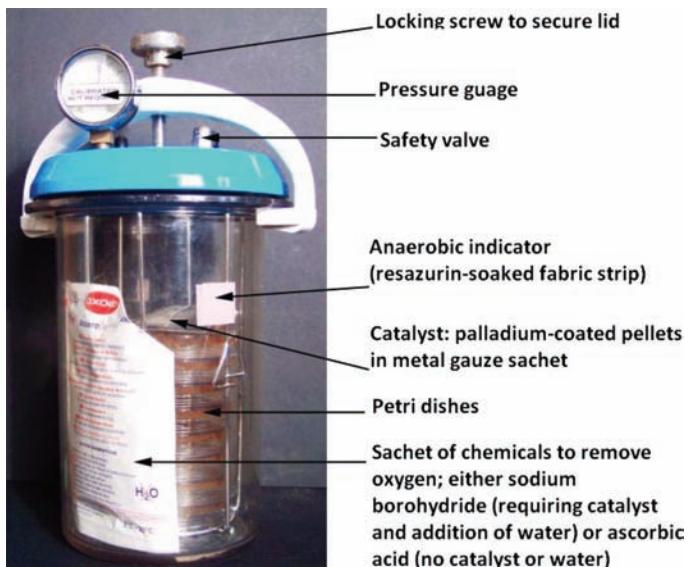


Figure 2.4 An anaerobic jar.

the oxygen has been removed (Figure 2.4). The features of an anaerobic jar are:

- The jar itself, which is usually made of thick polycarbonate with an airtight lid, and is large enough to hold up to 15 standard plates in a stainless steel rack. There is normally a pressure gauge and vent valve in the lid.
- A means of removing oxygen; this is achieved in one of two ways. The traditional method is to combine it with hydrogen by means of a low-temperature catalyst consisting of palladium-coated pellets in a wire gauze sachet that is placed in the jar. The hydrogen is generated *in situ* by addition of water to sachets containing sodium borohydride. The alternative is to combine the oxygen with ascorbic acid (also contained in opened sachets in the jar) – a process that does not require a catalyst.
- A means of confirming that anaerobic conditions have been achieved: normally a fabric strip soaked in resazurin as a redox indicator (see above).

Some pathogens, whilst not being strict anaerobes, nevertheless require gaseous conditions with either reduced oxygen or elevated carbon dioxide levels in order to grow on Petri dishes (e.g. species of *Campylobacter*, *Streptococcus* and *Haemophilus*). These organisms are usually grown in anaerobic jars and a range of sachets of chemicals are available to achieve the correct gaseous conditions.

2.4.2 Growing fungi

Fungi differ from bacteria by tolerating much higher osmolarities and preferring more acid conditions for growth, so fungal media often have high concentrations of sugars and a pH of 5–6. Antibacterial antibiotics are also used in some fungal media, particularly those employed for measuring the levels of fungal contamination in the atmosphere of pharmaceutical manufacturing areas; oxytetracycline and chloramphenicol are used in this way to prevent, or at least minimize, the growth of bacterial colonies.

2.5 The bacterial growth cycle

When bacteria are grown in a container of broth or streaked onto a Petri dish their growth usually follows the pattern in Figure 2.5 (which shows values for both cell concentration and time that would be typical of laboratory-grown cultures). This graph, in which the *logarithm* of the cell concentration is plotted against incubation time, is often seen to comprise four phases: the lag phase, logarithmic (log) phase (also sometimes called the exponential phase), the stationary phase and the decline phase.

For a laboratory culture, the starting concentration would be determined by how heavily the medium was

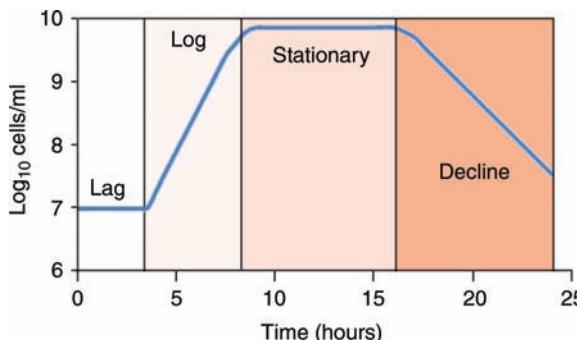


Figure 2.5 Bacterial growth cycle.

inoculated by the operator, but usually sufficient bacteria are added to a container of sterile broth to make the liquid just cloudy, so this would approximate to 10^7 CFU/ml. During the lag phase there is no increase in cell numbers; the bacteria are adjusting to the medium and synthesizing the enzymes and other materials that they will need to grow. Because the cells divide by binary fission (Section 1.3.2) their numbers double in a geometric progression (1, 2, 4, 8, 16... and so forth), which results in a straight line for the logarithmic phase of the plot. During this period the cells are typically breaking down readily digestible carbon sources like glucose, and generating organic acids, for example lactic acid; this is described as primary metabolism (see Chapter 20). Ultimately, the log phase stops due to a shortage of one or more essential nutrients, or to the accumulation of toxic metabolites, such as acids in bacteria or ethanol in yeasts (many of which show the same growth pattern). Typically, aerated cultures of common bacteria like *E. coli*, *Bacillus* species, or pseudomonads will reach a cell concentration approaching 10^{10} CFU/ml at the end of the log phase. The stationary phase is shown in the figure as being of 7–8 hours, although the duration can vary widely depending on the temperature, pH and nutrient status of the culture. Here, the bacteria are undergoing secondary metabolic reactions in which the organic acids are further modified into a wide range of metabolites, several of which, like antibiotics, are commercially valuable (Chapter 20). The rate at which the bacteria die in the decline phase is also very variable, as is the ‘final’ concentration. In some cases the culture completely dies out, though this may take days or weeks. In the case of bacteria that form spores the final, stable, concentration may be significantly higher than the starting value.

It is possible to calculate the cell generation time during the log phase of growth from the following equation where X is the number of generations achieved

during the time required for the population to increase from N_0 to N

$$\log_{10} N = \log_{10} N_0 + X \cdot \log_{10} 2$$

From this

$$X = \frac{(\log_{10} N - \log_{10} N_0)}{\log_{10} 2}$$

If, for example, the population increased from 5×10^7 CFU/ml to 1×10^9 CFU/ml in 3 hours

$$X = \frac{(\log_{10} 10^9 - \log_{10} 5 \times 10^7)}{\log_{10} 2} = \frac{9 - 7.699}{0.301} = 4.32 \text{ generations in 3 hours}$$

So the mean generation time would be 0.69 hours.

2.6 Environmental factors influencing microbial growth

Several of the factors that influence the rate and extent of microbial growth have already been mentioned in passing in the preceding sections; they include temperature, pH, redox potential, gaseous environment, osmotic pressure and nutrient availability. These will be considered in more detail in later chapters so it is sufficient here just to emphasize that each microorganism will have its preferred optimum for each of the above factors and will grow at a slower rate or to a lower population level when conditions are either side of the optimum. Some of the more important points about environmental effects on microbial growth and survival are listed below.

2.6.1 Water availability and osmotic pressure

All organisms need water to grow, but not necessarily to survive – many, particularly those forming spores, can survive well without water. The amount of ‘free’ water available for growth in a culture medium (water that is not bound by hydrogen bonding) is indicated by the *Water Activity* (symbol A_w , measured on a scale from 0 to 1, where 1 represents pure water with no dissolved solutes). Bacteria normally require higher water activity values than fungi. The osmotic pressure of a solution will rise as the amount of solute is increased so, generally, solutions containing large

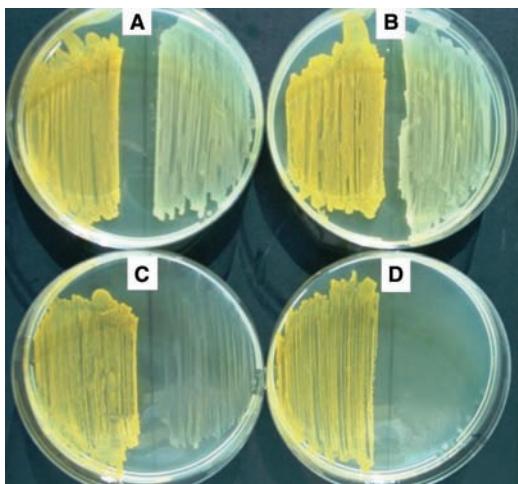


Figure 2.6 A comparison of salt tolerance in *Staphylococcus aureus* (on the left of each Petri dish) and *E. coli* (on the right) grown in media with different concentrations of sodium chloride (%w/v): A, 0.5; B, 2.5; C, 5.0; D, 7.5. Note that *E. coli* fails to grow at all in the highest concentration and only grows weakly at 5%w/v (plate C). The use of high salt concentration is the basis of the selective medium *mannitol salt agar* which is recommended in the pharmacopoeias for the detection of *Staph. aureus*.

amounts of dissolved sugars or salts will have low water activities. However, the two parameters water activity and osmotic pressure are not the same, because osmotic factors are not the only ones that influence water activity. Many organisms grow best at osmotic pressures similar to those in their 'natural' environment, so, for example, many pathogens prefer osmotic pressures similar to those at the sites in the body where they typically cause infections, for example staphylococci grow in relatively high salt concentrations because they are regularly exposed to salt from sweat on the skin (Figure 2.6).

2.6.2 pH

There are microorganisms that can survive in extreme pH conditions, such as hot acid springs in volcanic areas, but survival at extremes of pH is rare amongst organisms of pharmaceutical importance. *Helicobacter* species, the organisms associated with gastric ulcers, will grow at stomach pH (1–3) and *Vibrio cholerae*, the organism responsible for cholera, will grow at pH values between 8 and 9, but most pathogens will grow within a range of about 3 pH units and prefer values near neutrality because the pH of body fluids is usually close to 7.0. Fungi typically prefer, or tolerate, more acid conditions than bacteria.

2.6.3 Temperature

As with water availability, pH and all other environmental conditions, it is important to distinguish what is required for growth from that tolerated for survival. Most pathogens will prefer body temperature (37°C) for growth, and will grow more slowly at slightly lower and slightly higher temperatures too. Usually, though, the upper limit for growth is about 42–44°C because higher temperatures than that result in enzyme inactivation. Some pathogens will grow at refrigeration temperatures, for example *Listeria*, but all organisms require liquid water to grow, so the normal freezing point of 0°C is the lower limit. All microorganisms will survive at subzero temperatures provided that damage due to osmotic effects and ice crystals is prevented by cryoprotectant chemicals like glycerol. Survival at higher temperatures varies greatly between species, but spore-forming bacteria tolerate higher temperatures than any other organism – even boiling water.

Organisms may be classified according to their preferred growth temperature ranges: thermophiles are those with an optimum above 45°C; mesophiles have an optimum between 20°C and 45°C; and psychrophiles grow best at a temperature below 20°C.

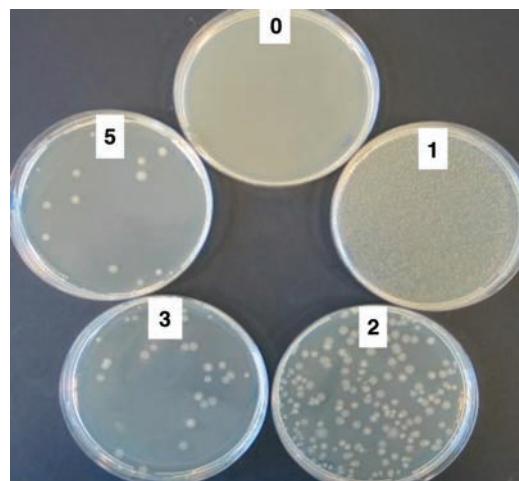


Figure 2.7 The effect of ultraviolet light (UV) on the survival of *Bacillus subtilis* spores. Equal numbers of spores were inoculated and spread over the agar surface of each plate and then exposed to UV for the number of minutes indicated. After this the plates were incubated to allow surviving spores to grow into colonies. On the plates exposed for zero and one minute the number of survivors is so high that the colonies developing after incubation form a complete surface covering of the agar and discrete colonies cannot be seen.

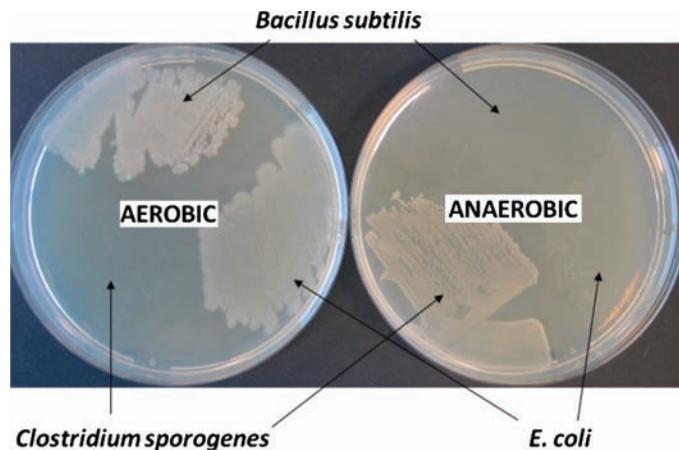


Figure 2.8 A comparison of bacterial growth under aerobic and anaerobic conditions. *Clostridium sporogenes*, a strict anaerobe, will not grow in air, whilst *Bacillus subtilis*, a strict aerobe will not grow under anaerobic conditions. *E. coli*, a facultative anaerobe, grows well in air, but to only a very limited extent without it.

2.6.4 Light, ultraviolet light and ionizing radiation

Microorganisms of pharmaceutical interest do not photosynthesize, so they grow equally well in light and dark. Like all other organisms, though, their DNA is damaged by ultraviolet (UV) light (Figure 2.7), so they may grow much less well if exposed to direct sunlight. Ultraviolet light is used in the pharmaceutical industry to decontaminate air in safety cabinets and for killing microorganisms in purified water to be used for manufacturing medicines (Chapter 19). Microorganisms are killed by X-rays, and killed even more effectively by electron beams and gamma rays, which are used as a means of sterilizing some drugs and, more particularly, medical devices like catheters, cannulas, valves, pacemakers and prostheses (Chapter 19).

2.6.5 Redox potential and the gaseous environment

These aspects have largely been covered in Section 2.4.1 but the following additional points are worthy of note. Most pathogenic microorganisms are either aerobes or facultative anaerobes (Figure 2.8); strictly anaerobic pathogens are less common. An aerobe is an organism that *requires* oxygen to grow, and a facultative anaerobe is one that can grow with or without oxygen but usually

grows faster and to higher population levels if oxygen is available. A few organisms are classed as microaerophiles, for example some species of *Helicobacter*, *Campylobacter* and *Streptococcus*: they do need oxygen to grow, but at a concentration lower than that found in the air.

The effects of several antibiotics are influenced by oxygen availability or redox potential. The aminoglycoside antibiotics (such as gentamicin, amikacin and tobramycin) are less effective in reducing conditions, whilst metronidazole is *only* effective when reduced. Because oxygen availability affects the growth rate of so many organisms it is common to find that antibiotics like penicillins and cephalosporins, which only act on growing bacteria, appear more effective in the laboratory than they do in the body. This is because bacteria at infection sites in the body usually grow much more slowly than those in the laboratory, both because they are competing with human cells for the available oxygen and because they are often covered either in slime layers that they manufacture themselves or in mucus secreted by the patient (as in the respiratory tract for example), both of which restrict oxygen diffusion.

Acknowledgement

Chapter title image: PHIL ID #7851; Photo Credit: Dr. Lucille K. Georg, Centers for Disease Control and Prevention.

