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The MicroBook
Clinical Microbiology for Medical Students

Oto Melter
Rute Castelhano
(eds.)

KAROLINUM

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PREFACE

Microbiology is a field which is in a constant state of change: with the discovery of new strains of organisms, many of them resistant to the antibiotics we typically use and new techniques helpful in the diagnosis of pathogens responsible for disease. It is also a field containing plenty of information which sometimes make a students' life harder, with endless names to know, which diseases are caused by which microorganisms or parasites, how to diagnose and treat those diseases, and so on.

Due to the difficulty of this subject, the idea of writing a book which could help the students during their studies came up. Within the book it is possible to easily find readable content about the most serious and frequent infections we deal with these days in the medical field. The relevant impulse for the writing of the current textbook is freely linked with the previous book Principles and Practicals in Medical Microbiology, Melter & Malmgren, Karolinum, 2014, for which there was a positive response from our students with the requirement to broaden the new textbook to cover all significant medical microbiology topics.

The main goal is to give actualised, organized and clear information to all the readers about microbiology, especially medical students in order to help them pass their exam. Even though it is good to remember that one source may not be enough, and that is why we recommend the addition of other textbooks and impacted professionals articles to complement ones knowledge.

The elaboration and reviewing of this book was influenced by current editions of prestigious publications in the field which are also called microbiology Bibles (Brooks et al: Jawetz, Melnik & Adelbergs' Medical Microbiology, LANGE; Lippincott's Illustrated Reviews, Microbiology, Lippincott Williams & Wilkins; Murray et al, Medical Microbiology, Elsevier Mosby). This book is special in the fact that it was written for medical students by medical students themselves. It was co-written by doc. MVDr. Oto Melter, Ph.D., who designed this book, reviewed, supervised, wrote many chapters and took most of the original photographs we find throughout the book. Chapters were also written and reviewed by smart enthusiastic students led by Rute Castelhano and the entire manuscript was reviewed for factual and grammatical discrepancies by Shenali Amaratunga.

Since different students wrote this book, we can see some heterogeneity among chapters but this heterogeneity is also a strong point as it will help students to stay motivated and attentive.

We decided that the usage of original diagrams and images in this book would be an advantage as the students would be able to improve their understanding. Majority of all the photographs and microphotographs were taken in our Department of Medical Microbiology, 2nd Faculty of Medicine, Charles University and University Hospital Motol, Prague but we are extremely pleased that we could also gather missing images from other experienced specialists in the field.

ACKNOWLEDGEMENTS

We would like to thank all of the medical students from the 2nd Faculty of Medicine of Charles University in Prague who took part in the creation of this book, who contributed their time, effort and help by writing and reviewing chapters and creating original diagrams to be integrated within the text. These students are current or former students, namely Annika Malmgren, Florian Merkle, Marketa Tolarova, Kiril Dimitrov, Marine Lopes, Adam Whitley, Elena Storaci, Irene Santos, Ana Ramos and Maxwell Cameron. We also thank MUDr. Vanda Chrenková and MUDr. Petr Hubáček, Ph.D, Department of Medical Microbiology, 2nd Faculty of Medicine, Charles University Prague for writing a few chapters. We would like to thank to Varun Kakkar for contributing with some original diagrams. At the same time, we thank Mgr. Jan Tkadlec, Ph.D, Department of Medical Microbiology, 2nd Faculty of Medicine, Charles University Prague and students Domenico Messina, Melvin Bae, Nicholas Pitto for their review of selected chapters. We would also like to thank student Shenali Amaratunga for carrying out the grammatical review of this entire textbook manuscript, and for her factual suggestions and revision as well.

A special thanks goes out to Senior consultant MUDr. Štěpánka Čapková, Department of Paediatric Dermatology, Motol University Hospital, Prague; prof. MUDr. Pavel Dundr, Ph.D Head of Institute of Pathology, 1st Faculty of Medicine, Prague; MUDr. Kamila Dundrová, Department of Medical Microbiology, University Hospital Motol, Prague, MUDr. Daniela Lžičařová, Department of Medical Microbiology, 2nd Faculty of Medicine, Charles University, MUDr. Dana Hrubá, Vidia Diagnostika, Prague and MUDr. Jan Krásný, Department of Ophthalmology, Královské Vinohrady Teaching Hospital, Prague; doc. RNDr. Roman Pantůček, Ph.D, Department of Experimental Biology, Faculty of Science, Masaryk University, Brno and Ing. Jan Urban, Ph.D, RNDr. Daniel Krsek and Mgr. Petr Pumann, Institute of Public Health, Prague for providing original images which contributed to the enrichment of this book.

Last but not least, we would like to especially thank prof. MUDr. Pavel Dřevínek, Ph.D, Head of the Department of Medical Microbiology, 2nd Faculty of Medicine and Motol University Hospital, who supported us in the writing of this textbook. A special thank you to both reviewers Emeritus Senior Consultant MUDr. Eliška Bébrová, Department of Medical Microbiology, 2nd Faculty of Medicine and Motol University Hospital, Prague, and doc. MUDr. Pavel Čermák, CSc., Head of the Department of Medical Microbiology, Thomayerova Hospital, Prague for their time spent to improve this textbook and their insightful comments.

1. GENERAL MICROBIOLOGY

1 GENERAL BACTERIOLOGY

Bacteriology is the study of bacteria. They are prokaryotic cells and this differentiates them from fungi and parasites which are eukaryotic and from viruses which are just protein encapsulated nucleic acid and not cells at all. As the name prokaryote suggests, they have no nucleus (pro = before, karyon = nucleus) but DNA is found freely in the cytoplasm of the cell, coiled in to a structure known as the nucleoid. This feature makes them the simplest cells on earth. It is therefore a valid guess, to estimate that **bacteria** are one of the **oldest organisms on the planet**. Marks in stromatolites, a type of sedimentary rock, show evidence of early prokaryotic life probably dating as far as 4.0 billion years ago. This means that they have had a lot of time to evolve into masterpieces, even if the general structure of a bacterial cell looks very simple (**fig. 1.1**), there are specialized structures like the mobile genetic elements (plasmids), capsules, flagellae, and spores. They are able to cope successfully and persist or propagate even in extreme physical and chemical conditions. This gives bacteria the opportunity to have an extraordinary impact on everyday human life not only in medicine, but also in fields such as agriculture, food industry, energy and the environment.

The goal of medical bacteriology is to diagnose bacteria as agents of infections using specific phenotypic and/or genotypic methods and to determine susceptibility to therapeutic agents.

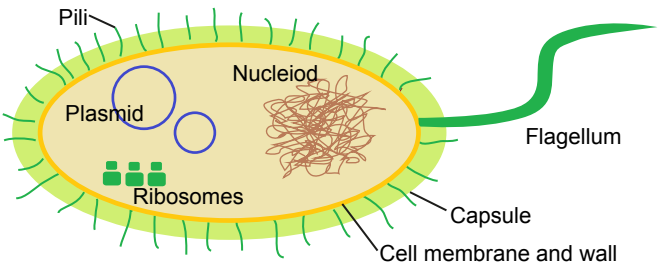

Figure 1.1: A schematic diagram of a typical bacterial cell. The bacterial chromosome consists of one circular molecule of DNA floating free in the cytoplasm, summed up in a coil known as the nucleoid. The bacteria are therefore considered prokaryotic (= before nucleus). The plasmids are smaller than the chromosome and are only present in some bacterial cells. Other significant structures are the ribosomes, the cytoplasmic membrane, the cell wall, the outer membrane (in Gram-negative bacteria) and the capsule. Sometimes pili (short hair-like structure) and flagellae (elongated organelles protruding from the cell) are present and may play a role to inhance virulence.

1.1 CLASSIFICATION AND NOMENCLATURE

You are probably familiar with the classification of the medically important bacteria into "good" versus "bad" bacteria. The "good" bacteria are the large number of **commensal bacterial species** which attach to host tissues and produce chemicals that are beneficial to the host. Did you know that around 90% of all cells existing on or in a healthy host are of bacterial origin? The "bad" bacteria on the other hand are those that, when they are present, cause illness. The presence of these **disease-causing bacteria** is known as a **bacterial colonization or infection**.

Bacteria could be divided into **Gram-positive** or **Gram-negative** bacteria. This division is done according to the staining of the bacterial cells depending on the structure of the bacterial envelope (**fig. 1.2**). This classification is medically important as many antibacterial drugs are directed against the cell envelope. The principle of Gram staining, introduced by Gram, is discussed in chapter 7. Some bacteria that do not fit into this classification, e.g., *Mycobacterium tuberculosis* are known as acid fast bacteria.

The bacterial nomenclature organizes bacteria according to the nature of the habitat, presence of certain enzymes, structure and so on. Firstly, we talk about the **order** *Eubacteriales* which include all medically important bacteria. Then we classify bacteria with similarities into certain **families**, for example *Enterobacteriaceae*. These bacteria can be found in similar environments, for example in the gastrointestinal tract. Amongst these bacteria, we distinguish **genus** (e.g., *Escherichia*) and **species** (e.g., *coli*). The outcome of this classification is a **binominal nomenclature** (e.g., *Escherichia coli*) which should be written in italic.

1.2 THE BACTERIAL STRUCTURE

As stated above, all bacteria are prokaryotes. This means that they do not contain membrane bound organelles, nor a true nucleus. The bacterial chromosome is instead coiled into a mass known as a **nucleoid**.

1.2.1 The Bacterial Envelope

The bacterial envelope consists of all the structures that surround the bacterial cytoplasm. These are the cytoplasmic membrane, the cell wall, the outer membrane (present in Gram-negative bacteria) and in some bacteria, there is a capsule as the outermost structure. It is, just as our human skin for us, the largest bacterial organ. And bacteria, as smallest prokaryotes, have an inversely larger surface than large cells do. Each of these structures have a distinct function.

The structure of the envelope is the difference between the Gram-negative and the Gram-positive bacteria. The **Gram-negative** bacteria have a small cell wall on top of the cell membrane followed by an outer membrane with porins and lipopolysaccharides (LPS), while the **Gram-positive** ones lack the outer membrane but have a very thick cell wall outside the cytoplasmic membrane (**fig. 1.2**).

Overall, the cell envelope can vary broadly depending on bacterial species or strain. The **cell membrane**, being the innermost layer in both Gram-positive and negative bacteria, consists of a phospholipid bilayer. As the cell membrane is selectively permeable, it transports

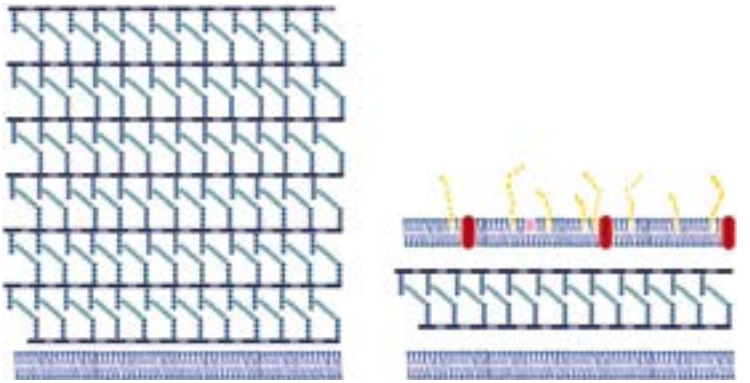

Figure 1.2: A schematic overview of the cell envelopes in Gram-positive (left) and Gram-negative (right) bacteria. The cell membrane, being the innermost part of the envelope protects the cytoplasm of both Gram-positive and negative bacteria. In Gram-positive bacteria this is covered by a thick peptidoglycan (cell) wall. In the Gram-negative bacteria only a thin cell wall is present, shielded by the outer membrane filled with lipopolysaccharides (LPS) (yellow) and porins (red). Additional proteins with diverse functions (pink) may also be inserted in the membranes. The space between the inner and outer membrane is known as the periplasmic space.

only some solutes, and functions as a protective barrier between the intra- and extra cellular environments. It enhances the excretion of hydrolytic enzymes and simultaneously preserves the enzymes and molecules that are responsible for the biosynthesis of DNA and other life sustaining elements of bacteria. In addition, it contains the proteins and enzymes necessary for electron transport and oxidative phosphorylation in aerobic species.

The chemical complex of the bacterial **cell wall** (**fig. 1.3**) contrasts with the chemically simple ones in eukaryotic cells. As seen in **fig. 1.2** it is usually present in both Gram-posi-

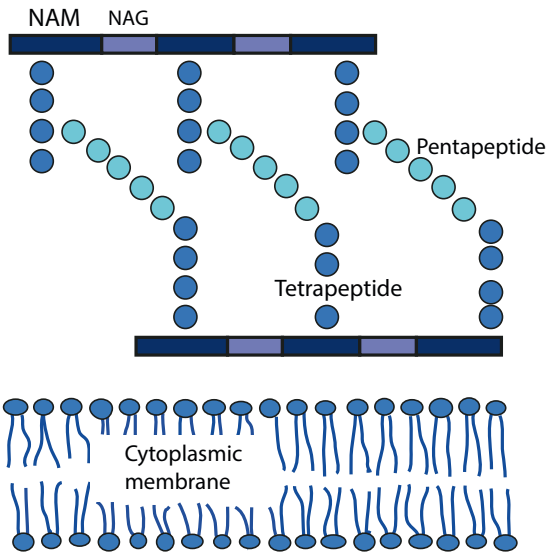

Figure 1.3: The peptidoglycan structure of the cell wall. The glycan backbone consists of a linear polymer of two monosaccharide subunits, N-acetylmuramic acid (NAM) and N-acetylglucosamine (NAG) that alters. The glycan layers are then cross-linked with identical tetrapeptides bound to the NAM and connected between each other by glycine pentapeptides.

tive and Gram-negative bacteria but only as very thin layer in the latter. The bacterial cell wall consists mainly of **peptidoglycans** (murein), where sugar backbones (glycan part of the name) made of repeats of N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) are held together with peptide cross-links (peptido–part of the name) consisting of tetra- and pentapeptides (**fig. 1.3**).

Outside the thin cell wall layer in the Gram-negative bacteria is a rather specialized **outer membrane**. Just as the cell membrane, it consists of a phospholipid bilayer, but it also contains porins, selective transport proteins, and lipopolysaccharides.

The **lipopolysaccharide** (LPS) (**fig. 1.4**) has a lipid portion (lipid A) that is inserted into the membrane. Attached to this is a core polysaccharide, followed by a long tail of saccharides that, as they are found at the exterior of the cell, are highly antigenic. They are therefore also known as the **O antigen**, and can be used in identification as they vary among species. Inserted into the membrane they cause no harm, but are easily recognized by our immune system. On the other hand, when the bacterial cell lyses, and the LPSs are released, they act as the feared **endotoxin** known for all Gram-negative bacteria. It activates the complement, cytokines and the coagulation cascades and thus, leads to clotting. Clot resolution and vasodilation in the whole system lead to a severe drop in blood pressure, causing a collapse of the circulatory system, and could, if not controlled, result in multiorgan system failure and septic shock.

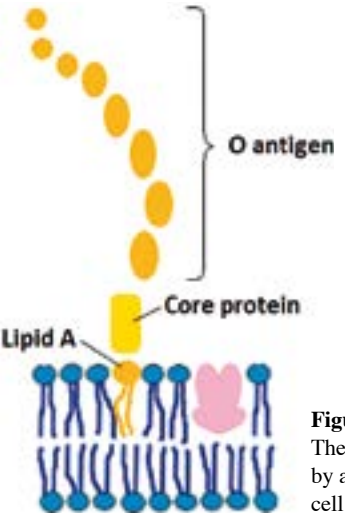

Figure 1.4: The lipopolysaccharide (LPS) of Gram-negative bacteria. The O-antigen, made of saccharides, is anchored in the outer membrane by a core polysaccharide and lipid A. Once the LPS is released from the cell membrane it acts as an endotoxin

The outermost layer in some bacteria is the **capsule**. It is made of externally secreted compounds, usually polysaccharides that form a coat around the bacterial cell (**fig. 1.1**). The capsule works usually as a virulence factor, as it can interact with its environment thus, deciding the human immune response towards the bacterium. The capsule of *Streptococcus pyogenes* for example consists of hyaluronic acid that mimics host intracellular connective tissue and therefore, no specific antibodies are produced by the host. The capsule of *Streptococcus pneumoniae* has the ability to bind host antibodies or part of the complement system (C3b) to the bacterial surface in such a way that fools the immune system and escapes opsonization and phagocytosis.

In addition to these well known structures, Gram-positive bacteria have extra molecules that are covalently linked to their membrane, also known to be a major cell surface antigen. It is a polymer of substituted glycerol units linked by phosphodiester bonds, and known as **teichoic acid**. It is suspected to be used by the bacterium in host tissue colonization and aids the spread of infection.

1.2.2 Flagella

The bacterial flagellum (**fig. 1.5**) is made up of several thousands of a protein subunit called **flagellin**. They are highly antigenic, and are therefore known as the **H antigen**. The flagellum is embedded into the bacterial cell by basal body (L-ring bound to LPS, the P-ring bound to peptidoglycan and the S-M ring to the cell membrane). The *Mot* proteins function as the flagellar motor using energy from proton motive force, whereas the *Fli* proteins switch the motor between counterclockwise (CCW) or clockwise (CW) direction in a liquid culture medium. The rotation of the flagellum propagates the bacterium towards (CCW) a higher concentration of attractants (chemotaxis). In the absence of a gradient it moves in a random manner. Flagella rotation can move bacteria in liquid media at speeds up to 60 cells lengths/second therefore it is extremely fast (a cheetah, the quickest mammal on Earth, moves 25 body lengths per second). Bacteria can be motile, they also **swarm** on solid culture media surface using flagella (e.g., *Proteus*). Bacteria could be **unflagellated** or contain one single (**monotrichous**) or multiple polar flagellum (**lophotrichous**) or have flagella (**flagellated**) distributed over the entire cell (**peritrichous**).

1.2.3 Pili (Fimbriae)

The pili (singular: pilus) (**fig. 1.1**) are hair-like structures which consists of protein subunits called pilins. They are shorter and thinner than flagella and many Gram-negative bacteria

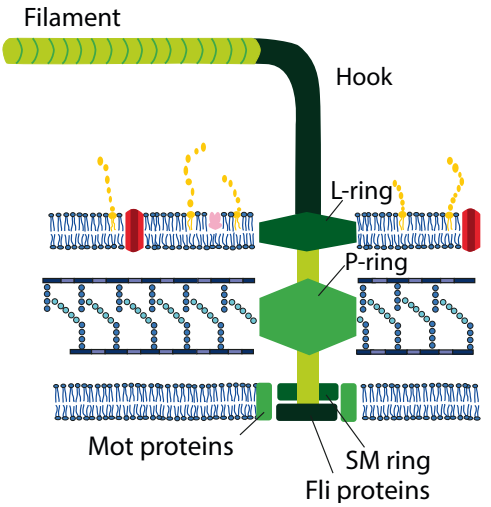

Figure 1.5: Structure of the flagellum of *E. coli*. The flagellum, consisting of protein subunits called flagellins attached to the cell envelope. It can rotate and thus bacterial cells can swim forward (counter-clockwise rotation) or stay (tumbling – cell stops and jiggles about). So the flagellum functions as a propeller.

possess them. The pili could be used to adhere to cell surfaces in the host. They could also be used to attach to other bacteria in order to exchange genetic information in the form of plasmids. In this case the pili are known as **sex pili**, encoded on F-plasmids (fertility). Minor proteins known as adhesins are located on the tips of the pili and are responsible for the attachment to host cells or other bacterial cells.

1.2.4 The Bacterial Chromosome

Bacteria usually have **one single chromosome of double stranded DNA** (**fig. 1.1**) but some bacterial genera have multiple chromosomes (e.g., *Burkholderia* – 3; *Vibrio* or *Leptospira* – 2). Chromosomal DNA is usually circular but there are some exceptions and bacteria with linear chromosome do exist, for example *Borrelia*. To put some numbers on this information, the chromosome of *E. coli* consists of one circular DNA with the length around 4 milion base pairs (bp), and containing 2000–3000 genes but its chromosome size can vary from 0.5 megabase pairs (Mbp) to 13 Mbp. Uncoiled, the DNA in a bacterium is around 1 mm in length, and therefore is extremely folded (supercoiled) to be able to exist in 1000-fold smaller cell as the **nucleoid**.

1.2.5 Mobile Genetic Elements

Some bacteria carry **extra-chromosomal nucleic acid** in the form of **plasmids** (**fig. 1.1**). These are self-replicating double strands of circular DNA. They can be smaller (1.5 kilobase pairs, kbp) or larger (around 150 kbp) and one bacterial cell can carry more than one type of plasmids. In contrast to viruses, they don't have an extracellular form and exist only inside cells simply as nucleic acid. Plasmids never carry genes that are essential for cell growth or cell replication, as they are present only in addition to the chromosome and not present in all bacteria, but all plasmids must carry genes that ensure their own replication. Frequently they contain **genes of antibiotic resistance** or **toxins**. The plasmids can be shared among the bacteria by means of **conjugation** which is a communication through sex pili (see below).

Another type of genetic element that could be found in some bacterial cells is the **transpozon**. Transpozons are small pieces of genetic information that can move between plasmids and the chromosome, or within the chromosome. They always carry genes responsible for their excision (resolvase) and insertion (transposase) into a plasmid or the chromosome and can be non-replicative (doesn't leave a copy of itself at the original location) or replicative (leaves a copy at the original site). They are often involved in the **transposition of antibiotic resistance genes**. Important to realize though, is that if they are inserted into a functional gene, the function of the gene affected could be destroyed. Therefore, transpozons are also known as mutagenic agents. Remember that while the plasmids are moving between the cells, the transpozons move within the cells.

Integrons are mobile DNA elements that can capture and carry multiple genes, particularly those responsible of antibiotic resistance. **Genomic islands** (GEIs) are parts of genome (>10 kbp) that have evidence for horizontal origins. GEIs associated with multiple antibiotic resistance genes are referred to as **resistant islands** (REIs) and the ones associated with pathogenesis are called **pathogenicity islands** (PAIs).

1.3 BACTERIAL VIRULENCE FACTORS

The virulence factors are those which make bacteria dangerous to us, either by destroying our tissues, over activating the immune system or by hiding the bacterium from our defenses, and thereby escaping clearance. Some of them are **structural components** of the bacterium, e.g., the endotoxin (LPS) which hyperstimulates the immune system. The peptidoglycan in the cell wall in Gram-positive bacteria function in a similar way as LPSs in Gram-negative species. Adhesin is another important virulence factor present on the pili of some bacteria, providing attachment to the host tissue. The pili in their own way are also virulence factors, as is the capsule. Other **virulence factors** are **produced by the bacteria**, encoded in the genetic material either in the chromosome or in the acquired plasmids. These are for example **exotoxins**, e.g., produced by staphylococci (enterotoxin) and *Corynebacteirum diphtheriae* (cytotoxin), and the **secreted enzymes** of, e.g., *Streptococcus pyogenes* (hyaluronidase). An important product of some plasmids is the antibacterial resistance which is a very strong virulence factor. Remember that almost everything present on the bacterium or secreted by it helps it to be virulent. These were only selected examples of some of the most important and most common virulence factors.

Virulence factors are **usually related to particular bacterial strains**. More detailed information about the virulence factors of specific species can be found in the chapters of special microbiology later in this book.

1.4 BACTERIAL ADAPTATION

The bacteria are highly flexible using number of sophisticated mechanisms to settle in various environments or hosts. There is a regulation of gene expression to produce only the proteins required at that moment. Moreover, there is an alternative to cell differentiate – sporulation in some bacteria when the environment is too harsh, the spores germinate again once conditions have improved (an extreme extension of the gene regulation).

1.5 REGULATION OF GENE EXPRESSION

To synthesize all proteins continuously would be very exhausting for small bacterial cells. The basic mechanism of regulation of gene expression is determined by RNA polymerase sensitivity to various promoters. The sensitivity is determined by a variable subunit of RNA polymerase known as sigma (σ) factor.

The control of gene expression enables individual bacteria to adjust their metabolism to **environmental changes** and to have a better use of the nutrient sources. To do this, the bacteria also uses **positive** (e.g., cyclic AMP complexes with a catabolite activator protein (CAP) to activate operons) or **negative** (the inducer, inactivates repressors and so allow gene expression) **control mechanisms**.

1.6 BACTERIAL SPORES AND SPORULATION

To survive in **extreme conditions**, e.g., nutrient starvation some Gram-positive species are capable of forming spores which are a **dormant stage** and are **resistant** to boiling, desiccation (drying), UV light, and treatment with chemicals, even antibiotics, for years or decades.

The process of sporulation (**fig. 1.6**) involves the production of many new surface structures, enzymes, and metabolites in parallel with disappearance of many vegetative components. Spores are the dormant (non-reproducing) stage in bacterial life. This process called **differentiation** is caused by the activation of a series of genes which determines the final composition of the spore, especially new surface proteins. The initiation of spore formation involves alteration of the transcriptional specificity of RNA polymerases associated with different promoter specific proteins (sigma factors).

Figure 1.6: Bacterial spore formation. At first the DNA becomes denser. A septum is then formed, known as the "forespore septum". The forespore is engulfed and a cortex is synthetized (purple). A coat is deposited (pink) and the spore is matured. Lysis of the bacterium releases the spore.

Germination is the process that "awakes" the spore, and turns it into a bacterium in its vegetative state again. More beneficial environments induce this process, but could also be because of exposure to certain agents, e.g., sublethal heat, extreme pH and so on which activate the spore. The spore constituents are degraded and a vegetative cell is released. This of course requires a supply of all nutrients essential for such active biosynthesis.

Bacteria of the genus *Clostridium*, feared for their ability to produce gas gangrene, tetanus, botulinum or pseudomembranous colitis are medically significant spore-formers. More information about these bacteria could be found in the chapter of anaerobic bacteria (chapter 22). *Bacillus anthracis*, an aerobic agent of sudden septicemic fatal human and animal infection also sporulates.

1.7 REPRODUCTION OF BACTERIA

To increase their fitness even more, not only by adapting their protein production and vegetative state, bacteria often try to acquire new genetic material which can help them to survive. The **acquisition of new genetic material** is known as bacterial recombination (e.g., not the binary fission). There are three basic ways of bacterial recombination – **conjugation**, **transduction** and **transformation**.

1.7.1 Conjugation

Conjugation (**fig. 1.7**) is the transfer of genetic information between two bacteria. The connection linking the **donor** and the **recipient** is established via a **sex pilus**, encoded on the F-plasmid in the male (F^+), which attaches onto the female (F^-). The DNA, which can be encoding the fertility gene (F), antibiotic resistance or other information, can then be passed through this pilus and then replicated inside the new cell.

Figure 1.7: Transfer of plasmid DNA by conjugation. A copy of the F plasmid (pink) of an F+ donor cell (on the left) is being transferred to an F– recipient through a sex pilus. After the conjugation process has ended, there are equal double stranded circular plasmids in both cells.

1.7.2 Transduction

Transduction means the transfer of genetic material from one bacterial cell to another by a bacterial virus – **bacteriophage**. They carry either DNA or RNA that replicate inside bacterial cells. The bacteriophage is called **virulent** if their genetic material is replicated, producing new virions that eventually will lyse the cell through the lytic cycle (**fig. 1.8**). It is called **temperate (prophage, lysogenic phage)** when they in a benign manner, insert their genetic material into the chromosome of the host bacterium. The lysogenic phages can be induced e.g., by UV light and thereafter they begin reproducing autonomously, turning into a virulent phage and breaking the host cell. During a lytic infection, the enzymes responsible for packing viral DNA into the bacteriophage sometimes (accidentally) pack host DNA (transducing particle). This means that the genetic material from one bacterium can be spread to other bacteria via bacteriophage. This phenomenon is called **phage conversion**.

An important gene that sometimes is carried in this manner is of the toxin produced by virulent strains of some *Corynebacterium diphteriae*. This could lead to conversion of a non-toxin producing bacteria into a toxin producing one.

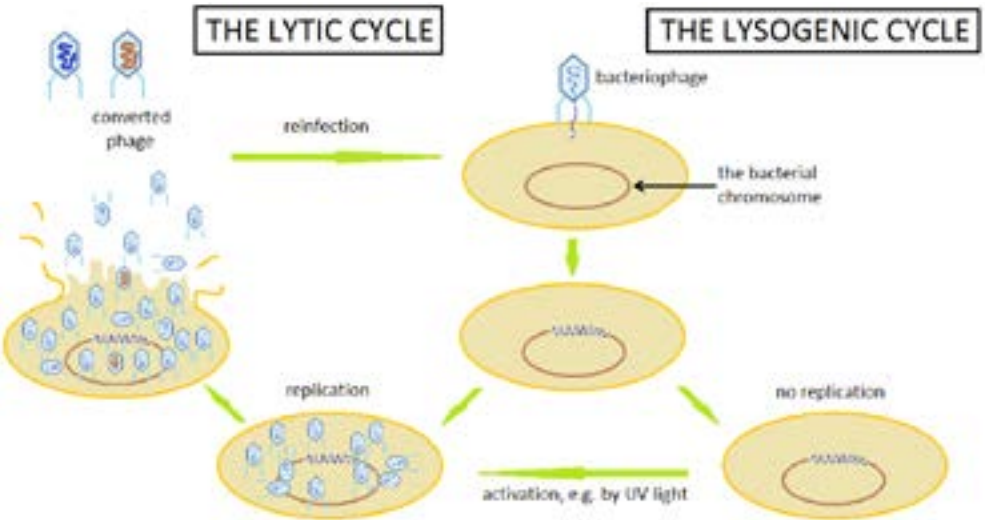

Figure 1.8: The lytic and lysogenic reproductive cycles of a bacteriophage. A bacteriophage injects its genetic information into the bacterial host, inserting it into its chromosome. Dependent on the position of the phage nucleic acid, the phage DNA can either replicate (enter the lytic cycle), or stay benign in the chromosome (entering the lysogenic cycle). The lytic cycle continues with production of new virions that eventually lyse the cell and release a new bacteriophage. Some virions collect during the assembly chromosomal DNA of the host bacterium, turning into converted phages. These will now spread the bacterial DNA. After activation, the cells in the lysogenic cycle can enter the lytic cycle.

1.7.3 Transformation

Transformation means alteration of the bacterial genotype by the uptake of naked, foreign DNA from the environment. This DNA is then recombined with a homologous region of the chromosome of the bacterium, and the information stored in the newly acquired DNA is then ready to be used. The ability to transform is unusual and many of the medically important bacteria (e.g., *Streptococcus pneumoniae*, *Neisseria gonorrhoeae*, *Haemophilus influenzae*) are transformable only in presence of **competence factors** produced only at specific points in the growth cycle. Uptake of naked DNA by *S. pneumoniae* can turn the avirulent, unencapsulated form into a virulent one with a capsule.

1.7.4 Mutation

The fourth way of acquiring new genetic information is of course through random mutation. This occurs in all living species, and is an important way of evolving, which then of course is followed by natural selection. Hence, a population of bacterial cells which better cope and propagate in a particular condition will finally prevail.

1.8 BACTERIAL COMMUNICATION

More than just being able to adapt to changing environments, bacteria have another function to increase their superiority in the harsh evolutional system. They do so by almost never existing as sole bacterial cells in the environment or in the host. On the contrary they try to and prefer to, exist as a macroorganism, where any bacterium acquires and specializes to a specific function, e.g., enzyme production. Like this, they can distribute and organize their metabolic needs much more efficiently. The simplest form of this type of macroorganism is a colony grown on an agar plate (**fig. 1.9**).

To be able to live a life as a macroorganism the cells of course need to communicate! There are two basic ways in which the bacteria communicate with each other. They both work by the bacteria sensing the presence of other bacteria of the same species by hormone-like substances secreted by the others. By sensing an increased level of these hormone-like substances, it knows when it can start specializing in its protein production. This type of cooperative communication is called **quorum sensing**. On the other hand by sensing a decreasing concentration of these substances the bacteria can calculate where there is a free niche to grow to expand the newly created macroorganism. This type of communication is called **diffusion sensing**. A combination of these two means of communication is known as **efficiency sensing**.

This knowledge has led to an idea of industrial production of a non-toxic version of these hormone-like molecules to be used in the post-antibiotic era to treat bacterial infections.

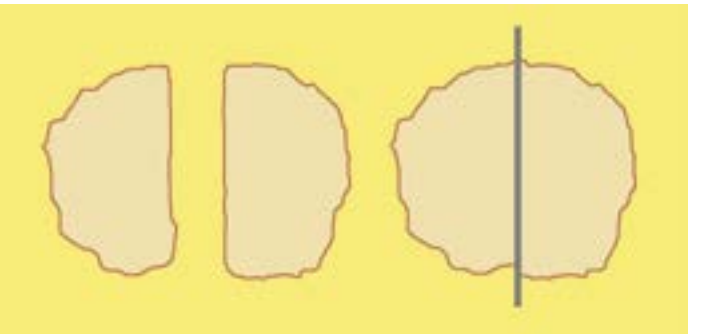

Figure 1.9: Evidence of bacterial communication. Colonies growing on nutrient agar will never "touch" each other, as seen on the left, due to their ability of diffusion sensing. If a mechanical barrier is positioned between them, as seen on the right, the communication by chemical signals is interrupted, and the colonies will grow up to the barrier.

1.9 BACTERIAL GROWTH AND METABOLISM

Growth and self-replication are key features of every living system including bacteria. Bacteria form **bacterial colonies** on solid culture media and **opacity** in liquid media, usually after overnight cultivation usually at 37 °C. To be able to grow they need a good supply of the right nutrients.

1.10 NUTRITION – SOURCES OF BIOGENIC ELEMENTS

The nutritional needs of the bacteria consist of an **energy source**, organic elements, inorganic ions and growth factors. The **organic elements** needed for bacterial growth are carbon (C), hydrogen (H), oxygen (O), nitrogen (N), phosphorus (P) and sulfur (S). The **inorganic ions**, needed for membrane potential and enzymatic function among others are potassium (K), sodium (Na), iron (Fe), magnesium (Mg), calcium (Ca) and chloride (Cl). The situation with **growth factors** differ from bacteria to bacteria, as some of them can carry out endogenous synthesis of the compounds they need. If it is not produced by themselves, they will need a supply of vitamins, amino acids, purines and pyrimidines in the growth medium.

These nutrients provide the building blocks for proteins and other bacterial structures, put together in the process of biosynthesis (**anabolism**) which requires energy. The bacterial cells use similar metabolic pathways to those in higher organisms.

Metabolic energy production. Bacteria generate metabolic energy from carbon sources (usually sugars) by one of the following mechanisms.

Many bacteria have the ability to acquire a lot of energy from carbon sources by the means of **aerobic respiration**. It functions similarly as in humans, where after breaking the carbon source down, a respiratory chain, where the last electron acceptor is oxygen, build up an energy gradient that when neutralized gives its energy to create ATP (adenosine triphosphate) from ADP and free phosphorus. ATP is created along the whole catabolic chain, but the largest contributor to the ATP pool is the respiratory chain. Those bacteria that work anaerobically break down the carbon source in a similar manner, but instead of reducing the product in the respiratory chain, another compound functions to complete the oxidation-reduction reaction. These alternative final electron acceptors could be, for example nitrate or sulphate. This way of escaping the respiratory chain but still using the energy of the original carbon compound is known as anaerobic respiration.

Obligatory anaerobes have no respiratory chain, and they find oxygen toxic. They use the pathway of anaerobic respiration or fermentation to produce ATP. **Obligate aerobes** use the respiratory chain only, and are not able to ferment. They die without oxygen. **Facultative aerobes** and **anaerobes** have preferences, as the name implies, but are capable of both mechanisms of catabolism.

2 GENERAL VIROLOGY

2.1 DEFINITION OF VIRUSES

Viruses (from latin word *virus*, which means poison) are strictly intracellular parasites. These pathogens do not have any metabolic proteins or proteosynthetic apparatus for its own survival and therefore have to "borrow" such proteins from the infected cell.

The viral particle (or virion) consists of proteins and nucleic acid (NA). Viruses may be either **enveloped** or **non-enveloped**. The envelope may be derived from different membranes in the cell, such as the nuclear membrane or the plasma membrane. The size of the viral particle is between 17 nm – 500 nm. The capsid is a protein coat surrounding the nucleic acid. It can have many different shapes, the most frequent are icosahedral and helical shapes. The capsid is composed of hexon and penton subunits. The nucleic acid contain viral genetic information in the form of either RNA or DNA. It may be either **single stranded** (ss) or **double stranded** (ds). The length of the nucleic acid is usually between 3–6 kb and up to around 200 kb. The NA may be either segmented or unsegmented. General virus cell cycle is depicted on **fig. 2.1**.

Cells must have receptors to which viral proteins can bind to, enabling the virus to begin the first step of infection. An example is the CD21 receptor on B cells, which the Epstein-Barr virus (EBV) can recognize and bind to. Such receptors make the cell permissive to infection. Infection is then characterized by doubling time, which is the time it takes for the number of viral particles to double.

2.2 HISTORY

The first signs of viral disease (poliomyelitis) were described for the first time in Egypt in 3,700 BC. In 1892, the Russian scientist Dimitriev Ivanovski discovered evidence that pathogens smaller than bacteria do exist. His tests described signs of tobacco mosaic virus infection in plants. In 1898, Dutch microbiologist Martinus Beijerinck gave the name 'virus' to the substance described by Ivanovski. Viruses were first detected directly by electron microscope in 1939. Detection of antibodies became one of the first techniques used for confirmation of previous infection. In 1941, G. Hirst observed haemato-agglutination of influenza virus for the first time. At present time, antibody detection is mainly based on the use of monoclonal

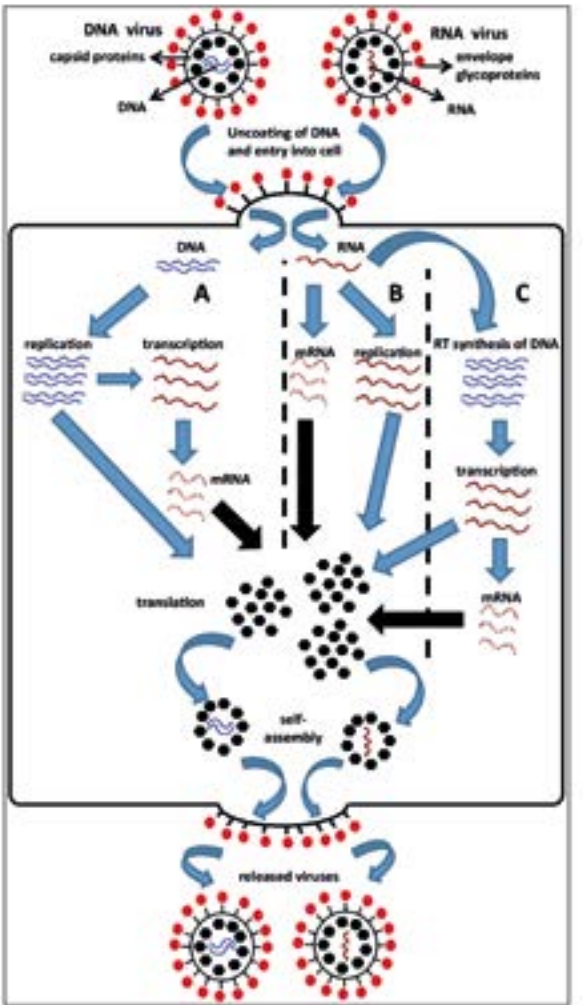

Figure 2.1: A simplified diagram of the viral reproductive cycle. A virus is an obligate intracellular parasite that uses its host cell's machinery to reproduce. After fusion of envelope proteins (in enveloped viruses) with the cell's membrane and removal of capsid proteins by cell enzymes, nucleic acid (DNA or RNA) is released into the host cell. DNA viruses (A). The viral DNA uses host nucleotides and enzymes to replicate itself (A). RNA is transcribed from DNA. Moreover, new capsid proteins and envelope glycoprotein are translated using mRNA. After self-assembly, new virus particles (virions) leave the cells coated with envelope glycoproteins (in the case of enveloped viruses). RNA viruses (B). RNA can be single or double-stranded. After cellular enzymes remove the capsid, the RNA serves as a template for synthesis of new copies of genome RNA, and it serves as a template for mRNA synthesis. Capsid proteins and envelope glycoproteins are translated using mRNA. A capsid assembles around viral genome particles and the virus buds from the envelope coated by glycoproteins. RNA viruses – retroviruses (C). Reverse transcriptase (RT) synthetases, a complementary DNA strand to the RNA template, and a second DNA strand complementary to DNA. The resulting double stranded DNA is incorporated as a provirus into the host genome. Proviral genes are transcribed into mRNA and are translated into the virus proteins. RNA transcribed from the provirus also serves as viral genome. The assembly of the capsid around genomes is followed by budding of the new viruses from the cell and their coating with cell mebrane glycoproteins.

antibodies. An additional possibility for viral detection is the cultivation on tissue monolayers, J. Enders used it for the first time for poliovirus in 1949. R. Dulbecco first used plaque-forming assays in 1952. Subsequently, plaque-forming units (PFU) were established. Nowadays, polymerase chain reaction (PCR) is the most useful method for the direct detection of viral agents in clinical material.

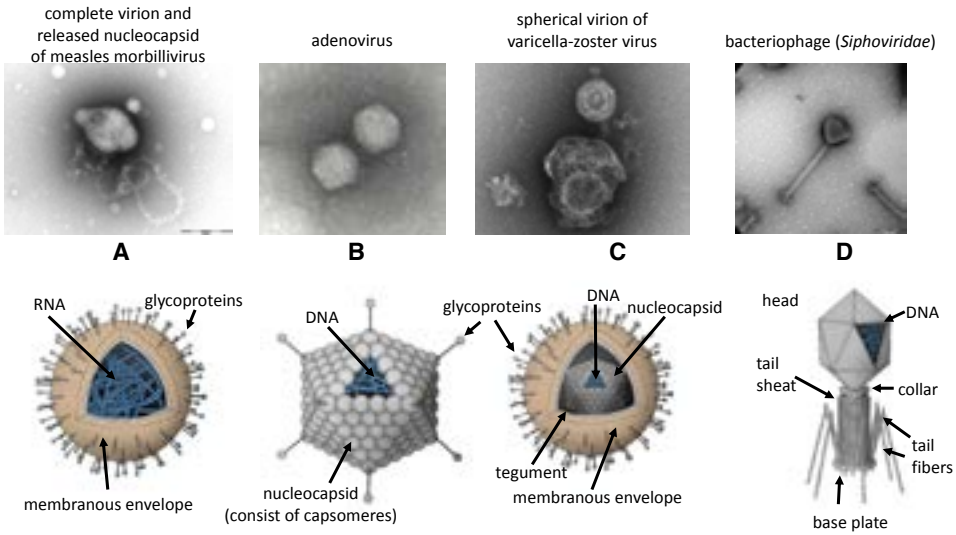

Figure 2.2: Different structure of viral capsids,
A. Diagram: spherical enveloped virion with glycoprotein spikes; fig.: transmission electron microscopy of a complete virion and released helical nucleocapsid of measles morbillivirus.
B. Diagram: polyhedral (icosahedral) nucleocapsid with glycoprotein spikes; fig.: transmission electron microscopy of adenovirus from a patient who suffered from acute gastroenteritis.
C. Diagram: herpesvirus – enveloped polyhedral (icosahedral) nucleocapsid with protruding glycoprotein spikes (fibres); fig.: spherical virion of varicella-zoster virus has an outer envelope with glycoprotein spikes and inner icosahedral proteinaceous nucleocapsid encapsulating viral DNA.
D. Diagram: complex capsid consists of a polyhedral head, tail and fibers; fig.: bacteriophage of the genus *Kayvirus*, representative of bacteriophages with contractile flagella from the family *Myoviridae* (therapeutic use for local *Staphylococcus aureus* infections)(courtesy of Roman Pantůček, Faculty of Science, Masaryk University, Brno).

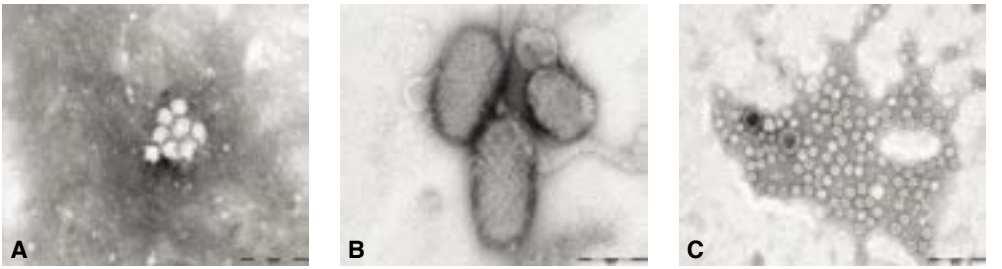

Figure 2.3: Electron microscopic photographs of different viruses. West Nile virus (A), parapoxvirus Orf virus (B), norovirus and rotavirus in patient stool suffered with acute gastroenteritis (C).

2.3 ELECTRON MICROSCOPIC EVIDENCE

Virions can be detected using electron microscopy (**fig. 2.3**).

2.4 VIRUS DETECTION BY OTHER METHODS

Colorless plagues are seen below as dots of lysed tissue culture cells caused by the cytopathic effect of the virus (**fig. 2.4**). The number of the plaques indicates the plaque forming unit (PFU).

Figure 2.4: Viral culture – plaques in the cell culture. Picture (A) shows the detail of the cell monolayer, picture (B) show details of the destroyed cells in the plaque.

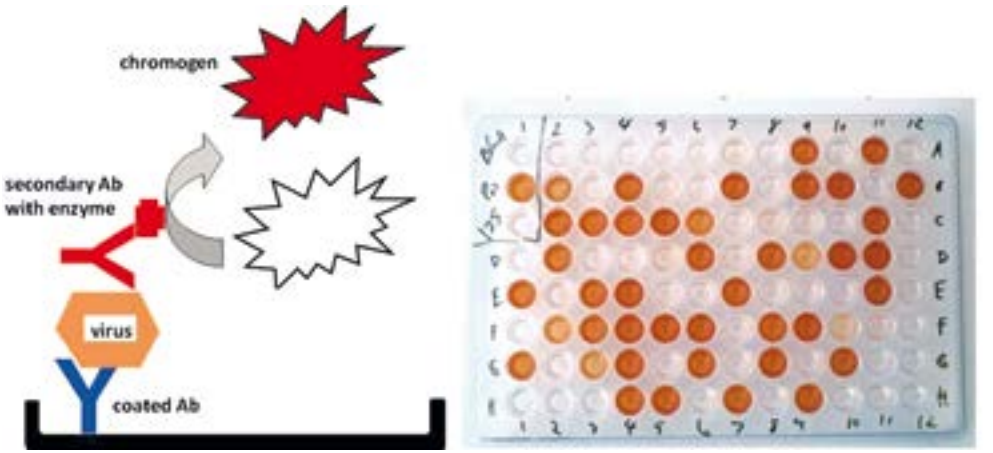

Figure 2.5: Enzyme-linked immunosorbent Assay (ELISA) is frequently used for the detection of viral particles, or antibodies (viral antigen is coated to the plate instead of first antibody) (A). Red/pink spots in panel B present the low/high positivity.

Principle and results of *Enzyme-linked ImmunoSorbent Assay (ELISA)* are depicted in **fig. 2.5**. This procedure can be used to detect viral particles (monoclonal antibodies are bound) or specific patient antibodies (specific viral antigens/proteins are bound).

Principles (on the left) and results (on the right) of complement-fixation reaction are depicted in **fig. 2.6**.

Detection of viral particles using immunofluorescence is depicted in **fig. 2.7**.

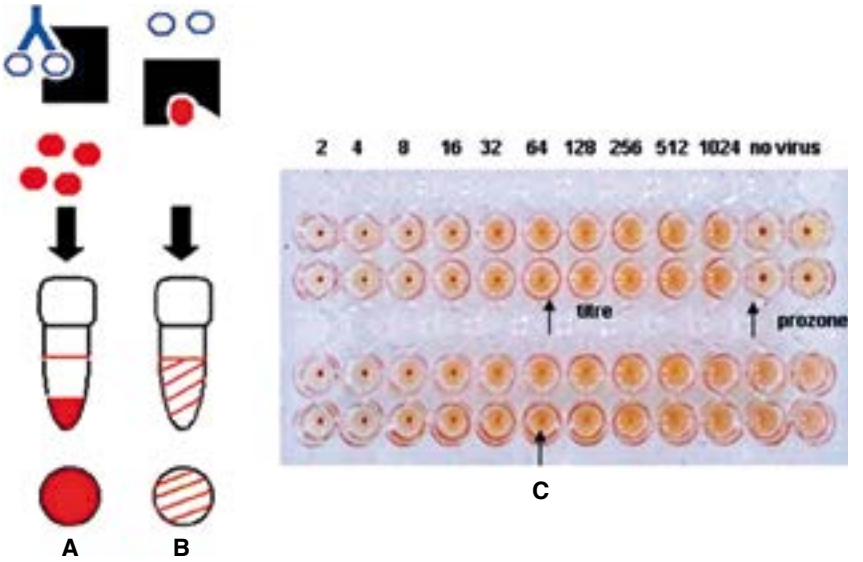

Figure 2.6: Complement-fixation reaction (CFR): A – positive result , where specific antibodies (Ab) bind to antigen (Ag – depicted as colorless circles in the fig.) creating a complex of antibodies and antigens (Ab-Ab). Complement binds to the Ab-Ag complex and cannot lyse red blood cells (depicted as full circles in the fig.), which subsequently sediment at the bottom of the tube. B – negative result, where complement lyses the red blood cells creating a pinkish colour from the reaction (view from side and above). C – titres of both the reactions are 1:64 (both reactions in duplicates).

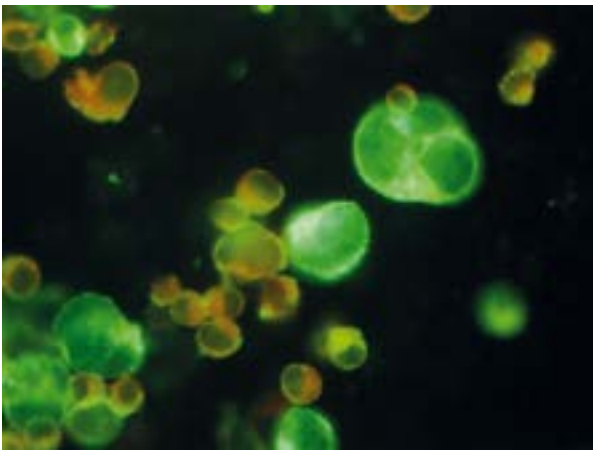

Figure 2.7: Examples of fluorescent microscopic detection of viral proteins. This case shows positivity of cytomegalovirus pp65 (CMV) in human cells.

PCR amplifies a specific target DNA or RNA. RNA first needs to be reversely transcribed by reverse transcriptase to create a DNA copy (cDNA). Thus, it is an useful tool for the direct viral detection of viruses in various clinical materials. Specific amplified products (amplicons) are detected by gel electrophoresis or, in the case of RQ-PCR (Real-time Quantitative PCR – RQ-PCR) using DNA probes that emit fluorescence, which can be quantitatively measured by a graph (**fig. 2.8**).

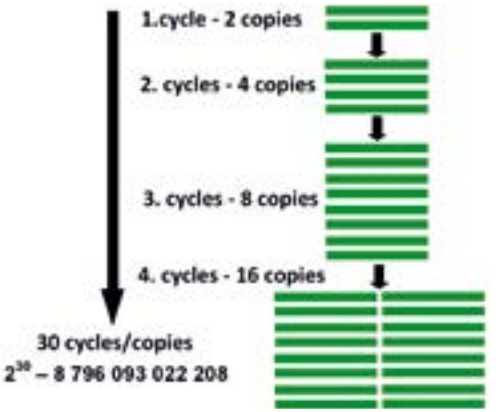

Figure 2.8: Principle of PCR.

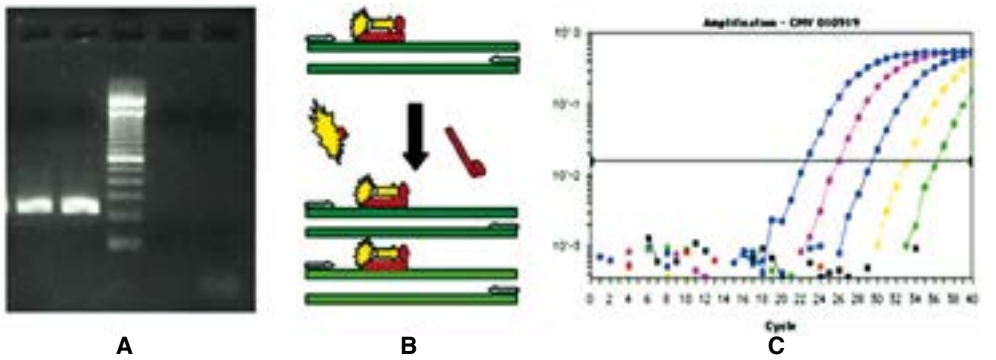

Figure 2.9: Detection of specific amplicon using gel electrophoresis (A) and RQ-PCR (B). Principle of RQ-PCR: In comparison to conventional PCR, RQ-PRCR uses fluorescein labeled DNA probes. If the probes are complementary to the tested target they hybridize to the target (template). When newly synthesized double stranded DNA gets close to the hybridized probe, the exonuclease activity of polymerase cleaves fluorescein from the probe and fluorescence is measured (C).

3 GENERAL MYCOLOGY

Mycology is the study of fungi, a kingdom which today contains more than 100,000 known species. They are eukaryotic microorganisms, which means that they have a nucleus that contains genetic information. The structure and function differs a lot from the prokaryotic bacterial cells, but they are also substantially different from other eukaryotic organisms (e.g., humans). Important structures in the fungi, which are frequent targets of antimycotic treatment, are their 80S ribosomes, the cell membrane (containing ergosterol) and their cell wall (containing chitin, mannan, glucan and chitosane (**fig. 3.1**).

For medical doctors, it is important to remember that majority of **medically significant fungi** are so called **opportunistic pathogens**. This means that they often do not cause disease in otherwise healthy people. People with reduced immunity, like HIV-patients and patients on anti-tumor therapy are susceptible. If you are not dealing with these kinds of patients, the diagnosis of a fungal infection is very rare. Anyhow, the aim of medical mycology is to identify pathogenic species (around 400 known) causing disease that bother our patients, and based on this, to determine functioning antimycotic therapy.

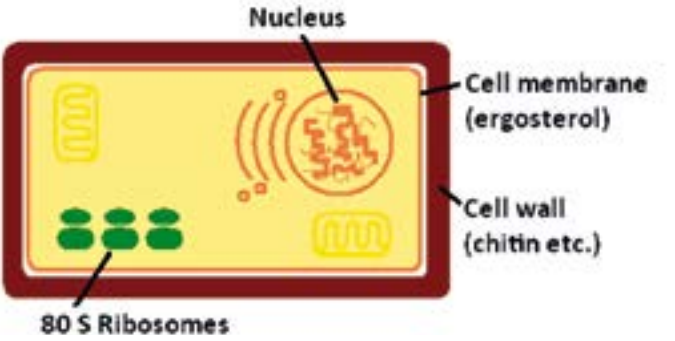

Figure 3.1: Schematic diagram of a typical fungal cell highlighting the targets of antifungal therapy. The nucleus is the characteristic feature of eukaryotic cells. The 80 S ribosomes, the ergosterol in the cell membrane and the many cell wall components are typical for fungal cells, and so are targets for antifungal therapy.

3.1 CLASSIFICATION OF FUNGI

The fungi can be classified into groups depending on their morphology. **Yeasts** are unicellular organisms that grow in small colonies on agar plates. **Molds** are multicellular organisms, showing a hairy and confluent appearance of their growth pattern. The fungi that switch between yeast and mold states are called **dimorphic fungi** (**fig. 3.2**).

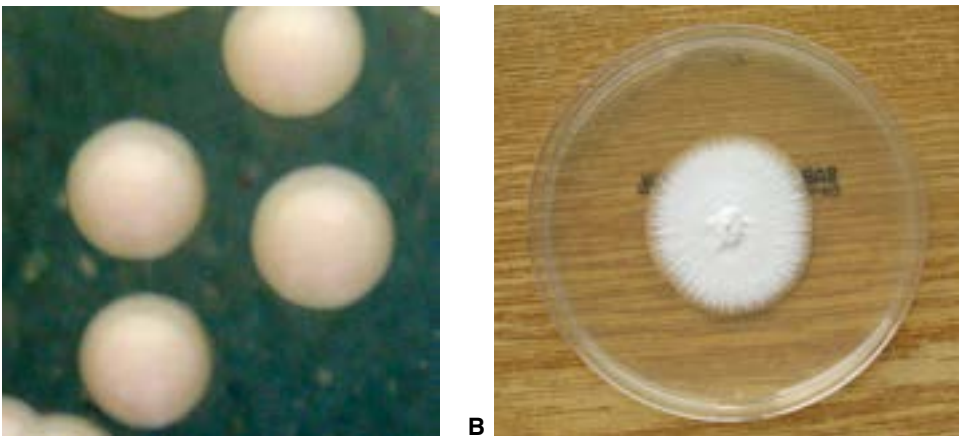

Figure 3.2: The difference between yeasts and molds by growth on culture media. The yeasts grow in small colonies – detail (A) while the molds show a hairy, confluent appearance – actual size (B). (Courtesy of Vanda Chrenková)

3.2 THE GROWTH OF FUNGI

Many fungi that appear like mold grow in a very different way from bacteria, human cells or other unicellular organisms. They extend by branching and reaching out by long filamentous extensions called **hyphae**. They consist of tubular cell walls that either surround separate cells, divided by septae (**septate**), or confluent cells without any division (**aseptate**, **fig. 3.3**). A collection of hyphae is called **mycelium**. The appearance of hyphae is sometimes specific of the different species of fungi, and therefore can be used in the identification process. Yeasts, being unicellular organisms do not grow in this fashion, but divide like the other unicellular organisms you have read about so far.

3.3 REPRODUCTION OF FUNGI

The fungi can reproduce either through mitosis in an asexual fashion, or via meiosis, sexual reproduction.

Asexual reproduction is very common, it occurs through the formation of vegetative spores (**conidia**, **fig. 3.4**) or the fragmentation of mycelium. These cells can develop into new organisms if the conditions are favourable. Important about the formation of conidia is, as