## MO001/3/2015

# Introductory Microbiology MIB2601

Semesters 1 & 2

**Department of Life and Consumer Sciences** 

## **IMPORTANT INFORMATION:**

This tutorial letter contains important information about your module.

BAR CODE



Learn without limits.

## CONTENTS

			Page	
Prefac	Preface			
Welcome message				
Unit 0	Welco	me and Introduction	5	
1.	Unit 1	The evolution of Microorganisms and Microbiology	15	
2.	Unit 2	The Study Of Microbial Structure: Microscopy and Specimen Preparation	23	
3.	Unit 3	Bacterial Cell Structure	30	
4.	Unit 4	Eukaryotic Cell Structure and Function	36	
5.	Unit 5	Microbial Nutrition	40	
6.	Unit 6	Microbial Growth	44	
7.	Unit 7	Control of Microorganisms in the Environment	48	
8.	Unit 8	Antimicrobial Chemotherapy	54	
Discussion forums and topics in MIB2601 62				

## PREFACE

#### **Dear Students**

Welcome to the module Introductory Microbiology (MIB2601).

This is an online module, but you could also use this printed document (MO001) to study for this module. This document is essentially a printed version of everything you will find on the module web site on myUnisa. It is a convenient document that you will be able to refer to at any time, page through, and make notes on. However, we would still like to encourage you to use the module web site, as this has several advantages. For example, you can easily access any part of your study material by clicking on the links in table of contents of the learning units, and you can interact with your lecturer and fellow students on the module's discussion forum.

This document starts with the message on the Welcome page of your module web site. It then goes on to the text of the learning units of the module. Be sure to read learning unit 0, as it contains important information about the module. Also remember to read your Tutorial Letter 101, which will provide you with essential details about the module and its assessment.

At the end of this MO001 document, there is a list of the Discussion forum topics that will be available for your use on the module site.

I wish you all the best with your studies.

Your lecturer

## INTRODUCTORY MICROBIOLOGY (MIB2601) WELCOME MESSAGE

## **Dear Students**



Welcome to the module Introductory Microbiology which is offered in the Department of Life and Consumer Sciences. I am Dr Monde Nyila, and I will be your lecturer for this module. I trust that this module will deepen your understanding of Microbiology and help you further your studies in general.

The purpose of this module is to provide you with knowledge and insight into Microbiology as a scientific discipline. You will learn about the development of Microbiology as a discipline, microscopy, prokaryotic and eukaryotic cells, the growth and development of microbes, and chemical and physical control of microorganisms. The knowledge and skills you will acquire in this module are fundamental to current research and new developments in food microbiology, medical microbiology, biotechnology, health and medicine.

This module is offered online, but as an alternative you will also be receiving a printed study pack. You will find more details on how to study this module in learning unit 0, as well as in Tutorial Letter 101.

If you are reading this online, you will see the different options that are available on this site on the lefthand side of the screen. The material that you must study is contained in the **learning units**. A printable pdf version of the learning units and Tutorial Letter 101 is stored in **Official Study Material**. From time to time you will receive announcements, for example to remind you of a due date for an assignment. We will use the **Discussions** tool during the course of your studies, and you can also use it to communicate with other students. Additional Resources is a folder that contains resources relevant to this course. The schedule will remind you of important dates in the semester, for example due dates for assignments. You will find more details about these different tools in learning unit 0.

After reading this page, you should read Tutorial Letter 101 (if you have not done so already). Online, you can access this in Additional Resources, in the subfolder 'Tutorial Material'. Then you should proceed to the learning units. Be sure to read learning unit 0, as this contains important information.

If you have any queries about the module, you are welcome to contact me by email or telephone. You may also make an appointment to see me in my office at the Unisa Science Campus in Florida.

I wish you all the best in your studies.

Dr Monde A. Nyila Pr.Sci.Nat. Tel.: (27) 011 471 2294 Email: <u>nyilama@unisa.ac.za</u> Fax: (27)011 471 2796 Office: Unisa Science Campus, Florida, Calabash Building, office 219

## INTRODUCTORY MICROBIOLOGY (MIB2601) Learning units

## **LEARNING UNIT 0**

#### Welcome and Introduction

## 0.1 Getting started

Welcome to Introductory Microbiology (MIB2601), a module that is offered in Unisa's Department of Life and Consumer Sciences. I would like to take this opportunity to wish you success with your academic year.

This is an online module, which means that you will find everything you need to complete the module on this site. Check this site regularly for updates, posted announcements and additional resources uploaded throughout the semester. Rapid communications throughout the semester(s) have been made possible through myUnisa. By using the myUnisa site, you can:

- submit assignments (please note: it is advisable that you submit your assignment online as this will ensure that you receive rapid feedback and comments),
- access your official study material,
- have access to the Unisa library functions,
- "chat" to your lecturer or to fellow students and participate in online discussion forums, and
- obtain access to a variety of learning resources.

Please take some time to familiarise yourself with the site so that you get to know where the different tools and resources are. I will give you more information about this later in this learning unit.

Although I would like to encourage you to study this module online, we also recognise that it might be impossible for some of you to get online at all, while others might only be able to get online infrequently. For this reason, you can also use the print-based study pack that you will receive to study for this module.

Your study material for this module includes the following:

- Your prescribed textbook
- These learning units
- Tutorial Letter 101
- Any other tutorial letters you may receive during the course of the year

Details of your prescribed book are given in the "Prescribed books" menu option that you can access on the left-hand side of this screen and also in Tutorial Letter 101.

Tutorial Letter 101 will be posted to you or given to you on registration, but you can also access it on the module web site. You can do this by clicking on "Official Study Material" in the menu on the left. As you will see, "Official Study Material" also contains a printable

version of these learning units (MO001).

Tutorial Letter 101 is just one of the tutorial letters you will be receiving during the year. It is extremely important that you read this tutorial letter carefully. You will also receive Tutorial Letter 201 during the course of the semester shortly after the due dates for submission of each assignment. Tutorial Letter 201 is closely linked to Tutorial Letter 101 and will provide you with a guide to the answers for the assignments.

In this learning unit, I will give you an overview and some general information about this module. I will also tell you more about how you can study this module, how to use myUnisa, and about the assessment in the module.

Click on "Next" below to go to the next screen, where you will find more information about contact details.

#### 0.2 Lecturer and contact details

In this section I will give you my own contact details, as well as details of the Department of Life and Consumer Sciences at Unisa, which is the academic department that offers this module. I will also give you the university's contact details, as well as some information about the student support services at Unisa that you are welcome to make use of.

Please note that whenever you contact the university, whether in writing or telephonically, you should always mention the **module code** and your **student number**.

Also note that if you write a letter to Unisa, you may enclose more than one letter in an envelope, but do not address enquiries to different departments (e.g. Despatch and Library Services) in the same letter. This will cause a delay in the replies to your enquiries. Please write a separate letter to each department and mark each letter clearly for the attention of that department. Letters to lecturers may not be enclosed together with assignments. Always write your student number and the module code at the top of your letter.

#### 0.2.1 Lecturer and department

Lecturer: Dr Monde A Nyila

Telephone number: +27 11 471 2294

E-mail address: nyilama@unisa.ac.za

Postal address: The Lecturer (MIB2601) Department of Life and Consumer Sciences Private Bag x6 Florida 1710

The department offering this module is the Department of Life and Consumer Sciences.

Telephone number (departmental secretary): +27 11 471 2230 Fax number: +27 11 471 2796

## 0.2.2 University

Should you need to contact the university about matters not related to the content of this module, consult the publication my Studies @ Unisa, which you received with your study material. This brochure contains information on how to contact the university (e.g. to whom you can write for different queries, important telephone and fax numbers, addresses and details of the opening and closing times of particular facilities). This brochure can be accessed at: <u>http://www.unisa.ac.za/contents/study2012/docs/myStudies-Unisa-2014.pdf.</u>

You can also make use of the following contact routes:

- Unisa website http://www.unisa.ac.za & http://mobi.unisa.ac.za
- E-mail (general enquiries) <u>info@unisa.ac.za</u>
   International students are urged to make use of the e-mail address <u>info@unisa.ac.za</u>
- <u>study-info@unisa.ac.za</u> for application and registration queries
- assign@unisa.ac.za for assignment queries
- exams@unisa.ac.za for examination queries
- despatch@unisa.ac.za for study material queries
- finan@unisa.ac.za for student account queries
- myUnisaHelp@unisa.ac.za for assistance with myUnisa
- myLifeHelp@unisa.ac.za for assistance with myLife e-mail accounts
- SMS 32695 South Africa only

You will receive an auto response SMS with the various SMS options. The cost per SMS is R1,00.

• Fax 012 429 4150

#### 0.2.3 Student support services

For information about the various student support systems and services available at Unisa (e.g. student counselling, tutorial classes, language support), consult <u>my studies @ Unisa</u> (<u>http://www.unisa.ac.za/contents/study2012/docs/myStudies-Unisa-2014.pdf)</u>.

#### • Fellow students

It is always a good idea to have contact with fellow students. You can do this by using the **Discussion** menu option on myUnisa. You can also use the discussion forum to find out whether there are students in your area who would like to form study groups.

#### Library

My Studies @ Unisa lists all the services offered by the Unisa library.

To log in to the library website (<u>http://www.unisa.ac.za/Default.asp?Cmd=ViewContent&ContentID=17</u>), you will be required

to provide your login details, i.e. your student number and your myUnisa password, in order to access the library's online resources and services. This will enable you to:

- request library material,
- view and renew your library material,
- use the library's e-resources.

## • Unisa Directorate for Counselling and Career Development (DCCD)

The DCCD supports prospective and registered students before, during and after their Unisa studies. There are resources on their website (<u>http://www.unisa.ac.za/Default.asp?Cmd=ViewContent&ContentID=15974</u>), and also printed beschetz and its have a spinted beautise.

booklets available to assist you with

- career advice and how to develop your employability skills
- study skills
- academic literacy (reading, writing and quantitative skills)
- assignment submission
- exam preparation

## • The Advocacy and Resource Centre for Students with Disabilities (ARCSWiD)

You will find more information about this centre on their web page at <u>http://www.unisa.ac.za/default.asp?Cmd=ViewContent&ContentID=19553</u>. You can also contact Ms Vukati Ndlovu on 012 4415470 in this regard.

#### 0.3 Purpose and outcomes of this module

The purpose of the module is to provide you with knowledge and understanding of Microbiology as a scientific discipline. On completing the module, you should be able to describe the methodologies used by microbiologists to study the organisation and structure of prokaryotic and eukaryotic cells; to explain the fundamental principles and consequences of bacterial growth and reproduction; and to describe the control of microorganisms by means of physical methods and chemicals.

More specifically, the outcomes of this module are that after completing the module, you should be able to do the following:

- 1) Explain the meanings of basic terms and concepts in microbiology.
- 2) Outline the development of microbiology as a science, and describe the scope and benefits of microbiology as a discipline.
- 3) Describe and distinguish between the working principles and use of different types of microscopes, and describe laboratory techniques relevant to their use.
- 4) Describe, compare and contrast prokaryotic and eukaryotic cell structure and function.
- 5) Identify the nutrients which promote microbial growth and explain how they are taken up from the environment, and describe microbial growth processes.
- 6) Identify, explain and compare the physical and chemical measures used for microbial control.

The next section will give you a better idea about how the content of the module is structured

and how the various ideas expressed in the learning outcomes are related.

#### 0.4 How the content of this module is organised

This module introduces you to the world of microbes and how this field/subject evolved over the years. Microorganisms are important contributors to the functioning of the biosphere. They may play both beneficial and harmful roles. They harm humans by causing diseases. But they also inhabit humans and help to digest their food.

The learning units are structured as follows:

- In learning unit 1 you will learn more about what the **science of microbiology** involves, and how it has evolved.
- Learning unit 2 will give you an overview of the various **microscopic and laboratory technologies** that microbiologists use to study microorganisms.
- In learning units 3 and 4 you will study the **cellular structure** of prokaryotes (bacteria and archea) and eukaryotes.
- Learning units 5 and 6 will give you an insight into microbial **nutrition and growth**.
- Learning units 7 and 8 deal with the **control of microorganisms**. Learning unit 7 will give you an overview of direct physical, chemical and biological control methods, while learning unit 8 focuses specifically on antimicrobial chemotherapy, that is, the control or removal of microbes in the living cells of humans and animals.

Now that you have a better idea of how the module is structured, let's look at what your studies will involve.

#### 0.5 Learning resources

Your main learning resources for this module will be your prescribed textbook and these learning units. These resources will be supported by tutorial letters.

The prescribed textbook to be used in conjunction with the online material is:

Willey, JM, Sherwood, LM & Woolverton, CJ. 2014. *Prescott's Microbiology*. 9th edition. New York: McGraw-Hill.

#### ISBN: 9789814581561

More details about the textbook are given in the menu option "Prescribed books" to the left of this screen, and also in Tutorial Letter 101.

The textbook is a comprehensive guide to the subject field. You will not be required to study the whole textbook, as I will guide you to what is needed while working through these learning units. You will need to study the chapters that are mentioned at the beginning of each learning unit and any recommended reading sections. If you find a topic particularly interesting then feel free to do further reading on that topic.

We will refer to the textbook as "Prescott" in the study guide.

## 0.6 Module-specific study plan

Use your my Studies @ Unisa brochure for general time management and planning skills.

This is a semester module over 15 weeks and requires at least 120 hours of study time; this means that you will have to study at least 8 hours per week for this module.

The following is a recommended time schedule that you could use as a **guideline** for studying this module.

ACTIVITY	HOURS
Reading and re-reading Tutorial Letter 101 and	3
Learning unit 0	
Skimming learning units and textbook, forming a	5
thorough general impression of the whole	
First reading of learning units 1-8 and textbook (@ 2	16
hours per learning unit)	
In-depth study of learning units 1-8 making mind maps	64
and summaries, and doing learning activities (@ 8	
hours per learning unit)	
Completing two assignments (Note: Assignment 1	10
should typically take less time than assignment 2)	
Examination revision	20
Writing the examination	2
Total	120

Below is an **example** of how you can schedule your study plan.

Week	Activity (each week represents 8 hours of study time)
1	<ul> <li>Read and re-read Tutorial Letter 101 and learning unit (LU) 0</li> </ul>
(January/	<ul> <li>Skim the learning units and textbook, forming a thorough general</li> </ul>
July)	impression of the whole
2	Read through the learning units and textbook and identify all key areas
3	
4	In-depth study of LU 1-3 (make mind maps and summaries and
5	complete learning activities)
6	Complete and submit assignment 1 (please note, depending on how
	you will submit the completed assignment, allow sufficient time for the
	assignment to reach Unisa before the due date)
7	<ul> <li>In-depth study of LU 4-6 (make mind maps and summaries and</li> </ul>
8	complete learning activities)
9	
10	Complete and submit assignment 2 (please note, depending on how you will submit the completed assignment, allow sufficient time for the assignment to reach Unisa before the due date)
11	<ul> <li>In-depth study of LU 7-8 (make mind maps and summaries and</li> </ul>
12	complete learning activities)
13	
14	Revision and preparation for exam
15 (April/	
October)	

## 0.7 How should you go about studying this module?

Distance learning is not easy and you should not underestimate the time and effort involved. Once you have received your study material, you need to plan how you will approach and complete this module. You can use the study plan in the previous section as a guideline to draw up a reasonable study schedule that can guide you through the whole module. Remember to take into consideration the due dates of the assignments as given in Tutorial Letter 101 for this module.

The assignments in this module will take the form of written work, and they should give you an idea of how well you are making progress in achieving the learning outcomes.

Your work on each learning unit should involve the following:

- Skim through the unit and draw your own basic mind map of the content of the learning unit. Then expand this map as your knowledge and understanding of the unit increases. If you have internet access, you can learn more about making mind maps on the following websites:
  - http://www.wikihow.com/Make-a-Mind-Map
  - <u>http://www.mind-mapping.co.uk/make-mind-map.htm</u>
- Make your own summary of every unit.
- Do a reflection exercise at the end of every unit. Most of the learning units contain some reflective questions that you should answer.

As you work, build up your own study and exam preparation **portfolio**. This portfolio will not be assessed, but it will be an extremely valuable tool for you in completing your assignments and revising for the examination.

**What is a portfolio?** A portfolio is a folder/file in which you gather and compile additional and/or summarised information during the year as you work through the learning material.

## Your portfolio should comprise the following:

- Answers to each activity in each learning unit
- A mind map/summary of each learning unit
- Your marked assignments (or a copy you made prior to submitting your assignment)
- Your reflections on each learning unit
- Where relevant, any extra reading material taken from the internet, additional books, medical and/or scientific journals
- A list of words or glossary of new terms in your own words

Compile and revise the contents of your portfolio to ensure that you achieve the learning outcomes of this module.

## 0.8 Orientation to using myUnisa

I have already outlined the advantages of online learning in section 0.1 of this learning unit. In the sections that follow, I will give you an orientation to using myUnisa. We will see how the Unisa menu options work, and we will refer to the "rules" or "etiquette" of online

communications. Finally, you will have the opportunity to try your own hand at using one of the most important tools on myUnisa, the Discussions tool.

#### 0.8.1 The myUnisa menu options

You need to be able to use the various menu options on this course site. They will enable you to participate actively in the learning process.

Click on the links below to see where the various options are located.

- Learning units: The learning units are your main learning resource in this module and contain the content and learning activities that you need to work through to achieve the module outcomes.
- **Official study material:** Here you will find the printable (pdf) version of the learning units, as well as your tutorial letters.
- **Discussions**: This tool allows us to hold discussions as if we were in a contact setting, and I hope that this will give you clarity on many of the issues that students tend to struggle with. I will set up a number of discussion forums that you can visit to discuss specific topics. There will also be a forum for students where you can discuss issues among yourselves, or just support one another.
- **Announcements**: From time to time I will use this facility to give you important information about this module. You should receive e-mail notification of new announcements placed on myUnisa.
- **Schedule:** This tool gives you access to important dates and details about events, such as examination dates and deadlines for your assignments. You will need this information to help you manage your time and plan your own schedule.
- Assignments: This tool allows you to submit your assignments electronically, and to monitor your results. If you can, please submit your assignments via myUnisa. If you do not know how to do this, consult Tutorial Letter 101.
- **Course contact:** If you want to send me e-mails in connection with this module, use this tool to communicate with me.
- Additional resources: This tool allows you to access any additional learning support material that might help you in your studies for this module. I will send an e-mail alert or announcement to inform you if I add anything to this folder.

#### 0.8.2 myUnisa etiquette

myUnisa is the university's online platform where lectures and students meet, interact and participate in an ongoing process of learning and teaching. In interacting online, always remember to be mindful of and respectful towards your fellow students and your lecturers. The rules of polite behaviour on the internet are referred to as **netiquette** – a term that means "online manners".

You can access the web sites below to learn more about netiquette.

<u>http://networketiquette.net/</u>

- <u>http://www.studygs.net/netiquette.htm</u>
- <u>http://www.carnegiecyberacademy.com/facultyPages/communication/netiquette.html</u>

Please observe the rules of netiquette during your normal, everyday online communications with colleagues, lecturers, and friends. In particular, remember to be courteous to your fellow students when using the Discussions tool.

#### 0.8.3 Activity 0.1: Introduce yourself

At this point, I would like you to do a first activity.

The purpose of the activity is the following:

- It will help you to get to know the myUnisa online environment.
- It will help you to get to know and connect with your fellow students.

To do the activity, click on the "Discussions" option in the menu on the left-hand side of the screen. From here, click on the forum "Module-related discussions", and then on the topic "Introducing yourself".

Once inside the topic, post a short entry in which you:

- tell us who you are and where you live; and
- share what Microbiology means to you, and why you chose to study it.

Also respond to at least one posting by one of your fellow students.

#### 0.9 Assessment in this module

Your work in this module will be assessed by the following:

- Two written assignments, which will be used to calculate a year mark that will count 30% towards your final mark.
- A third assignment, which is a Discussion question on myUnisa. This assignment will not be marked, but I encourage you to complete it, as this will add to your learning experience.
- One written examination of 2 hours, which will count 70% towards your final mark.

<u>Please consult Tutorial Letter 101</u> for details about the assessment in this module. Make sure to read the following information in the tutorial letter:

- How your assignment and exam marks will be calculated
- The due dates and unique numbers of your assignments
- How you should submit your assignments
- Examination periods, admission and marks

## Tutorial Letter 101 also contains the actual assignment questions.

Remember that while Tutorial Letter 101 will be sent to you, you can also access an electronic version by using the link on this page, or else by going to the menu option "Official Study Material".

Good luck and enjoy the course!

## **LEARNING UNIT 1**

#### The Evolution of Microorganisms and Microbiology

#### 1.1 Introduction

You leave your food overnight outside the refrigerator and when you come home the following day, your food does not look good. What do you think has caused the spoilage of your food? The culprit is microorganisms!

Microbes contain 50% of the biological carbon and 90% of the biological nitrogen on earth. They are found everywhere, from geothermal vents in the oceans, to the coldest arctic ice, to every person's skin. Microorganisms are important contributors to the functioning of the biosphere. They play both beneficial and harmful roles. They harm humans by causing diseases. But they also inhabit humans and help to digest their food and produce vitamins B and K.

Microorganisms play a huge role in industry – in the manufacturing of bread, cheese, beer, antibiotics, vaccines, vitamins, enzymes, and other important products. What do you think would happen on earth if there were no microorganisms?

## 1.2 Learning outcomes

When you have completed this learning unit, you should be able to:

- define microbiology as a scientific discipline and describe some of the methods commonly used in the study of microorganisms
- distinguish and compare the structure of prokaryotic and eukaryotic cells
- discuss the evolution and development of microbiology by referring to important events and the scientists involved
- describe the relationship between microorganisms and disease, and apply Koch's postulates to relate microorganism and disease
- explain the contributions that Pasteur, Winogradsky, Beijerinck and other scientists made to the field of industrial microbiology and microbial ecology
- distinguish between different sub-disciplines and areas of specialisation in microbiology

## 1.3 Textbook reference

Study chapter 1 (The evolution of microorganisms and microbiology) in your prescribed textbook (Prescott's Microbiology by JM Willey, LM Sherwood & CJ Woolverton, 2011). From now on we shall refer to your textbook as simply "Prescott".

You can also consult additional resources on this learning unit by visiting the McGraw-Hill Online Learning Center at the following website:

http://highered.mcgraw-hill.com/sites/0073375268/information\_center\_view0/.

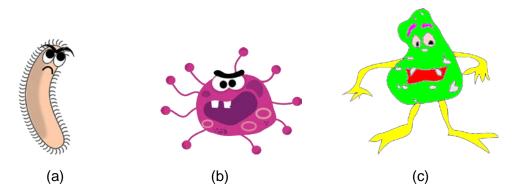
#### 1.4 The microbial world

Do you see the microorganisms on your skin? Most microorganisms are so small that they cannot be seen with the naked eye. You need a microscope to see them. Microorganisms are diverse organisms and fall into two groups of cells, namely prokaryotic cells and eukaryotic cells. For a detailed explanation and the differences between these two types of cells refer to Prescott. These two groups of cells led to the development of the five kingdoms system,

namely the Monera, Protisa, Fungi, Animalia and Plantae.

Figure 1.1 on page 3 in Prescott shows types of biological entities that are studied by microbiologists.

These days, you will find many representations of "germs" or bacteria in the popular media. Here are a few examples:

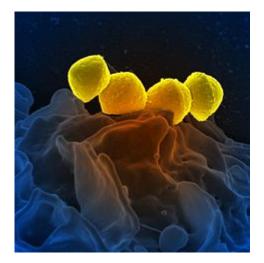


**Figure 1.1**: Bacteria in the popular media (<u>http://pixabay.com/en/bacteria-germ-pathogene-cell-funny-297171/; http://pixabay.com/en/germ-cartoon-frown-sick-nasty-303979/; http://pixabay.com/en/bacteria-amoeba-germ-microorganism-153373/)</u>

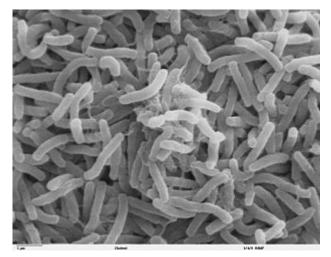
Do you think this is what bacteria really look like? If not, what is wrong with these pictures?

I'm sure you realised that the artists who drew figures (a) and (b) above were thinking of bacteria as fully-fledged little animals, or even little "men", with limbs and faces like animals or humans! This is a popular misconception. Of course, bacteria look nothing like this. The artist who drew figure (c) has provided us with a slightly more accurate picture, but even here the bacteria seem to have fully-formed "eyes" staring at us.

Bacteria and other microorganisms consist of only one cell and do not have limbs or complex sense organs like multicellular organisms. Under a microscope, they tend to look like balls, rods or spirals. Here are some examples of real bacteria, as seen under an electron microscope:



 (a) Streptococcus pyogenese (the colours are not real but have been added to make the bacteria more clearly visible)
 (http://commons.wikimedia.org/wiki/File:Streptococcus\_pyogenese\_Bacteria.jpg)



(b) *Vibrio cholerae*, the bacteria that cause cholera (<u>http://commons.wikimedia.org/wiki/File:Cholera\_bacteria\_SEM.jpg</u>)



(c) Campylobacter jejuni (http://commons.wikimedia.org/wiki/File:ARS\_Campylobacter\_jejuni.jpg)

Figure 1.2: Scanning electron images of bacteria

## 1.5 Activity 1.1: Different types of organisms

After studying the relevant sections in Prescott, answer the following questions:

- 1. Distinguish among bacteria, Archaea, fungi, protists and viruses.
- 2. To which of the aforementioned groups do algae belong? Give a reason for your answer.
- 3. Compare prokaryotic and eukaryotic cells. Write your answer in tabular form.

Note that in your table you should only include those characteristics that can actually be compared. This includes both differences and similarities between the two groups.

#### 1.6 Feedback on activity 1.1

Bacteria and Archaea lack many membrane-delimited organelles that are found in eukaryotic cells. The organelles that are not found in the former two groups are nucleolus, mitochondria, endoplasmic reticulum, Golgi body, and lysosomes. The genetic material of both the bacteria and the Archaea is not found in the true membrane-bound nucleus as compared to the

eukaryotic cells.

## 1.7 Microbial evolution

To learn more about microbial evolution, study pages 5 to 12 in Prescott.

Note that like any other science the field of microbial evolution is also based on the formulation of **hypotheses**. What does this mean? For detailed information on the formulation of hypotheses, see the discussion on the scientific method on page 5 of Prescott.

## **1.8** Microbiology and its origins

Do you think microorganisms existed before they were seen by scientists? Some scientists suspected the existence of microorganisms and their responsibility for disease. The physician **Girolamo Fracastoro** suggested that disease was the result of invisible living creatures. The first scientist to publish extensive, accurate observations of microorganisms was the amateur microscopist **Antony van Leeuwenhoek** of the Netherlands. Can you name other scientists who played a role in the early development of microbiology?

What do you think causes the growth of microorganisms? Do you know where microbes come from? In earlier times people believed that living organisms could develop from non-living matter. This concept was known as **spontaneous generation**. Earlier scientists, including Aristotle, even thought that some of the simpler invertebrates could arise through spontaneous generation. Spontaneous generation was challenged by the Italian physician, **Francesco Redi**. For detailed information on Francesco Redi's experiments to prove that spontaneous generation did not exist, read section 1.3 in Prescott.

Industrial microbiology largely developed from the work **of Louis Pasteur** and others on the alcoholic fermentation of wine and other alcoholic beverages. The leading chemists of the time believed that fermentation was due to chemical instability that broke down sugars to alcohol. Pasteur did not agree with that notion, and believed that the fermentations were due to the actions of living organisms. What is your opinion on this? For more information on this topic, read the relevant text in your prescribed textbook.

**Theodore Schwann** and others proposed that yeast cells were responsible for the conversion of sugars to alcohol.

**Sergei Winogradsky**, the Russian scientist, made contributions to soil microbiology. Winogradsky discovered that soil bacteria could oxidise iron, sulphur, and ammonia to obtain energy. For more information on Winogradsky's work read the relevant text in your prescribed book.

**Martinus Beijerinck** also made significant contributions to microbial ecology and many other fields. Beijerinck isolated the aerobic nitrogen-fixing bacterium Azotobacter, a root nodule bacterium (later named Rhizobium), and sulphate-reducing bacteria. For more information on Beijerinck's work, see Prescott.

## 1.9 The development of microbiological knowledge

The following are some of the most important events in the development of microbiological knowledge:

• Recognition of the relationship between microorganisms and disease: Read upon the suggestion made by Fracastoro, linking organisms and disease, the role played by Agostino Bassi through his demonstration of a silkworm disease, and other scientists who also showed that microorganisms cause disease.

- Koch's postulates: Do you know what causes disease? Robert Koch, a German physician, demonstrated the link between microorganisms and disease (see figure 1.15 in Prescott for "Koch's postulates applied to tuberculosis". Koch demonstrated this by using the criteria proposed by his former teacher, Jacob Henle, to establish the relationship between Bacillus anthracis and anthrax, and his findings were published in 1876.
- The development of techniques for studying microbial pathogens: To isolate and identify suspected bacteria that cause diseases, it became necessary to isolate the pathogens in pure cultures. A **pure culture** is a culture that contains only one type of microorganism.
- Box figure 1.1 (Techniques & Applications) on page 5 in your prescribed book shows the scientific method used when conducting research.

In your prescribed textbook (pp 13–19), thoroughly study the role played by microbiologists in the discovery of microorganisms, and the role played by Antony van Leeuwenhoek, Robert Hooke, Joseph Lister, Louis Pasteur and others in microbiology. Also see figures 1.11(a), 1.11(b) and 1.11(c) in the textbook, and get a clear picture of what the section is all about.

## 1.10 Activity 1.2: Koch's postulates

1. Discuss all the contributions Robert Koch made to microbiology from 1876 to 1884 and also discuss Koch's postulates.

2. Apply Koch's postulates to answer the following:

How would you prove that a particular organism was the cause of a rotting disease in an apple tree? How could you be sure you had found the correct microorganism and not just confused it with another of the millions of microorganisms that occur on an apple tree?

#### 1.11 Feedback on activity 1.2

Figure 1.11(a) (page 13 in Prescott) may help you with this activity. Figures 1.11(a), 11(b) and 1.10 **Figures** should also help to explain the events and development of microbiology from the 15th century to the 21st century. A lot has been achieved between 1546 (Fracastoro's "invisible organisms cause disease") and present (gene sequencing of antibiotic-resistant bacteria that can help doctors fight back)!

The discovery of penicillin by Alexander Fleming was an enormous contribution to the field of medicine and the sub-discipline industrial microbiology. Fleming discovered that the fungus *Penicillium* produced what he called penicillin, the first antibiotic that could control bacterial infections. Today microorganisms are used to produce products such vaccines, steroids, antibiotics, alcohol, etc.

Koch's experiments demonstrated that microorganisms are present in many diseases and that they are the source of the problem in those instances. The same microorganisms could be shown to be absent from the healthy organism.

#### 1.12 Microbiology today

Do you know any sub-disciplines of microbiology? If yes, could you mention them? The subdisciplines of microbiology are immunology, food and dairy microbiology, industrial microbiology, agricultural microbiology, and so forth. To get more information on the role of these sub-disciplines in microbiology, see the paragraphs "Molecular and Genomic Methods of Studying Microbes" and "Major Fields in Microbiology" in Prescott. In which of these fields would you be most interested, and why? Could you mention the challenges that microbiologists are facing with regard to the emergence of new diseases?

In answering this question you might have thought of such challenges as multi-drug-resistant tuberculosis, HIV/AIDS, etc. HIV/AIDS poses a serious threat to humans at present. The lack of a vaccine that prevents HIV/AIDS is a challenge to every nation. The only way to slow down the spread of HIV/AIDS is to change behaviour and educate communities about the danger of being involved in multiple sexual partnerships. What is your opinion on the spread of HIV/AIDS in sub-Saharan Africa? What do you think the governments in this region should do to curb the spread of the virus? Read up on the challenges we are facing with regard to the emergence of new diseases and the development of new techniques such as DNA-based techniques for studying microorganisms.

Remember that you can get more additional resources on this learning unit by visiting the McGraw-Hill Online Learning Center website (see section 1.3 above).

## 1.13 Activity 1.3: Revision questions

1. Briefly describe the major contributions of the following scientists in the development of microbiology:

- 1.1 Van Leeuwenhoek
- 1.2 Jenner
- 1.3 Pasteur
- 1.4 Lister
- 1.5 Bassi
- 1.6 Schwann
- 1.7 Beijerinck
- 1.8 Fleming
- 1.9 Koch
- 1.10 Tyndall
- 2. Define the following terms or concepts:
- 2.1 Spontaneous generation
- 2.2 Immunology
- 2.3 Prokaryotic cell
- 2.4 Eukaryotic cell

3 Compare and contrast a prokaryotic cell with a eukaryotic cell.

4 Give your own example to illustrate the application of Koch's postulates.

5 Explain how Louis Pasteur contributed to microbiology in his work with alcoholic fermentation.

6 What are the different roles played by microorganisms in our world? Give examples to illustrate your answer. Find at least two of your examples by referring to any two recent articles on microorganisms, whether from a newspaper, magazine or the internet.

7 What do you think are some of the challenges we are facing with regard to new diseases? Give at least two challenges, and give reasons why you think these challenges are serious.

#### 1.14 Feedback on activity 1.3

I will not give you any specific feedback on questions 1 and 2, as you should be able to check the information and your own definitions in Prescott.

To check your answer to question 3, refer to the table you drew in answering activity 1.1, and

the feedback on that activity.

There are any number of examples you could have selected to illustrate the application of Koch's postulates. As my example, I would select the disease "fireblight", which is a disease condition suffered by pear and apple trees. The disease kills tissues in these plants, making them shrunken, cracked and blackened, as if the tree had been scorched by fire (hence the name).



**Figure 1.3**: A tree affected by fireblight (<u>http://en.wikipedia.org/wiki/File:Apple\_tree\_with\_fire\_blight.jpg</u>)

A farmer who finds this condition in the orchard would have to apply Koch's postulates in the following way to determine the microbial cause of the disease with certainty, using the help of a technologist in the process:

- The technologist would have to isolate microorganisms from both the affected plants and healthy plants. The same microorganism would have to be found in all the affected plants, and not in any of the healthy plants.
- The technologist would then have to isolate the microorganism found in all the diseased plants and grow it in a pure culture.
- Afterwards they must then introduce the microorganism into one of the healthy trees (tree A), where it must cause the same symptoms as in the affected trees.
- The organism must then again be isolated from tree A, compared to the microorganisms found in the originally diseased plants, and found to be identical.

Fireblight is caused by the bacterium *Erwinia amylovora*. I have chosen this example because when we think of microbial infection, we tend to think of humans as victims first, and of animals next ... but remember that plants can suffer equally from these types of infections!

To check your answer to question 5, refer to Prescott, where this issue is clearly discussed.

You should have mentioned both the harmful and beneficial roles of microorganisms in your answer to question 6, as well as their role in industry. You might also have found information on their role in modern biotechnology. Some useful internet sites for finding information on scientific developments are the *Science Daily Magazine* and the *New Scientist Magazine*. Two relevant headlines I found were "Strain of MERS coronavirus engineered for use in a vaccine" and "Probiotics reduce piglet pathogens".

## 1.15 Reflection

Think about what you have learned in working through unit 1 and note down the three insights you gained that were the most interesting or newest for you. Has the way that you think about microorganisms changed in any way? If so, explain how.

## 1.16 Summary

In this study unit you have learned about the historical events in the development of microbiology as a discipline and how those events led to the knowledge of microorganisms that cause diseases as well as those that are beneficial. Think of scientists such as Robert Koch, Joseph Lister, Antony van Leeuwenhoek, Louis Pasteur and others that have contributed so much to the development of microbiology. Microscopes such as the transmission electron microscope (TEM), scanning electron microscope (SEM), confocal scanning laser microscope (CSLM) and other types of microscopy have made it possible for scientists to study the microbes in detail and be able to study the organelles of microbes' cells. Without these tools we would not know exactly how microorganisms cells are built and how they function. The knowledge of the different organisms has helped scientists and researchers to manufacture drugs that can destroy as well as prevent the growth of pathogenic microorganisms.

## LEARNING UNIT 2

#### The Study Of Microbial Structure: Microscopy and Specimen Preparation

#### 2.1 Introduction

As we all know, microbiology is usually concerned with organisms so small that they cannot be seen or viewed clearly with the naked eye (unaided eye). Because of this problem, microscopes are essential in the study of microorganisms.

Microscope technology has undergone a long evolution, just like our understanding of microorganisms that we discussed in learning unit 1. From simple light microscopes that function essentially like strong magnifying glasses, the technology has developed to use electrons as an alternative to light, and today microscopes are even being developed that can use laser light to take three-dimensional "snapshots" of living cells! (If you are interested. vou can read more about this on the following website: http://www.hhmi.org/news/new-microscope-produces-dazzling-3d-movies-live-cells)

If you decide to make a career in the field of life sciences, you may very well at some point have to examine organisms under a microscope, and in order to do so, prepare the relevant specimens for examination. It is therefore important for you to understand how a microscope works, and how specimens are prepared.

In this learning unit you will be introduced to the following:

- Light microscopy
- Specimen preparation (we discuss this directly after light microscopy, since the relevant specimens we will investigate are all prepared for light microscopes)
- Electron microscopy
- New types of microscopy

## 2.2 Learning outcomes

Once you have completed this unit, you should be able to:

- > give an overview of microscopy and specimen preparation
- > explain how lenses bend light rays to produce magnified images of small objects
- describe the preparation and simple staining of a specimen for observation under a light microscope
- > describe the Gram staining procedure and its use in the categorisation of bacteria
- Compare the mechanisms of a transmission electron microscope and a scanning electron microscope with one another and with that of a light microscope

#### 2.3 Textbook reference

Study pages 22 to 40 in Prescott in detail.

## 2.4 Light microscopy

In this section we will first investigate general principles related to lenses and the bending

of light. Then we will distinguish between different types of light microscopes.

The structure of a basic light microscope is shown in Figure 2.3 page 24. .

#### 2.4.1 Lenses and the bending of light

To understand how a light microscope works, one must know something about the way in which lenses bend and focus light to create images. **Refraction** occurs when a ray of light passes from one medium to another. During refraction, the ray of light is bent at the interface. To get a clear picture of this, see figure 2.1 on page 23 in Prescott. Also read pages 23 to 26 for detailed information on lenses and the bending of light. Look at table 2.2 on page 29 for the properties of objective lenses.

#### 2.4.2 Activity 2.1: Basic concepts in light microscopy

1. Define the following terms: refractive index, refraction, focal point and focal length.

2. Draw your own labelled diagram to differentiate between the focal point and focal length of a lens, and also explain the difference below your sketch.

#### 2.4.3 Feedback on activity 2.1

Refer to Prescott for the definition of the relevant concepts.

In answering question 2, you should have kept your drawing simple. Refer to Prescott.

You can also find many drawings of this type on the internet by going to Google Images and searching for "focal point focal length".

#### 2.4.4 Types of light microscopes

Microbiologists commonly use light microscopes to study microbes. Modern microscopes are all **compound** microscopes, in other words, the magnified image formed by the objective lens is further enlarged by one or more additional lenses.

Microscope **resolution** is the ability of a lens to separate or distinguish between small objects that are close together. What is the numerical aperture, the working distance and the Abbé equation? For more information on these concepts, study the section on microscope resolution on pages 24 to 26 in Prescott. See the properties of microscope objectives in table 2.2 on page 25 in Prescott.

The following are the main types of light microscopes:

- **The bright-field microscope**. This is the common, ordinary light microscope (fig 2.3, p 24). It forms a dark image against a brighter background. The light source is either a mirror or an electric illuminator.
- The dark-field microscope. This microscope allows the viewer to observe living, unstained specimens and organisms (fig 2.6 and fig 2.7, p 26). This microscope is also used to identify certain bacteria like the thin, distinctively shaped *Treponemapallidum* (fig 2.7a, Prescott), the causative agent of syphilis.
- The phase-contrast microscope. The phase-contrast microscope is used for viewing unpigmented living cells (fig 2.8, page 31 and fig 2.9, p 27).
- The differential staining interference contrast microscope (DIC). This microscope is used to observe live, unstained specimens. The specimen appears brightly coloured

and three-dimensional (fig 2.11, p 28).

- The fluorescence microscope. Fluorescence microscopes expose a specimen to ultraviolet, violet, or blue light and form an image of the object with the resulting fluorescent light. Epifluorescence microscopy is the most commonly used fluorescence microscopy. For more information on these forms of microscopy and the use of fluorochromes in staining (fig 2.12), see your prescribed textbook.
- **Confocal microscopes.** Confocal microscopy is used to view three-dimensional objects whose image under the normal light microscope is murky and not easily distinguishable. The confocal scanning laser microscope (CSLM) uses a beam of laser to illuminate a fluorescent stained specimen. For a more detailed explanation of how this is done check figure 2.15 on page 30 in Prescott.

## 2.4.5 Activity 2.2: Light microscopy

1. Draw your own basic, labelled sketch of a light microscope. Under the sketch, in table format, list each of the main parts of the microscope and explain their functions.

2. Compile a table to show the differences between the various types of light microscopy. In the first column, put the name of every type. In the second column, briefly and simply explain how the type works. In the third column, indicate what type of specimen can be viewed with the type, or any other relevant information about the specimen.

## 2.4.6 Feedback on activity 2.2

Check your answer to the first question in Prescott. If you would like to have a clearer idea of how a light microscope is used, and you have internet access, you could always search for a short video clip about this on YouTube (<u>www.youtube.com</u>). You could use a search phrase like "how to use a light microscope". Here is one example: <u>https://www.youtube.com/watch?v=bGBgABLEV4g</u> ("Using a microscope").

Different types of light microscopes are used in microbiology to study different specimens. The table you compiled in answering question 2 should have made it clear that one should choose the correct microscope for the type of specimen (cell or organism) in order to get the best image. For instance, for live, unstained cells the differential interference contrast (DIC) microscopy is appropriate. On the other hand, scientists use CSLM to study biofilms, and here the laser beam is used to illuminate a specimen that has been stained with dye.

## 2.5 The preparation and staining of specimens

Do you know why is it necessary to fix and stain microorganisms' specimen? Although we are able to observe microorganisms directly under the light microscopes, they do need to be fixed and stained. The reason the microorganisms' specimen are fixed and stained is to enlarge the visibility of microbes, show the specific structures and preserve them for later study in the future.

## 2.5.1 Fixation

This is a process by which the internal and external structures of cells and microorganisms are preserved and fixed in position. There are two types of fixation-heat and chemical fixation. Read more about this in Prescott.

## 2.5.2 Dyes and simple staining

Basic dyes are methylene blue, basic fuchsin, crystal violet, safranin and malachite green.

Acidic dyes are eosin, rose bengal and acid fuchsin. They have negatively charged groups like-COOH groups. For more information on this, see the prescribed textbook.

## 2.5.3 Differential staining

This is used to distinguish organisms based on their staining properties. Types of differential staining are Gram staining, acid-fast staining, the Ziehl-Neelsen method, endospore, capsule and flagella staining.

Look at figure 2.18 in Prescott for steps in the Gram-staining procedure.

You could also do an internet search for pictures or video clips of the various staining methods.

## 2.5.4 Activity 2.3: Specimen preparation

1. Define basic dye and acidic dye.

- 2. Which type of fixation would you use for prokaryotes and why would you choose it?
- 3. Explain the staining procedures you would use for endospores and flagella.

## 2.5.5 Feedback on activity 2.3

In this unit you have learned about stains and dyes. You have also learned about differential staining procedures and the purpose of their use. You have to know how all these dyes and stains are used in the preparation of specimens – for example, if you want to check for the presence on endospores in a microorganism, you will have to prepare your specimen in accordance with the endospore staining procedure. The same applies to all other staining procedures.

#### 2.6 Electron microscopy

Why was the electron microscopy developed although there was the light microscopy? What are the limitations of the light microscope?

The light microscope lacks the capability to study the internal morphology of microorganisms. For a detailed explanation on this matter see Prescott, pages 38 to 43.

#### 2.6.1 General principles of electron microscopy

The working principle of electron microscopes is very similar to that of light microscopes, except that instead of light, electron microscopes use a beam of electrons to illuminate a specimen and provide a magnified image. The electron microscope uses electron lenses to control the electron beam, and to focus it to form a visible image.

A basic electron microscope is illustrated in Figure 2.26 page 38.

#### 2.6.2 Types of electron microscopes

We distinguish the following types of electron microscopes:

• The transmission electron microscope (TEM). The TEM uses a beam of electrons

instead of light. The resolution is 1 000 times greater than that of a light microscope (figs 2.20 and 2.21). See the characteristics of light and transmission electron microscopes which are compared in table 2.4.

• The scanning electron microscope (SEM). The SEM produces an image from electrons released from atoms on the object's surface. Specimen preparation for SEM is simple. Samples that have been dried are mounted and coated with a thin layer of metal. The SEM provides 3-D images of the surfaces of microscopic objects (fig 2.27).

#### 2.6.3 Activity 2.4: Electron microscopy

- 1. Compare the light microscope with the transmission electron microscope.
- 2. Describe the morphology that is studied with the scanning electron microscope.

## 2.6.4 Feedback on activity 2.4

See the comparison between the light and transmission electron microscopes in table 2.4 on page 37 in Prescott. Now that you have learned about the TEM and SEM, you will realise that the preparation of specimens for the SEM is simpler when compared to the TEM. The SEM is used to study the surfaces of microorganisms because of its high resolution (7 nm or less).

## 2.7 New types of microscopy

New types of microscopy continue to develop although the initial microscopes were developed about four centuries ago. Why do you think is there a need to develop new microscopy?

Two of the main new types of microscopy are the following:

- Scanning probe microscopy. Scanning probe microscopes measure surface features of an object by moving a sharp probe over the object's surface. The scanning tunnelling microscope was invented in 1980 and is the best example of a scanning probe microscope. Do you have an idea what its magnification is? It can magnify objects a 100 million times (see fig 2.29 in Prescott).
- Atomic force microscopy. This is another type of scanning probe microscopy. It moves a sharp probe over the specimen's surface while keeping the distance between the probe tip and the surface constant (see fig 2.30 in Prescott).

There are also other new types of microscopy. If you are interested, you can do your own information search for these by entering a simple phrase like "new types of microscopes" in an online search programme like Google.

#### 2.8 Activity 2.5: Atomic force microscope

Explain how the atomic force microscope functions.

## 2.9 Activity 2.6: Revision questions

- 1. Define the following terms:
- 1.1 Refractive index
- 1.2 Refraction
- 1.3 Focal point

- 1.4 Focal length  $(4 \times 3)$
- 2. Differentiate between the focal point and focal length. (4)
- 3. Name the parts of a light microscope and briefly explain the function of each. (15)
- 4. Define the following terms:
- 4.1 Numerical aperture
- 4.2 Resolution
- 4.3 Working distance
- 4.4 Immersion oil  $(4 \times 3)$
- 5. Differentiate between dark-field, phase-contrast, differential interference contrast and epifluorescence microscopes. (12)
- 6. Differentiate between heat fixation and chemical fixation. (4)
- Explain the procedure of Gram staining, and indicate why this procedure is performed.
   (8)
- 8. Discuss the endospore staining procedure. (6)
- 9. Compare the characteristics of a transmission electron microscope (TEM) with that of a light microscope. (18)
- 10. Describe the function and mechanism of a TEM. (5)
- 11. Describe how confocal scanning laser microscopy works. (4)
- 12. Distinguish between the scanning tunnelling microscope and the atomic force microscope. (6)

## 2.10 Reflection

Make a brief list of the following:

- What were the most interesting aspects of this unit for you?
- What were the most difficult aspects to understand?

Consider what you can do to find help with the difficult aspects. For example, you might be able to e-mail your lecturer or pose a question on the discussion forum, or consult another textbook or internet site.

Also note down what you might do to learn more about those aspects you are interested in. Now that you have completed this unit, also think about the role of technology in a science like microbiology. What is the value of technology in this science, and what might be some of the limitations that technology imposes? Also, what does this mean for us here in South Africa? Write down a few of your key ideas about this.

## 2.11 Summary

You have now learned about all the different types of microscopes. You will also have a clear picture of why dyes and stains are essential in microscopy. You have also learned about the preparation and fixation of microorganisms' specimen. This unit has provided you with the knowledge and skills that are essential in order to study microbes in detail by using different types of microscopy. In the next learning unit you will learn about the prokaryotes (bacteria and Archaea).

#### LEARNING UNIT 3

#### **Bacterial Cell Structure**

#### 3.1 Introduction

It is thought that approximately 50% of the world population is infected with the *Helicobacterium pylori*, the ulcer-causing bacterium. The affected individuals either develop duodenal or gastric ulcers. How does the *H. pylori* survive in the harsh acid environment of the stomach? Do you know the answer? *H. pylori* produces the enzyme which hydrolyses urea and produces ammonia. The ammonia further neutralises the acid. This is one way the bacterium survives in the harsh environment. There are other ways in which the bacterium survives in hostile environments, for example environments with an extreme pH, etc.

In order to understand how bacteria survive in such extreme environments we need to examine their cell structure first and understand the function each cell structure performs. As we go on in this learning unit, we will learn more about this.

## 3.2 Learning outcomes

When you have completed this learning unit, you should be able to:

- describe and identify the main structures in prokaryotic cells
- distinguish a typical bacterial cell from a typical plant or animal cell in terms of shapes, arrangements, etc.
- compare the structure of Gram-positive and Gram-negative bacterial cell walls and explain how differences between the two groups of bacteria contribute to their Gram reaction
- describe the various external structures that typically occur in prokaryotic cells (such as capsules, pili, fimbriae and flagella)

#### 3.3 Textbook reference

Study chapter 3 (Bacterial Cell Structure) in your prescribed textbook (Prescott).

Online homework and assessments are available on Connect Plus Microbiology at the following website:

www.mcgrawhillconnect.connect.com

#### 3.4 **Prokaryotes**

As we have already explained in learning unit 1, bacteria are ubiquitous in nature and can only be studied in detail with the aid of powerful microscopes. In this learning unit we will talk more about prokaryotes.

The prokaryotes are formed by bacteria. It has been shown through the biochemical, genetic and genomical analysis that bacteria and Archaea form a distinct taxonomy group (taxa).

Below I have listed a number of internet sites where you can find more information about this topic. Those of you who do not have internet access should note, however, that while this information is enriching for you, it is not compulsory reading; all the information you need to succeed in this module is in the textbook.

- A diagram that shows the prokaryotic cell: <u>http://static.newworldencyclopedia.org/thumb/9/99/Prokaryote\_cell\_diagram.svg/320px-</u> <u>Prokaryote\_cell\_diagram.svg.png</u>
- Information on prokaryotic cells: <u>http://biology.about.com/od/cellanatomy/ss/prokaryotes.htm</u>

These links are not compulsory but for your personal enrichment. Most of the information is readily available in the prescribed textbook.

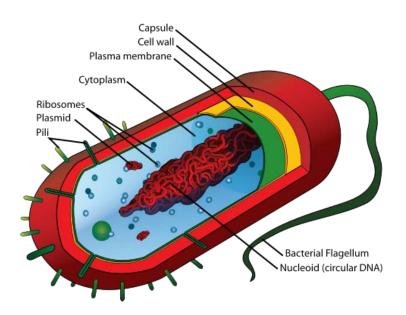


Figure 3.1. Example of a prokaryotic cell, a bacterium

(Source: <a href="http://commons.wikimedia.org/wiki/File:Average\_prokaryote\_cell-\_en.svg">http://commons.wikimedia.org/wiki/File:Average\_prokaryote\_cell-\_en.svg</a>)

#### 3.5 A typical bacterial cell

Bacterial cell morphology has a variety of shapes and the common ones are the cocci and rods.

The cells can exist alone (singly) or in a particular arrangement that can be very helpful in their identification. The arrangements are **diplococci** (singular- coccus) which is formed when cocci divide and remain together to form pairs. The long chains of cocci are formed when cells stick together after repeated divisions in one plane. Genera such as *Enterobacter*, *Streptococcus* and *Lactococcus* show this pattern.

Rods, also known as **bacilli** (singular-bacillus), differ in their length-to-width ratio. *Bacillus megaterium* is formed by long chains.

Other shapes are comma-shaped vibrios; rigid, spiral-shaped spirilla; flexible, spiral-shaped spirochetes. Pleomorphic bacteria have variable shapes and do not have a single characteristic form.

Look at the pictures of these shapes and arrangements of cells in Prescott.

#### 3.6 Activity 3.1: Structure of bacteria

After studying the relevant sections in Prescott, answer the following questions:

- 1. What characteristic shape can bacteria assume? Describe the ways in which the bacterial cells cluster together.
- 2. What advantages might a bacterial species have that forms clusters or chains that unicellular bacteria do not have?
- 3. What is the function of a gas vacuole?
- 4. With regard to the statement below, name the structures that are involved and explain why you have chosen them.

"They provide resistance to phagocytosis, adherence to surfaces; rarely found in the Archaea."

5. Discuss the role of periplasmic space with regard to both gram-negative and grampositive bacteria.

#### 3.7 Feedback on activity 3.1

Bacteria and Archaea lack many membrane-delimited organelles that are found in eukaryotic cells. The two groups of prokaryotes share similar features such as nucleoid, cell wall, gas vacuoles, flagella etc.

With the help of your textbook, you should attempt to answer the first two questions yourself. Regarding the third question, gas vacuoles are responsible for floating in water environments.

The structures that provide resistance to phagocytosis and adherences to surfaces are capsules and slime layers.

The periplasmic space of gram-negative bacteria consists of hydrolytic enzymes and binding proteins which are responsible for nutrient processing and uptake.

#### 3.8 Bacterial cell envelope

Bacterial cell envelope consists of a plasma membrane and all the external parts that cover it. It thus consists of the plasma membrane, cell wall and one additional layer. Refer to the image of a prokaryotic cell above to see these parts.

#### 3.8.1 Plasma membrane

The plasma membrane covers the cytoplasm of all cells. The plasma membrane of the bacteria and Archaea are crucial because they play important roles such as in the transport systems used for the uptake of nutrients.

For more detailed information on the plasma membrane you may want to consult link below:

http://micro.magnet.fsu.edu/cells/plasmamembrane/plasmamembrane.html

#### 3.8.2 Bacterial cell wall

The cell wall is found on the external part of the plasma membrane and forms a rigid part of

the cell that is essential for the following functions:

- Determines the shape of the cell
- Protects the contents of the cell from osmotic lysis
- Protects the cell from harsh substances (toxic)
- Plays a role in the pathogenicity of organisms

Optional further internet reading:

- http://www.fastbleep.com/biology-notes/35/112/654
- <u>https://www.boundless.com/microbiology/bacteria-archaea-and-eukaryote-cell-structure/cell-walls-of-prokaryotes/the-cell-wall-of-bacteria/</u>

## 3.9 Activity 3.2: Structure and functions of the bacterial cell envelope

- 1. Discuss the functions of bacterial and archaeal plasma membranes.
- 2. Discuss the function of the cell wall.
- 3. Describe the composition and structure of peptidoglycan.

## 3.10 Feedback on activity 3.2

Plasma membranes act as semipermeable barriers, they are responsible for respiration.

The role of plasma membrane is detecting and responding to chemical stimuli in the environment.

The cell wall is responsible for structural support, gives shape to the cell and protects the cell from osmotic lysis (osmotic stress). You can find more information here:

http://wiki.answers.com/Q/What\_is\_the\_primary\_function\_of\_a\_cell\_wall#slide=3

Consult Prescott to check your answer regarding petidoglycan.

#### 3.11 Cytoplasm of bacteria and Archaea

The cytoplasm is the internal part of the cell that is surrounded by the plasma membrane.

Structures such as inclusions, ribosomes, the nucleoid, and plasmids are found inside the cytoplasm.

Optional further internet reading:

- <u>http://micro.magnet.fsu.edu/cells/bacteriacell.html</u>
- http://samples.jbpub.com/9781449635978/05940\_PDFx\_CH04\_Pommerville.pdf

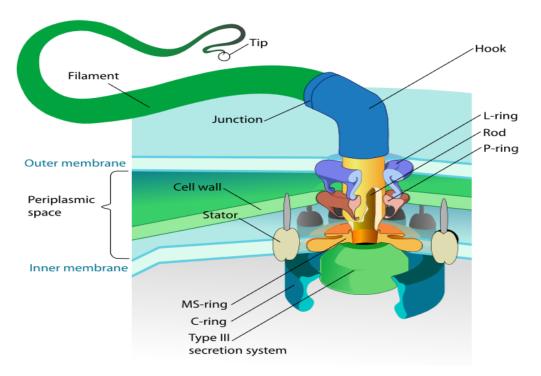
## 3.12 External structures

The external structures of bacteria and Archaea extend beyond the cell envelope (plasma membrane and cell wall). What do you think is the purpose of these external structures?

These external structures are pili, fimbriae and flagella.

You have probably realised that the role of the external structures is protection, attachment to surfaces, horizontal gene transfer and cell motility.

The diagram below shows the structure of a flagellum.



## Figure 3.2 Bacterial flagellum

#### Source:

http://upload.wikimedia.org/wikipedia/commons/1/15/Flagellum\_base\_diagram\_en.svg

Optional further internet reading:

- <u>http://www.microbiologytext.com/index.php?module=Book&func=displayarticle&art\_id=66</u>
- https://www.princeton.edu/~achaney/tmve/wiki100k/docs/Flagellum.html

## 3.13 Motility and chemotaxis

In section 3.7 above we learned about external structures outside the cell that contribute to motility.

Four ways of movement have been observed in bacteria:

- The swimming movement through flagella
- The cockrscrew movement through spirochetes
- The twiching movement assocoaited with type IV pili
- Gliding motility

Optional further internet reading:

- http://iai.asm.org/content/72/8/4905.full
- http://onlinelibrary.wiley.com/doi/10.1046/j.1365-2958.1998.01061.x/pdf

#### 3.14 Bacterial endospore

Why do you think are some bacteria able to withsatand harsh, unbearable environments?

Several genera of gram-positive bacteria are able to form resistant dormant structures that are able to withstand unfavourable environments such as lack of nutients, heat, ultra violet radiation, etc. These structures are known as endospores.

Optional further internet reading:

- https://micro.cornell.edu/research/epulopiscium/bacterial-endospores
- <u>http://www.tutorvista.com/content/biology/biology-iii/kingdoms-living-world/reproduction-bacteria.php#endospore-formation</u>

## 3.15 Activity 3.3: External structures

- Suppose you are working as a laboratory assistant and and you Gram-stain two samples of bacteria. One sample shows up purple under the microscope, and one pink. Explain what this is called and what causes this with reference to bacterial cellular structure. What are the implications if some bacteria have a tough endospore for the transmission/control of certain diseases?
- 2. Distinguish briefly between fimbriae and pili, and the function of each.
- **3.** Explain in a general way how bacteria move towards substances such as nutrients and away from toxic material or harsh environments.

## 3.16 Reflection

Think about what you learned in working through unit 3 and note down any insights that you may have gained. What are some of the implications of the particular structure of bacteria for their survival – and our survival? Is this a field of research that you might be interested in pursuing? Write down a few key points to express your thoughts on these questions.

#### 3.17 Summary

This learning unit highlighted the structure of prokaryotic cells. We often ask ourselves why microorganisms are resistant to some antibiotics and disinfectants and keep on thriving. It is because the cell envelopes of some bacteria have protection that does not allow the passage of substances to the cell. Think of the peptidoglycan in Gram-positive as well as in Gram-negative cells. We also learned about the role of pili, fimbriae, flagella and endospores.

## **LEARNING UNIT 4**

## **Eukaryotic Cell Structure and Function**

#### 4.1 Introduction

In learning unit 3 we learned about prokaryotes (bacteria and Archaea). Besides the prokaryotic microbes, there are eukaryotic microbes which can be divided into two groups, namely protists and fungi.

The broad class of eurkaryotes includes most life forms that we are familiar with, including ourselves. In this module we are going to concentrate only on eukaryotic microbes.

For more information in this regard, you can visit the following websites:

http://www.ucmp.berkeley.edu/alllife/eukaryotamm.html

http://evolution.berkeley.edu/evolibrary/article/ 0/endosymbiosis 03

In this unit we will first look at the general characeristics of eukaryotic cells, and then we will examine protists and fungi in more detail.

#### 4.2 Learning outcomes

When you have completed this learning unit, you should be able to:

- describe the main structures in the eukaryotic cell
- discuss the various elements of the cytoskeleton in terms of structures and function in the cell
- discuss the composition, structure and function of each of the internal organelles (such as the endoplasmic reticulum, Golgi apparatus, lysosomes, ribosomes, mitochondria, chloroplasts, nucleus and nucleolus)
- discuss the mechanism of endocytosis and the differences between phagocytosis and pinocytosis
- compare and contrast prokaryotes and eukaryotic cells

#### 4.3 Textbook reference

Study chapter 4 (Eukaryotic Cell Structure and Function) in Prescott.

You can also consult additional resources by visiting the McGraw-Hill Online Learning Center at the following website:

http://highered.mcgraw-hill.com/sites/0073375268/information\_center\_view0/.

#### 4.4 Common features of eukaryotic cells

Consult the following websites for the structure of the eukaryotic cell:

http://commons.wikimedia.org/wiki/File:Eukaryotic\_Cell\_(animal).jpg

http://en.wikipedia.org/wiki/Eukaryotic\_cell

Eukaryotic cells have membrane-bound nuclei. They consist of organelles such as

ribosomes, mitochondrion, endoplasmic reticulum, nucleus, etc. Have you noticed the differences between the eukaryotic and prokaryotic cells?

Eukaryotic cells also have a cell envelope. The cell envelope of eukaryotic cells consists of the plasma membrane and all coverings external to it.

Most eukaryotic microbes do not have a cell wall.

Optional further internet reading:

http://www.ck12.org/book/CK-12-Biology/section/14.1/

## 4.5 Activity 4.1: Structure of eukaryotic cells

- 1. What is an organelle? How are organelles similar to organs of a multicellular organism?
- 2. What advantages does compartmentalisation have for eukaryotic cells?

## 4.6 Feedback on activity 4.1

As we have explained earlier, eukaryotic microbes consist of organelles that have distinct functions. They are intracellular structures that do specific tasks in cells similar to the functions of organs in the body of a multicellular organisms.

The advantages of compartmentalisation are discussed in Prescott, pages 94 to 95.

## 4.7 Overview of protist structure and function

These animal-like microbes are also known protozoa. The unicellular microbes are chemoorganotrophs, organisms that use organic compounds as sources of energy, electrons, and carbon for biosynthesis. Other chemoorganotrophs are the slime molds and the water molds.

Beside the chemoorganotrophs there are photosynthetic organisms such as algae.

Optional further internet reading:

- <u>http://www.biology.ualberta.ca/parasites/ParPub/text/text/proto01b.htm</u>
- Some valuable information on protozoa: <u>http://www.webpages.uidaho.edu/bionet/biol116/o2/presentations/t3l3\_early\_eukaryotes</u> .pdf

#### 4.8 Overview of fungal structure and function

The vegetative structure of a fungus is known as a thallus.

A **yeast** is a unicellular fungus. It has a single nucleus and its mode of reproduction is either sexually or by budding and transverse divisions or asexually by forming spores.

How does a fungus reproduce? You can read more about this in your textbook. For more information you can also read the links mentioned above and those mentioned below.

https://www.boundless.com/biology/fungi/characteristics-of-fungi/reproduction/

http://mb0804mycology.wordpress.com/2008/07/29/reproduction-of-fungi/

## 4.9 Activity 4.2: Fungal structure and reproduction

Describe each of the following types of asexual fungal spores: sporangiospore, arthrospore, conidiospore, and blastospore.

#### 4.10 Feedback on activity 4.2

A fungus can range from a single cell microscopic yeast to multicellular molds, macroscopic puffballs, and mushrooms. Are you aware that the mushrooms we eat are a type of fungus?

The formation of asexual spores is a result of dispersal. These asexual spores differ in the way they are formed and they thus have different names such as sporangiospore, arthrospore, conidiospore, and blastospore. You can often tell more about the meaning of a word by understanding where the various parts of a word come from. For example, you could look up the word parts "arthro-", "blasto-" "conidio-" and so forth in a dictionary or on the internet. This will help you to make more sense of these technical terms.

## 4.11 Activity 4.3: Discussion on the role of protists and fungi

Please go to the Discussions tool on the website for this module and then answer the following question. (If you do not have internet access, please answer the question on your own in writing.)

What are some of the positive and negative effects of protists and fungi for animals and humans?

Post your own answer and also try to respond to at least one other student's post.

#### 4.12 Feedback

The discussion in the Discussions tool will serve as feedback for this question. For those of you who do not have regular internet access, I will communicate some main points that emerged from the discussion later in the semester.

#### 4.13 Activity 4.3: Revision questions

- 1. Discuss the roles of the following organelles:
- 1.1 Chloroplasts
- 1.2 Lysosomes
- 1.3 Cell wall
- 2. How do RER and SER differ from one another in terms of structure and function?

## 4.14 Feedback

Each and every organelle that is found in the eukaryotic microbe functions in the same way as in eukaryotic cells of plants and animals. For example, chloroplasts in plant cells are responsible for photosynthesis, and the cell wall protects and gives shape to the cell.

# 4.15 Summary

In this learning unit you have learned about the eukaryotic microbes such as protists and fungi.

You have learned that eukaryotic cells have a complex structure compared to prokaryotic cells.

Eukaryotic cells have organelles such the mitochondrion, endoplasmic reticulum, Golgi apparatus, lysosomes, etc. The nucleus of the eukaryotic cell is membrane bound. The cell envelope of many eukaryotic microbes lacks a cell wall. The protists are divided into two groups: protozoa and algae. The protozoa are chemoorganotrophs, i.e. they use organic molecules as energy sources.

The algae are photosynthetic organisms.

With regard to fungi, they vary in size and complexity, starting from a single-cell microscopic yeast to a multicellular mold, macroscopic puffballs, and mushrooms.

Reproduction in fungi can be asexual or sexual.

# **LEARNING UNIT 5**

## **Microbial Nutrition**

## 5.1 Introduction

A newborn infant must consume food immediately after birth. In the same way newborn microbes need food too. The microbe needs food to survive, thrive and reproduce. As we all know food is a source of energy.

In this unit we will explore how microbes feed in order to grow and reproduce.

You can obtain more information about this topic by visiting the following website:

http://bugs.bio.usyd.edu.au/learning/resources/CAL/Microconcepts/Nutrition/introduction. html

## 5.2 Learning outcomes

When you have completed this learning unit, you should be able to:

- explain the meaning of appropriate terms
- identify the elements which microorganisms require in large quantities (macronutrients) and the elements they require in trace quantities (micronutrients)
- describe and compare the various processes by which microorganisms take up nutrients from the environment (passive and facilitated diffusion, active transport, group translocation)
- describe the various culture media for microorganisms and indicate their use in the study of microorganisms
- describe the techniques used to obtain pure cultures

## 5.3 Additional material

You can also consult additional resources for this learning unit by visiting the McGraw-Hill Online Learning Center at the following website:

http://highered.mcgraw-hill.com/sites/0073375268/information\_center\_view0/.

## 5.4 Elements of life

What are the ten **macroelements (macronutrients)** that are required in large quantities by the microbes?

The macroelements are carbon, oxygen, hydrogen, nitrogen, sulphur, and phosphorus which are found in organic molecules such as lipids, proteins, carbohydrates, and nucleic acids. Other macroelements are potassium, calcium, iron and magnesium and they exist as cations. They are associated with and contribute to the activity and stabilisation of molecules and cell structures.

Beside macronutrients and micronutrients there are **growth factors** that are needed by microorganisms. Metabolically limited microorganisms require specific growth factors to support growth. These are usually vitamins such as lipoic acid, etc.

Further optional internet reading:

http://textbookofbacteriology.net/nutgro.html

## 5.5 Activity 5.1: Elements of life

After studying the relevant sections in Prescott, answer the following questions:

- 1. What are nutrients? On what basis are nutrients divided into macronutrients and micronutrients (trace elements)?
- 2. Describe some of the ways in which a microorganisms utilise micro- and macroelements.
- 3. Discuss the classification of microorganisms according to their energy and electron requirements.

## 5.6 Feedback on activity 5.1

Nutrients are materials that are used in **energy conservation** and **biosynthesis** by microorganisms.

Nutrients that are needed in large quantities are known as macronutrients (macroelements), and those that are needed in small quantities are known as micronutrients (microelements/trace elements).

Carbon, a macroelement, is needed to synthesise the organic molecules from which organisms are built. Hydrogen and oxygen are also essential elements found in many organic molecules such as proteins, carbohydrates, lipids and nucleic acids.

Microelements such as manganese, zinc, cobalt, molybdenum, nickel, and copper are needed by most cells as part of certain enzymes and co-factors in the catalysation of reactions and maintenance of protein structure.

For more information on the classification of microorganisms according to their energy and electron requirements refer to Prescott, table 11.2 on page 231.

#### 5.7 Uptake of nutrients

The microbial cell's first step in nutrient use is its uptake of it. Microorganisms use different mechanisms for nutrient uptake. The nutrient uptake by microbes is by means of

- 1. passive diffusion
- 2. facilitated diffusion
- 3. active transport
- 4. group translocation
- 5. iron uptake

For more information on the nutrient uptake by microorganisms see Prescott.

Further optional internet reading: http://biology.about.com/od/cellularprocesses/ss/diffusion\_2.htm

http://www.textbookofbacteriology.net/structure\_8.html

## 5.8 Culture media and isolation of pure cultures

Do you know how microbes are prepared, transported and grown? A **culture medium** is used for the preparation, growth, transport and storage of microbes.

Different media are used for cultivation, isolation and identification (i.e. three different types of microorganisms). If you want to isolate or identify a specific group of microorganism you will use an **enrichment media** which will promote the required species of microorganisms. Think of how *Streptococcus pyogenes* is isolated and identified (see Prescott, table 7.7 on page 156). Cultured media can be prepared completely from chemically-defined constituents such as peptones and yeast extract.

Further optional internet reading:

http://i1.ytimg.com/vi/RuRgCRASzpo/hqdefault.jpg

http://www.microbiologyonline.org.uk/teachers/preparation-of-media-and-cultures

The links below have information on the isolation of pure culture techniques:

http://upendrats.blogspot.com/2010/02/microbial-pure-culture.html

http://delrio.dcccd.edu/jreynolds/microbiology/2420/files/pure%20cultures.pdf

http://amrita.vlab.co.in/?sub=3&brch=73&sim=213&cnt=1

## 5.9 Activity 5.2: Nutrient uptake and culturing techniques

- 1. Describe the facilitated diffusion, active transport and group translocation in terms of their distinguishable properties and mechanisms of action. Write your answer in tabular form.
- 2. What is a pure culture and why it is important?
- 3. How are spread plates, streak plates and pour plates prepared?

#### 5.10 Feedback on activity 5.2

For the uptake of nutrients to be a success, microbes use different methods such as passive diffusion or facilitated diffusion. The movement of nutrients and substances in and out of the bacterial cell is controlled by the bacterial plasma membrane. In passive diffusion, substances move down the gradient (from a higher concentration gradient to a lower concentration gradient), and does not require energy. Passive diffusion only allows few substances to enter the cells.

With regard to facilitated diffusion, transport proteins assist to move substances in the direction of decreasing concentration and metabolic energy is needed.

# 5.11 Activity 5.3: Revision questions

- 1. Describe active transport and group translocation in terms of their distinctive characteristics and mechanisms.
- A microbe is placed in an environment where oxygen is in abundance, temperature is above 100°C and the pH is below 3. Will the microbe survive? Give reasons for your answer.

## 5.12 Feedback on activity 5.3

Active transport is divided into two types, namely primary active transport and secondary active transport. Primary active transport involves primary carriers called active transporters which use the energy provided by ATP hydrolysis to move substances against a concentration gradient without modification. Primary active transporters are known as uniporters (move single molecule across the membrane).

Secondary active transport connects the potential energy of an ion gradient to move substances without modifying them. They are called **cotransporters**.

The movement of ions or other substances both move in the same direction, which is known as **symport**. **Antiport** occurs when they move in opposite directions.

#### 5.13 Reflection

1. Think about what you have learned in working through this unit and note down the five insights you gained that were the most interesting or newest to you. Has the way you think about microorganisms changed in any way? If so, explain how.

2. Consider the following statement: "Learning how to isolate microbes has been an enormous step forward in medicine, and may be the reason many of us are still alive and healthy today".

In your own words explain why this is so.

## 5.14 Summary

In this learning unit you have learned about the microbial nutrition and culture media.

Microbes need both macro- and micronutrients for energy conservation and biosynthesis.

There are ten macronutrients (macroelements) that are needed in large quantities by microbes.

In addition to the macronutrients there are other nutrients that are also essential but are needed in small quantities (microelements/trace elements) by microorganisms.

In addition to macronutrients and micronutrients there are also growth factors that are needed by microorganisms. Metabolically limited microorganisms require specific growth factors to support growth.

With regard to the nutrient uptake, there are various mechanisms which microbes use to uptake nutrients. These are facilitated diffusion, active transport, etc.

When dealing with culture media it is important to know which type of media and pure culture technique to choose when isolating and identifying the specific microorganisms.

## **LEARNING UNIT 6**

## **Microbial Growth**

### 6.1 Introduction

In learning unit 5 we learned about microbial nutrition and how microbes use nutrients from the environment. In this learning unit we will discuss how other factors can affect the growth and reproduction of microbes. These factors are temperature, pH, oxygen concentration, etc.

#### 6.2 Learning outcomes

When you have completed this learning unit, you should be able to:

- explain the meanings of appropriate terms
- describe the various growth phases in a closed system and describe what happens during each phase
- describe the various types of continuous culture systems and explain their applications
- differentiate and categorise microorganisms in terms of the environmental factors that promote optimal growth of the organisms

## 6.3 Textbook reference

Study chapter 7 (Microbial Growth) in your prescribed textbook.

You can also consult additional resources for this learning unit by visiting the McGraw-Hill Online Learning Center at the following website:

http://highered.mcgraw-hill.com/sites/0073375268/information\_center\_view0/.

#### 6.4 Growth curve and closed system

When microbes are cultured in broth they bare normally gown in a **batch culture**; that is they are grown and incubated in a closed culture with a single batch of medium. During incubation there is no fresh medium is provided and this lead to the decline in concentration of nutrients and the increase in concentrations of wastes. Microbial growth refers to an increase in cellular constituents. It also leads to growth in length and in size usually coupled with cell division. Growth also involves the increase in the size of a population. The growth curve consists of four phases: the lag phase, exponential (log) phase, stationary phase and death phase. Check Figure 7.27 page 161 for the diagram of the Microbial Growth Curve in a Closed System.

Further optional internet reading:

http://microbiology.okstate.edu/faculty/demed2/Notes/Microbial%20growthdoc.html

http://textbookofbacteriology.net/growth\_3.html

## 6.5 Continuous culture of microorganisms

The **closed systems** are also known as batch cultures which are discussed in section 6.4

In closed systems the nutrients are limited and growth eventually ceases, (added when

finished) and wastes are not removed. In the closed system the nutrients are soon depleted and there is an increase in the concentration of waste products.

The closed system has **four phases**. When microorganisms reach the stationary phase growth ceases.

However, it is possible to grow microorganisms continuously in a system where environmental conditions are established that maintain the growth of microorganisms whereby nutrients are continually provided and the wastes are also removed on a regular basis. This system is known as a **continuous culture system**.

There are two types of continuous systems, namely the **chemostats** and the **turbidostats**. You can read more about these in Prescott on pages 160 to 162 and 168 to 169.

#### 6.5 Activity 6.1: Growth curve

- 1. Define growth.
- 2. Discuss the four phases of microbial growth in a closed system.
- 3. Compare and contrast the closed system to a continuous system.

#### 6.6 Feedback on activity 6.1

Growth in microorganisms entails a lot of things such as an increase in the number cells through reproduction and the formation of biofilm.

With regard to the lag phase, this occurs when microorganisms are initially introduced into the new culture medium. There is no immediate increase in the cell numbers. During the lag phase the microbial cells begin to synthesise new components that are essential for their growth.

#### 6.7 Influence of environmental factors on growth

Microorganisms are affected by the chemical and physical nature of their environment (surroundings). The environmental factors influence microbial growth. By understanding environmental factors and their effect on microorganisms, we can control microbial growth and thus prevent the unwanted growth of microbes in foods, kitchens, bathrooms, etc. The environmental factors are

- 1. solutes and water activity
- 2. pH
- 3. temperature
- 4. oxygen concentration
- 5. pressure
- 6. radiation

Study these in detail in Prescott.

Further optional internet reading:

http://upendrats.blogspot.com/2010/01/factors-affecting-microbial-growth.html http://www2.hawaii.edu/~johnb/micro/m140/syllabus/week/handouts/m140.9.1.html http://www.mona.uwi.edu/biochem/courses/bb10b/documents/lecture06.pdf

## 6.7 Activity 6.2: Environmental factors

- 1. What are cardinal temperatures?
- 2. Define psychrophile, psychrotroph, mesophile, thermophile, and hyperthermophile.
- 3. Why is oxygen toxic to some microorganisms? How do these microorganisms protect themselves against the harmful effect of oxygen?
- 4. What are barotolerant and piezophilic bacteria? Where would you expect to find them?

## 6.8 Feedback on activity 6.2

Psychrophile is an organism that grows at a temperature of between 0 °C and 15 °C. Examples of pyschrophile microorganisms are *Bacillus pyschrophilus* and *Chlamidomonas nivalis*.

Psychrotrophs grow at temperatures of between 0 and 7 °C. Psychrophiles, on the other hand, grow at an optimum temperature between 20 and 30 °C with a maximum close to 35 °C. Examples of pyshrotrophs are *Listeria monocytogenes* and *Pseudomonas fluorescens* 

Mesophiles grow at an optimum temperature between 20 and 45 °C. Examples of mesophiles are Escherichia coli and Thrichomonas vaginalis.

#### 6.9 Microbial growth in natural environments

Nutrients and environmental factors affect the growth of microorganisms in their habitat at any particular time. If microbes grow at a fast rate where nutrients are abundant, they soon deplete them and this results in the release of toxic substances which will limit further growth. In the food industry this can lead to a loss of income because food is spoilt due to food poisoning by microorganisms. Food that has undergone spoilage can no longer be consumed and needs to be removed from the shelves.

**Biofilms** are complex slime-encased microbes that are found attached to surfaces, for example in water or inside the gut. Read more about this in Prescott, pages 150-151.

Further optional internet reading:

http://bacteriality.com/2008/05/26/biofilm/

http://www.coe.montana.edu/biofilmbook/MODULE\_01/Mod01\_Grn/Mod01\_S02\_Grn.htm

http://mpkb.org/home/pathogenesis/microbiota/biofilm

## 6.10 Activity 6.3: Revision questions

- 1. What is the advantage of biofilms for microbes? Name two.
- 2. What medical challenges are posed by biofilms?

## 6.11 Feedback on activity 6.3

Biofilms are complex, slime-encased communities of microbes growing on surfaces such as in metal tongue studs (stainless steel and titanium), hulls of ships, medical devices, food-handling facilities, kitchen counters, hospital rooms, hospital equipment, etc.

It is important to understand how microbes produce in order to determine the environmental factors that are conducive to their growth or to eradicate their growth. It is also important to know and understand how biofilms differ from ordinary cells (planktonic cells) of the same species.

## 6.12 Reflection

With the knowledge you gained in this unit, think about your attitude to bacterial infections. You should be aware of how microorganisms may potentially infect other organisms, and how to handle potential microbe infested places. Presumably your attitude will have changed on how to handle food or store it in such a way that you do not promote the growth of various types of microbes. Write down two or three thoughts about this.

## 6.13 Summary

In this module you have learned about microbial growth and how it is affected by nutrition and other environmental factors. You have learned about the batch culture (closed system) and its four phases, namely the lag phase, exponential, stationary and death phase. In the closed system there is no addition of nutrients or removal of wastes. In the continuous system nutrients are continually added and the wastes are removed.

In addition to nutrients there are other factors that affect the growth and distribution of microbes such as pH, temperature, etc. By knowing what these factors are you can control the growth of microorganisms. In the next learning unit you will learn about the physical and chemical control of microorganisms. We can prevent the spread of diseases if we know how to prevent the growth and spread of microbes.

## **LEARNING UNIT 7**

## **Control of Microorganisms in the Environment**

#### 7.1 Introduction

In learning unit 6 we learned about microbial growth. In this learning unit we will learn about how microorganisms that are pathogenic can be **controlled** and be **prevented** from spreading further. Did you know that microbes can be found in water supplies, bathrooms or even soaps used for hand washing? You may have asked yourself why we should always wash our hands with soap before touching food. The culprit microbe that is found hiding in water supplies is *Pseudomonas aeruginosa*. *Paeruginosa* can infect humans and is resistant to conventional antibiotics.

The control of microorganisms is a very hot topic as there are super bugs that are becoming resistant to most antibiotics.

In this learning unit we will explore mechanisms that are applied to control microorganisms. We will look at agents that control microorganisms such as physical, chemical and biological agents. These are also known as antimicrobial agents.

You can read more about microbial control on the following websites:

- <u>http://www.elmhurst.edu/~bio/mittermeyer/BIO216/control.html</u>
- http://microbiology.mtsinai.on.ca/fag/cleaning.shtml

## 7.2 Learning outcomes

When you have completed this learning unit, you should be able to:

- explain the meanings of appropriate terms
- compare and contrast the actions of disinfection, antisepsis, chemotherapy, and sterilisation
- distinguish between cidal (killing) and static (inhibitory) agents
- explain the mechanism by which filtration removes microorganisms
- describe the application of heat and radiation to control microorganisms
- describe the use of phenolics, alcohols, halogens, heavy metals, aldehydes, quartenary ammonium chlorides, and oxides
- select an appropriate method for controlling a certain microorganism in a situation of microbial growth

## 7.3 Textbook reference

Study chapter 8 (Microbial Growth) in your prescribed textbook.

You can also consult additional resources by visiting the McGraw-Hill Online Learning Center at the following website:

http://highered.mcgraw-hill.com/sites/0073375268/information\_center\_view0/.

## 7.4 Principles of microbial control

Microbial control is deeply rooted in microbial nutrition, growth, and development. If the **growth is prevented or inhibited**, or **replication is prevented** one can control microorganisms. Figure 8.1 in Prescott shows microbial methods.

What do you know about the following terms: sterilisation, disinfection, sanitisation, antiseptics, chemotherapy?

These terms all explain the various methods of inhibiting, killing or removing microorganisms. Study the terminology in Prescott.

Further optional internet reading:

http://quizlet.com/2512010/basic-principles-of-microbial-control-flash-cards/

http://faculty.taftcollege.edu/dsheehy/includes/courses/Microbiology8/documents/micro% 20ch7DCS.pdf

## 7.5 Activity 7.1: Principles of microbial control

- 1. What is the difference between bactericidal and bacteriostatic?
- 2. To which category do you think do most household cleaners belong? Why?
- **3.** Compile a table in which you include the following terms and an explanation of each: disinfection, antisepsis, chemotherapy, and sterilisation. Once you have completed your table, also write a short paragraph in which you summarise the similarities and differences between these methods.

#### 7.6 Feedback on activity 7.1

The difference between bacteriocidal and bacteriostatic substances is that bacteriocidal types of substances kill bacteria, whereas bacteriostatic substances merely inhibit them.

See Prescott, pages 173 to 174, for the differences between disinfection, antisepsis, chemotherapy and sterilisation.

#### 7.7 Mechanical removal methods

What is mechanical removal of microorganisms? Mechanical removal involves the use of apparatus such as **depth filters** and **membrane filters** in the filtration process of removing microorganisms. The filter does not directly destroy contaminating microorganisms but simply **removes** them.

See **figure 8.4** in Prescott for the membrane filter sterilisation.

#### 7.8 Physical control methods

Heat is one of the most commonly used physical methods to control microorganisms. Recently (May 2014) in Bloemhof, a town in North-West province in South Africa, the tap water was contaminated when there was a raw sewage spill into the dam that supplies water to the town. After drinking the contaminated water, the population in Bloemhof suffered from diarrhoea. After testing the water, it was found that enteropathogenic microorganisms (*Shigella*) that are usually found in sewage were the cause of the

diarrhoea outbreak. The people were advised to boil the water before using it.

As suggested by the example above, heat was used to control the microorganisms - a method that is easy to use and affordable.

The most common method of heat that is used to control microorganisms is **moist heat**. Moist heat is used to destroy viruses, bacteria, and fungi. Moist heat is also used to destroy endospores at temperatures above 100 °C and the steam under pressure is used to do this. A device known as an **autoclave** is used to carry out moist heat sterilisation.

See table 8.2 in Prescott on the moist heat sterilisation of different microorganisms.

Besides heat, the other physical control mechanism to deal with microorganisms is radiation. The most effective type of radiation is **ionising radiation** which penetrates deep into objects. For more detail on how ionising radiation works refer to Prescott.

Further optional internet reading:

http://www.hccfl.edu/media/574949/15-control%20of%20microorganisms.pdf

## 7.9 Activity 7.2: Mechanical and physical control methods

- 1. Explain the mechanism by which filtration removes microorganisms.
- 2. Describe how heat and radiation can be applied to control microorganisms.
- 3. Give one example each of a situation in which filtration, heat and radiation would be suitable control methods for microorganisms.

#### 7.10 Feedback on activity 7.2

You can find more information on the answers to questions 1 and 2 in Prescott, pages 175 to 179.

Regarding question 3, there are a variety of possible correct answers. Here are three simple scenarios that I thought of:

- Water that is obtained from the soil and still contains some soil particles with possible accompanying soil bacteria can be filtered.
- River water can be boiled to make it suitable for drinking.
- Fruit or vegetables that may contain harmful microorganisms can be irradiated. This would kill the organisms without causing any noticeable damage to the fruit and vegetables.

## 7.11 Chemical control agents

You have now learned about mechanical and physical control. These physical agents are mostly used to sterilise objects. When are chemical agents used to control microorganisms?

Chemicals are used in disinfection and antisepsis. To get more detailed information on this see Prescott.

The main chemicals that are used are the following: phenolics, alcohols, halogens, heavy

metals, quaternary ammonium compounds, aldehydes and sterilising gases.

## 7.12 Activity 7.3: Chemical control agents

- 1. Why are most antimicrobial chemical agents disinfectants rather than sterilants? What general characteristics should one look for in a disinfectant?
- 2. Compile a table in which you include the following substances and summarise the use of each: phenolics, alcohols, halogens, heavy metals, aldehydes, quaternary ammonium chlorides, and oxides.
- 3. Which disinfectants or antiseptics would you use to treat a patch of skin before surgery, and for small medical instruments (probes, forceps, etc.)? Explain why.

#### 7.13 Feedback on activity 7.3

Chemical agents are essentially used in laboratories and for hospital safety. Some chemicals are used to prevent microbial growth in food, and certain other chemicals are used to treat infectious diseases. When the chemicals are used as disinfectants they must be effective against a wide range of infectious microorganisms (Gram-positive as well as Gram-negative bacteria, acid fast bacteria, bacterial spores, fungi and viruses) at a very low concentration and in the presence of organic matter. There should be a balance between the toxicity of the chemical against infectious microbes on the one hand, and the safety of the agent to people or corrosive effect on common materials on the other hand. More detailed information can be found in Prescott, pages 180 to183.

For more information, look at the following link:

http://classes.midlandstech.edu/carterp/Courses/bio225/chap07/lecture5.htm

### 7.14 Evaluation of antimicrobial agent effectiveness

The assessment of antimicrobial agent effectiveness is a complex process controlled by two different US federal agencies, namely the Environmental Protection Agency (EPA), which regulates disinfectants; and agents used on humans and animals are under the control of the Food and Drug Administration (FDA). In South Africa the **Medicines Control Council** performs the task.

The destruction and inhibition of microbial growth is affected by six factors, namely:

- 1. Population size
- 2. Population composition
- 3. Concentration or intensity of an antimicrobial agent
- 4. Contact time
- 5. Temperature
- 6. Local environment

For more detailed information on the above six factors see Prescott, page 185.

## 7.15 Biological control

This is a relatively new field in the control of microorganisms that involves natural control processes such as predation of one microorganism on another, viral-mediated lysis, and toxin-mediated killing.

## 7.16 Activity 7.4: Discussion on selection of control method

Please go to the Discussions tool on the website for this module and then answer the question below. (If you do not have internet access, answer the question on your own in writing.)

A very large population of *Pseudomonas aeruginosa* is growing inside a pipe system that supplies water to a large village. It is summer and it is hot and moist. In this situation, would you recommend the use of quaternary ammonium chloride as a disinfectant in the pipe system? Give reasons for your answer.

Post your answer on the discussion forum and also try to respond to at least one other student's posting.

## 7.17 Feedback on activity 7.4

The discussion in the Discussions tool will serve as feedback to this question. For those of you who do not have regular internet access, I will communicate some main points that emerged from the discussion later in the semester.

#### 7.18 Activity 7.5: Revision questions

- 1. Give an example to illustrate how each of the six factors listed in 7.14 above may affect the destruction and inhibition of microbial growth.
- 2. Draw your own mind map to summarise the application of mechanical, physical and chemical methods of microbial control.
- 3. What do you think might be a suitable microbial control method to use in the following situation: Your son was playing and got injured and developed a wound on his leg. The wound needs to be dressed immediately to prevent further infection. How are you going to attend this problem so that you do not promote infection on the wound? At home you already have an emergency first-aid kit. Give reasons for your answer.

## 7.19 Feedback on activity 7.5

Here are a few examples for the six factors that may affect the destruction and inhibition of microbial growth:

- Population size A larger population would require a longer time to die than a smaller population. Therefore it is very important to make sure one eliminates the microbial population immediately when it starts to grow.
- 2) Population composition Depending on the type of the organisms that are treated,

bacterial spores exhibit more resistance to most antimicrobial agents than vegetative forms. Some species are able to withstand harsh conditions as compared to others, for example *Mycobacterium tuberculosis*. M. tuberculosis is more resistant to antimicrobial agents than most other bacteria. Therefore it is important that tuberculosis patients take their medication for six months as prescribed.

 Contact time - The longer a microbial population is exposed to a microbicidal agent, the more microorganisms are killed. This is achieved through sterilisation where contact time should be long enough to reduce the chance of survival by at least a million (6 logs).

Go to the following websites to obtain more information on microbial control methods:

http://www.thesurvivaldoctor.com/2014/01/13/sterilize/

http://www.saintelizabeth.com/getmedia/bcdbb617-0a58-4306-9660-40132f742b2e/cleaning-woundcare-instruments.pdf.aspx?ext=.pdf

## 7.20 Summary

In this leaning unit you learned about the control of microorganisms in the environment. You learned about different control methods and how these methods are employed and why they are employed. The information you have gained in this unit will equip you with the skills and knowledge to use disinfectants and antiseptics in the control of microorganisms at home, in the laboratory or any other place.

## **LEARNING UNIT 8:**

#### Antimicrobial Chemotherapy

#### 8.1 Introduction

In learning unit 7 we learned about the control of microorganisms by physical, chemical and mechanical agents. These control mechanisms are used in laboratories, on hospital equipment, in the food industry, in homes, and so on to remove or control the growth and spread of microbes.

Antimicrobial chemotherapy is about the use of antimicrobial agents in the control or removal of microbes in **living cells** of humans and animals. The antimicrobial agents that are selected should not be toxic to the cell. Bacteria such as streptococci, staphylococci, mycobacteria, and *Escherichia coli* develop biofilms that help them resist antibiotics. We will learn more in this unit about the principles of antimicrobial chemotherapy. You will also learn why different antimicrobial agents are used.

For more information on this topic you can look at the following web link:

http://textbookofbacteriology.net/antimicrobial.html

## 8.2 Learning outcomes

When you have completed this learning unit, you should be able to:

- explain the meanings of certain terms
- trace the historical development of chemotherapy
- discuss the general characteristics of antimicrobial agents
- explain the difference between a narrow- and broad-spectrum drug
- · correlate drug action with cidal and static effects
- discuss the mechanism of antiprotozoan drugs
- explain how one determines the level of antibacterial drug activity using the dilution susceptibility test, the disk diffusion test, and the Etest®
- relate side effect of toxicity of antibacterial drugs to mechanism of action
- discuss the use, specific mechanisms of action, advantages and disadvantages of antibacterial agents and compare them with those of others
- compare antifungal drug mechanisms of action
- explain why there are far fewer antifungal agents than there are antibacterial agents
- compare antiviral drug mechanisms of action
- provide a rationale for combination drug therapy
- explain why there are far fewer antiviral agents than there are antibacterial agents

### 8.3 Textbook reference

Study chapter 9 (Eukaryotic Cell Structure and Function) in your prescribed textbook.

## 8.4 The development of chemotherapy

Do you know how the era of modern chemotherapy started? The era started when the German physician Paul Ehrlich was working with dyes that specifically bind to microbial cells. He found that the dye trypan red was active against the trypanosome that was the cause of African sleeping sickness.

The first antibiotic formed from a natural microbial product was penicillin. It was first discovered in 1896 by the medical student Ernest Duchesne. His work on penicillin was however forgotten and penicillin was then rediscovered by Alexander Fleming in 1928. For more information on the discovery of penicillin, study the prescribed textbook. You may also want to consult relevant web links.

Further optional internet reading:

http://bsac.org.uk/wp-content/uploads/2012/02/Chapter 1.pdf

http://jac.oxfordjournals.org/content/48/suppl\_1/1.full.pdf

## 8.5 General characteristics of antimicrobial drugs

When Paul Ehrlich began his work on chemotherapeutic agents he found that a successful agent has a selective toxicity, meaning it kills or inhibits the microbial pathogen while doing little damage to the host cell.

Look at table 9.1 in Prescott which lists the properties of some common antibacterial drugs. Some of the properties of antibacterial drugs are listed below:

- Cell wall synthesis inhibition
- Protein synthesis inhibition
- Nucleic acid synthesis inhibition, etc.

Further optional internet reading:

http://microbiology.free.fr/Presentations/antimicrobialchemotheray.pdf

# 8.5 Activity 8.1: General characteristics of antimicrobial agents

- 1. Draw a simple timeline to illustrate the historical development of chemotherapy.
- 2. How do semisynthetic antibiotics commonly differ from their parent molecule?
- 3. Define the following:
  - a. Selective toxicity
  - b. Therapeutic index

4. Compile a table in which you list the general characteristics of antimicrobial agents in the first column, explain each characteristic in your own words in the second column, and in the third column indicate whether each characteristic has a cidal or static effect.

#### 8.6 Feedback on activity 8.1

Semisynthetic drugs are structurally modified by the addition of chemical groups to make them less susceptible to stomach acids and inactivation by pathogens. The examples of semisynthetic antibiotics are ampicillin and methicillin. For more information on this look at the following websites:

http://www.cs.stedwards.edu/chem/Chemistry/CHEM43/CHEM43/Antibiotics/Antibiotics.HTM

https://www.boundless.com/microbiology/antimicrobial-drugs/overview-of-antimicrobial-therapy/antibiotics-and-selective-toxicity/

http://dictionary.reference.com/browse/therapeutic+index

http://www.wisegeek.com/what-is-a-narrow-therapeutic-index.htm

## 8.7 Determining the level of antimicrobial activity

For a proper therapy to take place the effectiveness of the antimicrobial agent against a specific pathogen should be determined. The testing will show which antimicrobial agents are most effective against a particular pathogen and will also give an estimate of the appropriate therapeutic dose.

There are three tests that are used to determine the level of antimicrobial activity:

- Dilution susceptibility tests.
- Disk diffusion tests: The Kirby-Bauer method is the most often used disk diffusion and was developed by William Kirby, AW Bauer and their colleagues in the early 1960s.
- The Etest®: The Etest from bioMérieux SA is used in sensitivity testing under a range of conditions. It is conveniently use for use with anaerobic microorganisms that do not grow well in broth culture but quite well on agar. For detail information on this test check page 194.
- For more information look at the following links:

https://www.boundless.com/microbiology/antimicrobial-drugs/measuring-drugsusceptibility/kirby-bauer-disk-susceptibility-test/

http://amrls.cvm.msu.edu/microbiology/detecting-antimicrobial-resistance/testmethods/examples-of-antibiotic-sensitivity-tesing-methods

#### 8.8 Activity 8.2: Antimicrobial activity tests

Explain how the dilution susceptibility test, the Kirby-Bauer test and the Etest® can be used to determine sensitivity to antimicrobial agents.

## 8.9 Feedback on activity 8.2

The Kirby-Bauer method is the most often used disk diffusion test. This technique is used to save time and media. An antibiotic-impregnated disk is placed on the Petri dish plate with agar that has been previously inoculated with the test organism (bacterium). After this the plate is incubated for 24 hours. A clear zone around the antibiotic disk is an indication that the antibiotic inhibits bacterial growth or the bacteria is susceptible to the antibiotic agent.

## 8.10 Antibacterial drugs

In this section we are going to look at the mode of action of antibacterial drugs against bacterial cells and how they target the cells.

The following are modes of action of antibacterial drugs:

- 1. Inhibitors of cell wall synthesis examples are penicillins and cephalosporins.
- 2. Protein synthesis inhibitors examples are aminoglycosides and tetracyclines.
- 3. Metabolic antagonists examples are sulphonamides and trimethoprim.
- 4. Nucleic acid synthesis inhibitors examples are quinolones.

To get detailed information on these antibacterial drugs refer to **table 9.1** in the prescribed textbook. You may also consult relevant web links for more information.

## 8.11 Antifungal drugs

The therapy of fungal infections has been less successful compared to bacterial infection because the fungi are eukaryotes and their cells are similar to human cells. The similarity of fungal cells to human cells has made it difficult to treat fungal infection because drugs that are used in the treatment of fungal infections become toxic to human cells.

Fungal infections are subdivided into three groups - **superficial** mycoses, **subcutaneous** mycoses, and **systemic** mycoses.

You will notice that the treatment of mycoses needs many drugs. The three drugs containing imidazole-miconazole, ketoconazole, and clotrimazole are broad-spectrum agents available in the form of creams and solutions for the treatment of infections such as athlete's foot, oral and vaginal candidiasis.

For more on fungal drugs study the prescribed book. You may also want to consult the following relevant web links.

http://courses.washington.edu/medch401/pdf\_text/401\_06\_VI\_Antifungal.pdf

https://www.us.elsevierhealth.com/media/us/samplechapters/9781416056805/Ch07.pdf

## 8.12 Activity 8.3: Antibacterial and antifungal agents

1. Discuss the use, specific mechanisms of action, advantages and disadvantages of antibacterial agents and compare them with each other. Under "disadvantages", refer to their side effects.

- 2. Compare antifungal drug mechanisms of action.
- 3. Explain why there are far fewer antifungal agents than there are antibacterial agents.

## 8.13 Feedback on activity 8.3

Refer to Prescott to ensure that your answer to question 1 is sufficiently detailed. Did you mention that some antimicrobial drugs cause side effects?

Your answer to question 2 you should have included the following:

- Antifungal drugs generally have lower therapeutic indexes as compared to antibacterial drugs.
- Antifungal drugs produce more side effects compared to antibacterial agents.

In your answer to question 3 you should have referred to the fact that both fungi and humans are eukaryotes, and explained why this makes the development of antifungal drugs difficult.

## 8.14 Antiviral drugs

Viruses enter the host cells and make use of the host cell enzyme and contents. Earlier it was thought that a drug that blocked virus multiplication would be toxic for the host.

Through the discovery of inhibitors of virus-specific enzymes and replication cycle processes, antiviral drugs could be developed and manufactured.

The Tamiflu antiviral drug inhibits the viral molecule neuraminidase, which is needed for the release of newly synthesised influenza A virus particles from host cells. For more on Tamiflu and other antiviral agents such as amantadine, rimantadine see the prescribed textbook.

The treatment of HIV/AIDs includes the use of drug combinations. To see how anti-HIV agents block HIV replication see figure 9.16 in Prescott.

For more information look at the following links:

http://www.cdc.gov/flu/antivirals/whatyoushould.htm

http://www.cdc.gov/flu/professionals/antivirals/summary-clinicians.htm

http://www.niaid.nih.gov/topics/HIVAIDS/Understanding/Treatment/pages/arvdrugclasses.asp

http://www.health24.com/Medical/HIV-AIDS/Management-of-HIV-AIDS/Antiretroviraltreatment-20120721

http://www.avert.org/hiv-and-aids-treatment-care.htm

# 8.15 Antiprozoan drugs

Just like fungi, protozoa are eukaryotes and the potential for drug action on host cells and tissues is greater than it is when targeting bacteria (prokaryotes). There are very few antiprotozoan drugs that are available.

*Plasmodium falciparum,* a protozoa that causes malaria, is inhibited by the antimalarial drugs containing quinine such as chloroquinine and qualaquin.

Further optional internet reading:

http://antiprotozoal.wikispaces.com/Antiprotozoal+-+Mechanism+of+action

http://www.uptodate.com/contents/antiprotozoal-therapies

## 8.16 Activity 8.4: Antiviral and antiprotozoan agents

- 1. Compare the different antiviral drug mechanisms of action.
- 2. Explain why there are fewer antiviral agents than antibacterial agents.
- 3. What special considerations must be taken into account when treating infections caused by the protozoan parasite?

## 8.17 Feedback on activity 8.4

Antiviral drug mechanisms of action involve the interference of the drug with critical stages in the life cycle of the virus.

They also involve the inhibition of the synthesis of virus-specific nucleic acids, e.g. Adenine arabinoside, acyclovir and zidovudine.

Cocktails seem to be more effective than single drug therapy. Cocktails involve the combination of drugs.

## 8.18 Factors influencing antimicrobial drug effectiveness

**Drug effectiveness** - You may wonder why some drug therapies are not effective in the elimination of pathogens. The answer is not that simple because the drugs do not always spread rapidly throughout the body or immediately kill all the invading pathogens. Understanding the factors that control drug activity, stability, and metabolism in vivo are crucial in drug formulation. You may also wonder why some drugs are not administered orally but intramuscularly or intravenously. The reason is that some antibiotics are not well absorbed by the gut and must be given intravenously or injected intramuscularly. For more details, study the prescribed textbook. You can also look at relevant websites.

**Overcoming drug resistance -** Drug resistance is a serious challenge that we are facing today. Think of the XDR – TB resistance strain in South Africa. The overuse, misuse and abuse of antimicrobial chemotherapeutic agents have contributed to the increase in drug resistance.

There are several approaches in overcoming drug resistance. These are as follows:

- 1. Give a dose of the drug high enough to eliminate all susceptible microbes and all spontaneous mutants that might arise during treatment.
- 2. In some cases two or three different drugs can be given simultaneously to prevent the emergence of resistance to the other.
- 3. Some drugs are administered over a long period for 6 to 9 months to decrease the possibility of the pathogen developing drug resistance. This is done when treating tuberculosis.

There are other strategies that are used to prevent resistance. For more information, read the prescribed textbook. You may also wish to consult relevant web links.

#### Activity 8.5: Revision questions

- 8.19 These revision questions will help you to prepare for assignments and the examination.
  - 1. Describe five mechanisms of chemotherapeutic agents for killing or inhibiting bacterial pathogens.
  - 2. You are a medical practitioner treating a 13-year-old boy with an upper respiratory infection caused by a virus .The boy's father insists that you prescribe antibiotics he's not leaving without them! How do you persuade the boy's father that antibiotics will do more harm than good?
  - 3. What factors do you think must be considered when treating an infection present in a biofilm on a medical implant (e.g. an artificial hip) versus a skin infection caused by the same microbe?
  - 4. What are the primary medical practices that result in antimicrobial drug resistance? How can these be overcome?
  - 5. What is parenteral administration of a drug? Why it is used?
  - 6. Why is malaria, like tuberculosis, now treated with several drugs simultaneously?
  - 7. Suggest why drugs that inhibit bacterial protein synthesis are also effective against some protists.
  - 8. Summarise the mechanism of action and the therapeutic use of the following antifungal drugs: miconazle, nystatin, griseofulvin, amphotericin B, and 5-flucytosine.
  - 9. Summarise current HIV treatment. Briefly describe the action mechanism of the two most common classes of HIV drugs that are currently used in the treatment of HIV.

## 8.20 Feedback

With regard to question 2, issues such as drug resistance and how to overcome drug resistance are important. For ailments than are not caused by bacterial infection antibiotics should not be used at all.

With regard to question 3, it is very important to ascertain first what type of bacteria are found in the skin as well as on the medical implant. The treatment of a bacterial infection that is of biofilm origin will require vigorous treatment/strong antibiotics or a combination of antibiotics to remove the biofilm.

#### 8.21 Activity 8.6: Reflection

- Now that you have completed this learning unit, think about how your knowledge of antimicrobial chemotherapy may in future influence your use of chemotherapeutic agents such as antibiotics, antifungal and antiviral drugs. Are you now in a position to take a more informed decision when your medical doctor prescribes antimicrobial chemotherapeutic agents for you? Write down what you would do when this happens.
- 2. You have now reached the end of this module. Has the way you think about microbes about both their benefits and their harmful effects changed in any way? Write down some

ideas on how you now see the role of microbes in our lives, and also what actions you might take in your personal life to take advantage of this knowledge.

## 8.22 Summary

In this learning unit you have learned a lot about key concepts such as the following:

- The development of chemotherapy: You have learned about the pioneers of modern era chemotherapy and how it has influenced the development of effective chemotherapeutic agents that have minimum side effects and do not cause major harm to the host cell.
- General characteristics of antimicrobial drugs: Selective toxicity and therapeutic index are explained. The value in the therapeutic index gives you an indication of the effectiveness of a chemotherapeutic agent. The greater the number of the therapeutic index, the more effective the agent is against the pathogen. In table 9.1 (prescribed textbook) there is information on the classification of antibiotics and how they target various microbes.
- **Determining the level of antimicrobial activity:** The three tests used are the Kirby-Bauer test (a disk diffusion test), the dilution susceptibility test and the Etest®.
- Antibacterial drugs: Table 9.1 shows the action of antibiotics. Antibiotics such as penicillin disrupt the bacterial cell wall synthesis. In addition to penicillin there are other antibiotics that also disrupt pathogens (see table 9.1).
- **Antifungal drugs:** Miconazole, ketoconazole, clotrimazole, tolnaftale, nystatin, and griseofulvin are used in the treatment of superficial mycoses.
- Antiviral drugs: When treating viral infections, drug combinations (cocktails) are favoured as they appear to be more effective than monotherapies. Think of the treatment of HIV/Aids where cocktails are used to interfere with the replication of viral processes.
- Antiprotozoan drugs they interfere with the critical steps such as the following:
  - 1) Nucleic acid synthesis
  - 2) Protein synthesis
  - 3) Electron transport
  - 4) Folic acid synthesis

The mode of action of most antiprotozoan drugs used to treat protists infection is unknown.

# **Discussion forums and topics in MIB2601**

The list below shows the discussion forums and topics that have been opened on the module web site on myUnisa. I would like to encourage you to participate in the various forums and topics.

## Forum 1: Module-related discussions

## Topic: Activity 0.1: Introducing yourself

Post a short entry in which you:

- tell us who you are and where you live;
- share what Microbiology means to you, and why you chose to study it.

Also respond to at least one posting by one of your fellow students.

## *Topic: Learning units 1–2*

Use this topic to discuss issues related to learning units 1–2

## *Topic: Learning units 3–4*

Use this topic to discuss issues related to learning units 3–4

## Topic: Activity 4.3: The role of protists and fungi

Answer the following question:

What are some of the positive and negative effects of protists and fungi for animals and humans?

Also read the postings by other students, and respond to at least one of these. Mention anything you found particularly interesting about the other posting, or ask for clarification or more details of any aspect if you wish.

#### *Topic: Learning units* 5–6

Use this topic to discuss issues related to learning units 5-6

#### Topic: Learning units 7–8

Use this topic to discuss issues related to learning units 7-8

#### Topic: Activity 7.4: Selection of control method

Post your answer to the question below here, and also respond to at least one other posting by a fellow student.

A very large population of *Pseudomonas aeruginosa* is growing inside a pipe system that supplies water to a large village. It is summer and it is hot and moist. In this situation, would you recommend the use of quaternary ammonium chloride as a disinfectant in the pipe system? Give reasons for your answer.

Also read the postings by other students, and respond to at least one of these. Mention anything you found particularly interesting about the other posting, or ask for clarification or more details of any aspect if you wish.

**Forum 2: Assessment discussions** *Discussions about the assignments and the exam* 

Topic: Assignment 01

Use this forum to raise queries about Assignment 01.

Topic: Assignment 02

Use this forum to raise queries about Assignment 02.

*Topic:* Examination Use this forum to raise queries about the examination.

# Forum 3: Student lounge

Use this forum to discuss general matters among yourselves.