I

COURSE CODE: **MCB 212**

COURSE TITLE: GENERAL MICROBIOLOGY

NUMBER OF UNITS:3 Units/Compulsory

COURSE DURATION: Three hours per week

COURSE LECTURER: **EZEANYA, Chinyere C.**

INTENDED LEARNING OUTCOMES

At the completion of this course, students are expected to:

1. Define the concept of microbial cell structure.
2. Demonstrate knowledge on the TWO main types of microbial cell structure.
3. Define and list the different phases of growth; factors affecting microbial growth.
4. Apply the role of micro-organisms in soil, food and to solve simple practical problems.
5. Carry out projects to:
    - Identify and differentiate between the different microbial structures.
    - Demonstrate the concept of microbial growth resulting from isolation from food or soil.

COURSE DETAILS:

Week 1-2: *Introduction to concept of microbial cell structure.*

Week 3-4 *Concept of cell structure: emphasis on prokaryotic and eukaryotic cell structure.*

Week 5: *Introduction to the microbial growth and parameters for measurement.*

Week 6-7*: Compare and contrast the different phases of microbial growth, understanding the factors that affect microbial growth.*

Week 8-9: *Introduction to the concept of microbial role in soil, food and discussion of the various microbial groups and their roles.*

Week 10-11: *Projects.*

Week 12 *Revision*

RESOURCES

• **Lecturer's Office Hours:**

• **EZEANYA, Chinyere C.**. Mondays: 12:30-2:30pm.

• **Course lecture Notes: http://www.edouniversity.edu.ng/oer/micro/mcb212.pdf**

• **Books:**

•*General Microbiology* by Hans G. Schlegel, C. Zaborosch Cambridge University Press. 1993 (recommended).

Course Project:

• Multiple parts (2 or 3).

• Must be done in the Microbiology Laboratory.

• Home works + Project: ~ 30% of final grade.

• **Exams:**

• Final, comprehensive (according to university schedule): ~ 70% of final grade

Assignments & Grading

• **Academic Honesty:** All group work should be done in teams, otherwise stated.

• General solution to problems should be discussed extensively in groups but must have individual write ups.

NO LATE HOMEWORKS ACCEPTED

• All home works are to be submitted online on the class group platform.

• All home works are due at the time stated.

• Late projects will not be accepted.

CELL

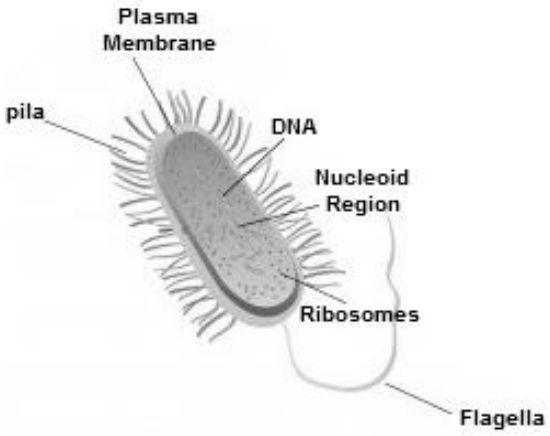
The cell is the smallest, functional unit of life. The cell was first observed by Robert Hook and English physicist in 1665. The cells can only be seen with the aid of a microscope. Microscope is an instrument which is used to observe microscopic living and non living organisms which maybe a single cell or multicellular cell organisms too small to be seen clearly by the unaided eyes. The study of microorganisms is known as microbiology. They are generally one millimeter or less in diameter.

TYPES OF CELL STRUCTURE

Microbes are divided into two main cell structures: Prokaryotic and Eukaryotic cell structure. Organisms with the prokaryotic cell structure are known as Prokaryotes which implies absence of a nucleus. Organisms with the eukaryotic cell structure are known as Eukaryotes.

PROKARYOTIC CELL STRUCTURE

The prokaryotic cell structure is simplest type of cell structure with organisms known as Prokaryotic organisms or Prokaryotes. Bacteria are typically example of microorganism with prokaryotic cell structure. These cell structures are single-celled with the absence of nucleus and comprises of capsule, cell wall, cell membrane, cytoplasm, nucleoid, ribosome, plasmid, pili, flagella.



PROKARYOTIC CELL

FEATURES OF PROKARYOTIC CELL

1. They are generally one millimeter or less in diameter.
2. They are single celled or unicellular
3. The shape of the cell ranges from spherical, rod or cocci.
4. They reproduce asexually by binary fission; exchange genes via conjugation, transformation and transduction.

DIFFERENT ORGANELLES AND FUNCTIONS OF A PROKARYOTIC CELL

CELL WALL

A rigid structure that surrounds the cell.

Function

1. Provides shape for the cell.
2. It protects the cell.
3. It aids in classifying bacteria into two groups: Gram positive and gram negative.

CELL MEMBRANE

The structure that surrounds the cell cytoplasm.

Function

1. The key function of the cell membrane is selective permeability and transport of solute. The cell membrane achieves this function via three mechanisms: Passive transport, Active transport and Group translocation and special transport process in the case of siderophores.
2. Electron transport and oxidative phosphorylation
3. Excretion of hydrolytic exoenzymes
4. Biosynthetic functions for example; phospholipid synthesis.

NUCLEIOD

It is a cytoplasmic region harbouring genetic material of the cell.

Function

1. It provides the location of genetic material-DNA (deoxyribonucleic acid) plays a key role in cell division as well as stores the genetic information of the cell.

GLYCOCALYX

It is a glyco-protein polysaccharide

Function

1. It surrounds the cell membranes.

RIBOSOME

They are the smallest membrane found in the cell

Function

1. It aids in protein synthesis

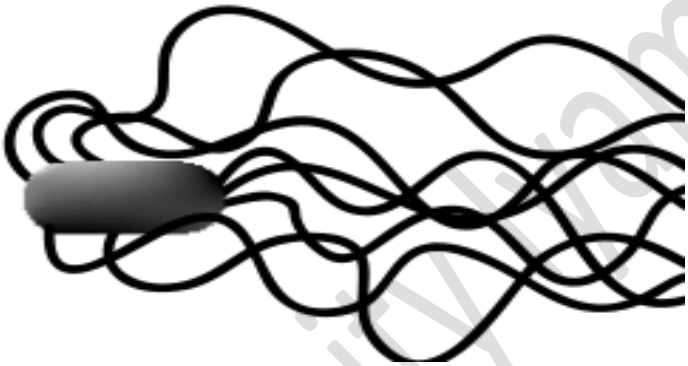
FLAGELLA

There are external to the cell wall. They exist four main flagellation arrangements: Monotrichous, Lophotrichous, Amphitrichous and Petrichous. Atrichous is the absence of flagella. The flagella are of three main parts: The basal structure, Hock-like structure and a long filament outside the cell wall.

Monotrichous: Bacteria with one polar flagellum

Lophotrichous: When a tuft of several polar flagella is present

Amphitrichous: Presence of flagella at both ends of the organism



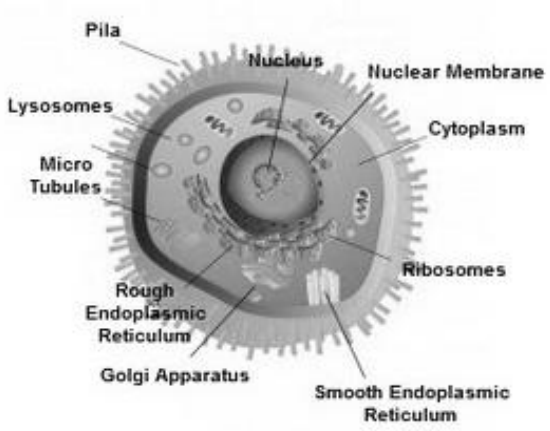
Petrichous: When flagella are distributed all over the surface of the organism

Function

They are responsible for motility.

EUKARYOTIC CELL STRUCTURE

Organisms with the eukaryotic cell structure are known as eukaryotic organisms or eukaryotes. They are known with the presence of a defined or true nucleus. Fungi is a typical example of microorganisms that are eukaryotes. The eukaryotic cell structure is a more complex cell structure compared to the prokaryotic cell structure.



EUKARYOTIC CELL STRUCTURE

FEATURES OF EUKARYOTIC CELL

1. They have membrane bound organelles thus are multicellular.
2. They can reproduce sexually and asexually.
3. They are ten times larger in size than prokaryotic cell structure.
4. Fungi, algae and protozoa are typical examples.

DIFFERENT ORGANELLES AND FUNCTIONS OF A EUKARYOTIC CELL

CELL WALL

They are thick protective structure.

Function

- Provide support
- Maintain shape of the cell

- Prevent the cell from taking in too much fresh water and bursting: Osmotic balance

CYTOPLASM

It is found between the nucleus and the plasma membrane. It contains both lipids and suspended organelles.

Function

Keeps cell organelles apart from one another.

PLASMA MEMBRANE

It is a barrier which is made up of phospholipids and proteins.

Function

- Regulates movement of materials inside and outside of the cell.

NUCLEUS

It is the largest and most visible organelle. It contains the cell's genome. It is surrounded by a nuclear envelope which is a double layered phospholipid bilayer.

NUCLEAR MEMBRANE

It surrounds the nucleus.

Function

Regulates the movement of materials in and out of the nucleus.

NUCLEOLUS

This organelle which is non-membranous bound is found within the nucleus.

Function

It regulates all cellular activities of the cell.

GOLGI BODY

A flat-like membrane.

Functions

- Generates Lysosomes
- Modifies cellular molecules
- Coordinates the packaging and shipment of materials out of the cell

ENDOPLASMIC RETICULUM

This membrane organelle which is connected to the nucleus aids in partitioning the cell. They are of two types: Rough and Smooth endoplasmic reticulum.

Function

- Synthesis and transportation of proteins and lipids

MITOCHONDRIA

This double smooth membrane is the power house of the cell.

Function

- Extracts energy from food to produce ATP.

GROWTH OF MICROORGANISMS INCLUDING THE EFFECT OF ENVIRONMENTAL FACTORS ON GROWTH, SURVIVAL, INHIBITION AND DEATH OF MICROORGANISM.

GROWTH

Growth is defined as the orderly increase of all the chemical constituent of an organism. Cell multiplication is the consequence of growth.

MEASUREMENT OF GROWTH

Growth can be measured using two parameters: CELL MASS OR CELL NUMBER. Cell mass and cell number are not necessarily equivalent.

CELL MASS

Determination of Cell Mass

This is done by determination of the following:

- Chemical composition
- Carbon or nitrogen or protein complex
- Enzyme
- Light scattered by the suspension of cells

CELL NUMBER

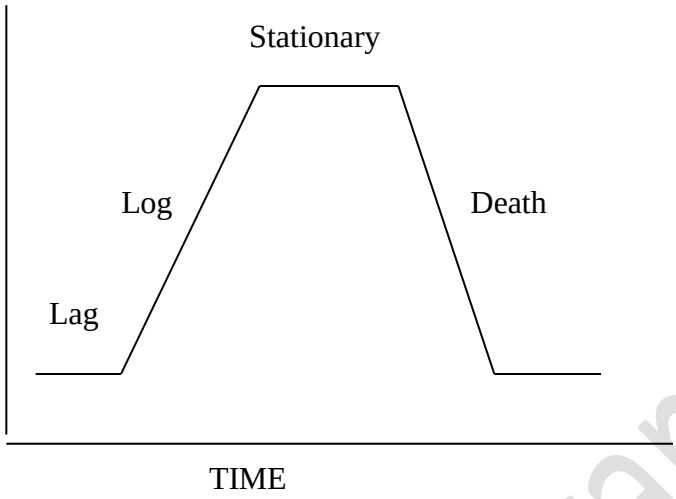
Determination of Cell Number

This is done by microscopically counting the individual cells in an accurately determined volume known as Total Cell Count or direct count. Devices that can be used are a COUNTING CHAMBER.

PHASES OF GROWTH

- **LAG PHASE:** This involves no increase in cell number.
- **EXPONENTIAL OR LOG PHASE:** After the lag phase proceeds the exponential phase. Here the microorganisms are in a steady phase. New cell materials are being synthesized at a constant rate and the mass increases in an exponential manner.
- **STATIONARY PHASE:** Here, nutrients are used up or toxic products accumulate from cells metabolism. Here, cells either form spores like in *Bacillus* or they reduce their size and increase their resistance to harmful chemicals.
- **DEATH PHASE:** The total number of viable cells begins to decrease.

GROWTH CURVE



FACTORS AFFECTING MICROBIAL GROWTH

The factors that affect microbial growth are basically categorized into two: Environmental and Nutritional Factors.

ENVIRONMENTAL FACTORS

The environmental factors that influence growth are:

- Temperature
- Oxygen
- pH
- Osmotic pressure
- Others: radiation, atmosphere pressure

Temperature

Most microorganisms grow within a temperature range. Bacteria are divided into four groups based on their optimum temperature requirement. They are;

Psychrophiles: Optimum temperature between -5^{0}C and 20^{0}C.

Mesophiles: Optimum temperature range between 20^{0}C and 50^{0}C. Most pathogenic bacteria are found in this group.

Thermophiles: Optimum temperature range between 50^{O}C and 80^{o}C.
Hyperthermophiles: Optimum temperature above 80^{0}C.

OXYGEN

Bacteria can be divided into five major groups based upon their oxygen requirement.

Obligate Aerobes: They have strict requirement for oxygen.

Obligate Anaerobes: They experience stunted growth in the presence of oxygen.

Facultative Anaerobes: These organisms can survive in the presence and absence of oxygen.

Microaerophilic Organisms: They require very minute amount of oxygen for growth.

Aerotolerant Organisms: They derive no benefit in the presence of oxygen unlike facultative anaerobes.

PH:

Most bacteria grow within pH 6.5 and 7.5 (neutral). Molds and yeasts grow in pH 5 and 6 (acidic). Some are called ACIDOPHILIC.

OSMOTIC PRESSURE

It is the pressure that is required to prevent the net flow of water across a semi-permeable membrane. Organisms can be grouped based on their osmotic pressure requirement.

Extreme or obligate halophiles require high osmotic pressure.

Facultative halophiles permit high osmotic pressure.

NUTRITONAL ASPECT OF GROWTH

Microorganisms require some nutrients in large amount (Macronutrients) and some others in small amount (Micronutrient). Carbon, oxygen, nitrogen, sulfur and phosphorus (component of carbohydrates, lipids, proteins) are macronutrients required by all organisms. Iron, copper, zinc needed for the activities of certain enzymes are examples of micronutrients.

Organisms can be divided into a number of groups based on:

- Source of energy.
- Source of carbon for synthesis of all organic molecules.

SOIL MICROBIOLOGY

Soil microbiology is the study of microorganisms in soil, their roles and the impact of their activities on the soil.

IMPORTANT SOIL BACTERIA

Actinomycetes

They are large group of bacteria that grow as hyphae like fungi. A number of antibiotics are produced by actinomycetes such as Streptomyces.

ROLE

They are responsible for the characteristically "earthy" smell of freshly turned, healthy soil.

They decompose recalcitrant (hard-to-decompose) compounds, such as chitin and cellulose, and are active at high pH levels

Nitrogen fixing Bacteria

ROLE

They form symbiotic relationship: the plant (roots of legumes like clover and lupine, and trees such as alder and locust) supplies simple carbon compounds to the bacteria, and the bacteria convert nitrogen (N_2) from air into a form the plant host can use.
Visible nodules are created where bacteria infect a growing root hair. The plant supplies simple carbon compounds to the bacteria, and the bacteria convert nitrogen (N2) from air into a form the plant host can use. When leaves or roots from the host plant decompose, soil nitrogen increases in the surrounding area.

Denitrifying Bacteria

Denitrifies are anaerobic, meaning they are active where oxygen is absent, such as in saturated soils or inside soil aggregates.

ROLE

They convert nitrate to nitrogen (N_2) or nitrous oxide (N_2O) gas.

Nitrifying Bacteria

Nitrifying bacteria are suppressed in forest soils, so that most of the nitrogen remains as ammonium.

ROLE

They change ammonium (NH4+) to nitrite (NO2-) then to nitrate (NO3-) – a preferred form of nitrogen for grasses and most row crops. Nitrate is leached more easily from the soil, so some farmers use nitrification inhibitors to reduce the activity of one type of nitrifying bacteria.

FUNGI

Decomposers

ROLES

These fungi generally use complex substrates, such as the cellulose and lignin, in wood, and are essential in decomposing the carbon ring structures in some pollutants

Convert dead organic material into fungal biomass, carbon dioxide (CO_2), and small molecules, such as organic acids.

Mutualist

Colonize plant roots

ROLES

Solubilize phosphorus and bring soil nutrients (phosphorus, nitrogen, micronutrients, and perhaps water) to the plant

Groups of Mycorrhizae

- The *Ectomycorrhizae*, grow on the surface layers of the roots and are commonly associated with trees.
- The *Endomycorrhizae* that grow within the root cells and are commonly associated with grasses, row crops, vegetables, and shrubs.

Parasitic Fungi

ROLES

Cause reduced production or death when they colonize roots and other organisms.

They help control diseases

PROTOZOA

Protozoa are organisms that have only one cell, and are microscopic, but larger than bacteria. They are grouped by the ways they move:

- Amoebae use a psuedo (fake) foot, ciliates have cilia (short hair) and move them very fast.
- Flagellates have one or more flagella (whips) and move them very fast.

Roles

- Mineralizing nutrients, making them available for use by plants and other soil organisms.
- They release the excess nitrogen in the form of ammonium (NH4+).
- Regulates bacteria populations
- Serves as food source for other soil organisms.
- Suppress disease by competing with or feeding on pathogens.

Advantage of Fungi over Bacteria in the soil

- In dry conditions, fungi can bridge gaps between pockets of moisture and continue to survive and grow, even when soil moisture is too low for most bacteria to be active.
- Fungi are able to bring nitrogen up from the soil, allowing them to decompose surface residue which is often low in nitrogen.

ROLE OF MICROORGANISMS IN FOOD

PATHOGENIC ROLE

Pathogenic micro-organisms such as viruses, bacteria, parasites and moulds can cause food-borne infections or intoxications.

FOOD BORNE INFECTION

This is caused by the ingestion of food with increased multiplication of pathogenic microorganisms in it. Example of bacteria; *Salmonella spp, Listeria monocytogenes* and *Escherichia coli.*

FOOD INTOXICATION

This is the production of toxins by pathogen microorganisms in food. Example: *Staphylococcus aureus* enterotoxin.

FOOD BORNE INFECTIONS AND INTOXICATION

Bacteria

- ***Campylobacter jejuni*:** Is a conventional cause of diarrhea among humans. The spread could be via direct contact amid humans and infected animals or their feces. In addition, it is spread by the ingestion of contaminated food or water
- **Non-typhi salmonellosis:** There exist approximately morethan 2000 serotypes of *Salmonella spp*, of which a small number cause Salmonella gasteroenteritis in humans. The symptoms consist of acute watery diarrhea complemented by nausea, cramps and fever. Blood in stool sometimes occur. Animal which is the principal reservoir and transmission occur via

ingestion of contaminated products. High risk foods are poultry, meat, eggs and milk.

- ***Salmonella typhi* and *paratyphi*:** Produce typhoid fever and paratyphoid fever in that order. Subsequently the reservoir for both these bacteria is usually humans, transmission occurs chiefly via person-to-person contact or food contamination by food handlers.

- ***Staphylococcus aureus*:** Here, infection is caused in humans. The bacteria which has its natural habitat in the nose and on the skin of healthy people. Elevated amounts can be found in lesions of skin such as infected boils or any pus draining lesion. Food poisoning caused by this bacterium results from the production of heat resistant staphylotoxin, which consequently result in diarrhea, vomiting, cramps and fever. The incubation period is usually 6 to 24 hours.

- ***Escherichia coli*:** There are several serotypes which have been documented, of which few are harmless to humans while others cause gastroenteritis. Enterotoxigenic *E.coli* is the utmost common cause of traveller's diarrhea. The source of infection is humans, and transmission usually occurs via contaminated food and water.

- ***Listeria monocytogenes*:** This bacterium is greatly associated with food storage. Storage for long periods of time in the fridge for the reason that it is ubiquitous, and holds the ability to grow slowly, stable at low temperatures.

Can be serious in immune compromised, where it causes septicemia and meningitis.

- **Shigella:** The infection sources are humans and primates. Since it has low infectious dose, the chief mode of transmission is person-to-person contact. Furthermore, it is transmitted through infected food and water. The symptoms of shigellosis include: fever and watery diarrhea. The infections sometimes demonstrate as a dysenteric syndrome which includes fever, abdominal cramps and tenesmus and bloody stools containing mucous.

- **Vibrio Cholerae 01:** The source is humans. The main mode of transmission is as a result of contaminated water and food, or person-to-person most likely in overcrowded, unhygienic conditions. It causes acute watery diarrhea, which most likely stretch up to 20 liters per day.

- **Clostridium botulinum:** Source: intestinal tract of fish, birds, and mammals. It is cosmopolitan. The bacterium is a spore-forming anaerobe, with a substantially potent heat labile toxin that upsets the nervous system.

VIRUSES

Viruses cannot reproduce in food. The main mode of transmission is thus by food handlers and the use of unclean utensils, which therefore introduce the virus to food which is then ingested by humans.

- **Rotaviruses and Norwalk virus** are the most important causes of gastroenteritis

- **Viral hepatitis A** epidemics are primarily caused by asymptomatic carriers which most likely handle food.

PARASITES

A lot of parasites like helminths, have a multifaceted lifecycle involving more than one host. The key channel of transmission for parasites to humans is via food. For example; the consumption of undercooked pork or beef, or the consumption of raw salads rinsed with contaminated water.

- ***Taenia solium* and *T. saginata*:** also called pig and beef tapeworms. Their cysts which is predominating in the muscle of the animal are consumed and the adult worm grows in the gut. The ova may progress into larvae that may possibly infect other tissues, such as the brain, forming cysticercoids and severe neurological disorders as a consequence.

- ***Trichinella spiralis*:** is seen in undercooked pork. The larvae colonize tissues and cause a febrile illness.

- ***Giardia lambila*:** This infection can either be foodborne, waterborne. It produces acute or sub- acute diarrhea, with abdominal pain and bloating.

- ***Entamoeba histolytica*:** The mode of transmission is mostly food- or waterborne. The cysts are highly resistant to chemical disinfectants, including chlorination. The infection is usually asymptomatic, but may appear as either a persistent mild diarrhea or a fulminant dysentery when symptomatic.

SAPROPHYTIC ROLE

Saprophytic micro-organism plays a role in biodegradation and cause food spoilage.

Bacteria: Examples of action of bacteria involved in food spoilage:

1. Lactic acid formation: *Lactobacillus, Leuconostoc*
2. Lipolysis: *Pseudomonas, Alcaligenes, Serratia, Micrococcus*
3. Pigment formation: *Flavobacterium, Serratia, Micrococcus*
4. Gas formation: *Leuconostoc, Lactobacillus, Proteus*
5. Slime or rope formation: *Enterobacter, Streptococcus*

Moulds: Mycotoxins production

1. *Aspergillus* produces aflatoxin, ochrtoxin, citrinin and patulin
2. *Fusarium*
3. *Cladosporium*
4. *Alternaria*

Mycotoxins can infiltrate the part of food that is not visibly mouldy. It is for that reason necessary to dispose food if any part of it is mouldy. In addition, they are difficult to destroy as they are stable to both heat and chemicals. Below are mycotoxins with specific mode of action:

- Hepatotoxins: aflatoxins, sporidesmins, luteoskyrin
- Nephrotoxins: ochratoxin, citrinin

- GIT toxins: trichocetens
- Neuro- and myotoxins: tremorgens, citreoviridin
- Dermatotoxins: verukarins, psoralen, sporidesmins, trichocetes
- Respiratory tract toxins: patulin

FOOD PROCESSING ROLE

Cultured micro-organisms like probiotic bacteria are used in food processing.

Yeast in food production

- Leavened diet and pasteries: *Saccharomyces cervisiae* ferments sugars to produce carbon dixoide, the end product which is gas production gives the porous structure of bakery products. It also adds to the flavor by formation of alcohols, aldehydes, esters etc.
- Beer: aids alcoholic fermentation.
- Wine: aids fermentation.
- Vinegar: aids fermentation
- Pickles

Bacteria in food production

- Probiotics: are live food supplements applied in yoghurt and other fermented milk products. It includes *Lactobacillus acidophillus and Bifidobacterium bifidum*. A minimum of 108 bacteria per 1ml is required to reach the colon alive so as to have a significant effect. These bacteria enhance the microbial spectrum in the gut thereby contributing to the following effects:

  1. Influence immunity.

2. Decline the risk of colon cancer,

3. Reduce cholesterol absorption

4. Yield acids that decrease the pH in the gut and consequently increase the absorption of minerals such as calcium and phosphorous.

Mould in food production

- Cheese: *Penicillium roqueforti* and *Penicillium camemberti*. Temperature requirement is a key factor.
- Dry salami: *Penicillium* and *Scopulariopis moulds*.
- Soy sauce: *Aspergillus spp*, especially *A. oryzae*, are included in production.
- Sake: is made with a blend of the mould *Aspergillus oryzae* and yeast.