MODEL ANSWER

Course : Integrated UG/PG Biotechnology
Semester : III Semester
Subject : Microbiology
Subject Code : LBTC-301

A. Objective type questions

(i) (b) Leeuwenhoek
(ii) (a) disease
(iii) (a) Obligate parasite
(iv) (c) Virion
(v) (b) Eubacteria
(vi) (a) Gram stain
(vii) (b) Cotton blue
(viii) (c) Glycogen
(ix) (d) Protein or polysaccharides
(x) (b) Methanogenes, Halobacteria, Sulfur dependent archeobacteria and Thermoplasma

B. Attempt any four of the followings

Ans. B.1 Classification is based on the types of light source used and consists of two main categories; optical microscopes utilizing visible light and microscopes that utilize sources other than visible light.

Microscopes utilizing a visible light source:

Microscopes that utilize the visible light as their source of illumination are of the following types;

Light (Optical) Microscope:

Basically it acts as a two stage magnifying device. An objective lens provides the initial enlargement and an ocular lens is placed so as to magnify the primary image a second time. Total magnification is obtained by multiplying the magnifying power of the objective and ocular lenses. An additional condensing lens is normally employed beneath the stage of microscopes to concentrate the light from its source into a very bright beam illuminating the object, thus providing sufficient light for inspection of the magnified image.

Polarizing Microscope:

Many natural objects including crystals & fibers exhibit special optical property known as double refraction or birefringence. In histological material, birefringence is caused by asymmetric particles, too small to be resolved even by best possible lenses. The polarizing microscope is a conventional microscope in which a nickel prism or Polaroid sheet is interposed in the light path below the condenser. This “Polarizer” converts all the light passing through the instrument into plain polarized light (light which vibrates in one optical plane only). A similar second prism termed “analyzer” is placed within the barrel of the microscope above the objective lens.

Phase contrast Microscope:

Lack of contrast has always been a problem in biological work because the refractive indices of cytoplasm and its inclusions are similar. In normal microscopy the problem is solved by staining differentially but this is subject to numerous limitations

Interference Microscope:

It depends upon the ability of an object to retard light. However, unlike the phase microscope, which depends upon the specimen diffracting light, the interference microscope send two separate beams of light through the specimens, which are then combined in the image plane. After recombination, difference in retardation of light results in interference that can be used to measure the thickness or refractive index of the object under investigation.

Dark field Microscope:

This microscope utilizes a strong, oblique light that does not enter the objective lens. A special dark field condenser, in which no light passes through the center of the lens, is employed. Light thus reaches the object to be viewed at an angle so oblique that none of it can enter the objective lens. The field is therefore dark. However small particles present in the specimen will reflect some light into the objective lens and will appear as glistening spots. Thus, it is possible to visualize particles far below the limits of bright light resolution. The effect is similar to phenomenon of dust particles seen in a beam of sunlight entering a darkened room.

It is useful in the examination of small transparent objects such as chylomicron (particles of fat in the blood) which are invisible in the glare of bright field examination.

Microscopes utilizing a non-visible light source:

Ultraviolet Microscope:

Since ordinary optical lenses are practically opaque to ultraviolet rays of light, quartz lenses are used throughout the lens system of this microscope.
This microscope depends upon the differential absorption of ultraviolet light by molecules within the specimen and the results are recorded photographically. In principle, this system allows an improvement in resolution about twice that of light microscope. This system is useful for detecting proteins that contain certain amino acid and in detecting nucleic acids.
Ultraviolet light is also employed in fluorescence microscopy. Many substances have the property of emitting visible light when irradiated by invisible rays. When ultraviolet light is focused upon such a specimen it glows and can be observed by its emitted fluorescence. Fluorescence may be naturally occurring within the specimens or it may result from the introduction of fluorescent dyes that bind to certain specific components of these specimens.

Electron microscope:

Commonly, two types of electron microscopes are in use:

- TEM (Transmission Electron Microscope)
- SEM (Scanning Electron Microscope)

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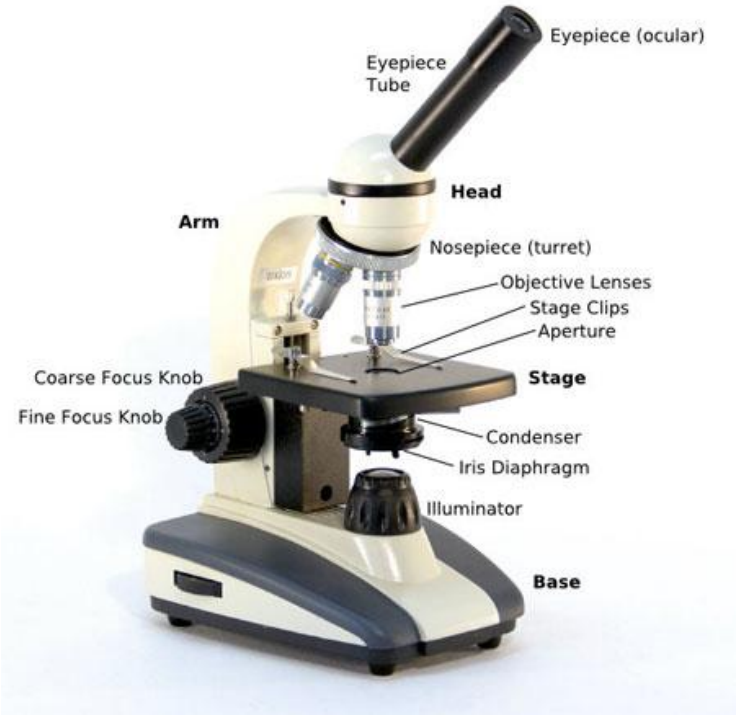


Compound Microscope

A high power or compound microscope achieves higher levels of magnification than a stereo or low power microscope. It is used to view smaller specimens such as cell structures which cannot be seen at lower levels of magnification. Essentially, a compound microscope consists of structural and optical components. However, within these two basic systems, there are some essential components that every microscopist should know and understand. These key microscope parts are illustrated and explained below.

Structural Components

The three basic, structural components of a compound microscope are the head, base and arm.

Head/Body houses the optical parts in the upper part of the microscope

Base of the microscope supports the microscope and houses the illuminator

Arm connects to the base and supports the microscope head. It is also used to carry the microscope.

When carrying a compound microscope always take care to lift it by both the arm and base, simultaneously

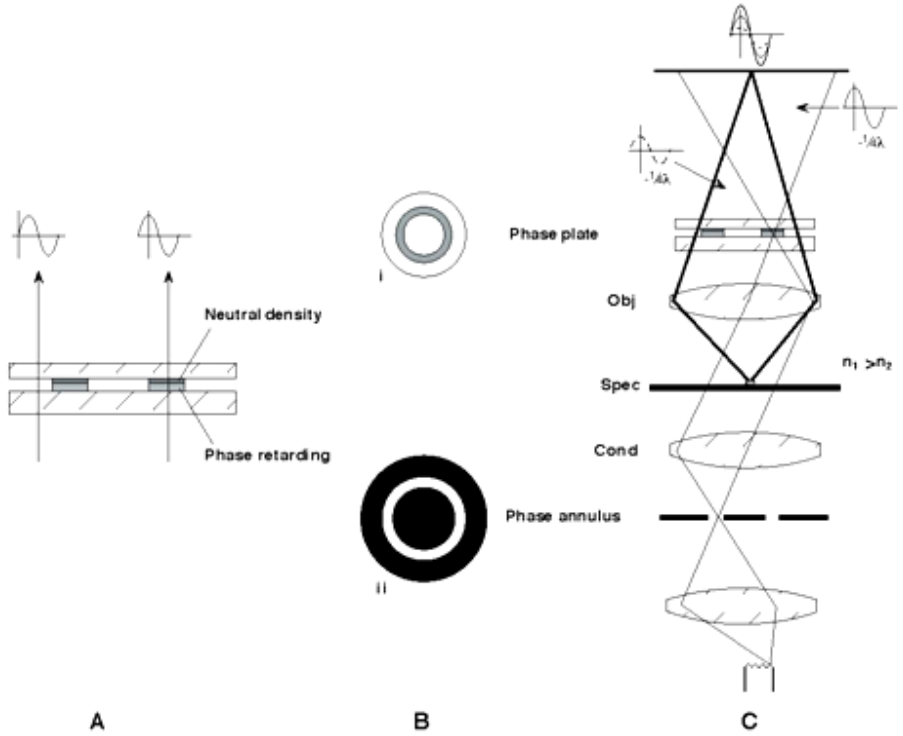
OPTICAL COMPONENTS

There are two optical systems in a compound microscope: Eyepiece Lenses and Objective Lenses:

Eyepiece or Ocular is what you look through at the top of the microscope. Typically, standard eyepieces have a magnifying power of 10x. Optional eyepieces of varying powers are available, typically from 5x-30x.

Eyepiece Tube holds the eyepieces in place above the objective lens. Binocular microscope heads typically incorporate a diopter adjustment ring that allows for the possible inconsistencies of our eyesight in one or both eyes. The monocular (single eye usage) microscope does not need a diopter. Binocular microscopes also swivel (Interpupillary Adjustment) to allow for different distances between the eyes of different individuals.
Objective Lenses are the primary optical lenses on a microscope. They range from 4x-100x and typically, include, three, four or five on lens on most microscopes. Objectives can be forward or rear-facing.
Nosepiece houses the objectives. The objectives are exposed and are mounted on a rotating turret so that different objectives can be conveniently selected. Standard objectives include 4x, 10x, 40x and 100x although different power objectives are available.
Coarse and Fine Focus knobs are used to focus the microscope. Increasingly, they are coaxial knobs - that is to say they are built on the same axis with the fine focus knob on the outside. Coaxial focus knobs are more convenient since the viewer does not have to grope for a different knob.
Stage is where the specimen to be viewed is placed. A mechanical stage is used when working at higher magnifications where delicate movements of the specimen slide are required.
Stage Clips are used when there is no mechanical stage. The viewer is required to move the slide manually to view different sections of the specimen.
Aperture is the hole in the stage through which the base (transmitted) light reaches the stage.
Illuminator is the light source for a microscope, typically located in the base of the microscope. Most light microscopes use low voltage, halogen bulbs with continuous variable lighting control located within the base.
Condenser is used to collect and focus the light from the illuminator on to the specimen. It is located under the stage often in conjunction with an iris diaphragm.
Iris Diaphragm controls the amount of light reaching the specimen. It is located above the condenser and below the stage. Most high quality microscopes include an Abbe condenser with an iris diaphragm. Combined, they control both the focus and quantity of light applied to the specimen.
Condenser Focus Knob moves the condenser up or down to control the lighting focus on the specimen.

Ans. B.2



phase contrast. A: Construction of the phase plate showing the relative phase shifts induced by the phase rings. B: Face view of the phase annulus (*ii*) and phase plate (*i*). C: The optical path for phase contrast illumination. **Cond:** condenser lens; **Spec:** specimen plane; **Obj:** objective lens; n_1, n_2: refractive index of the sample and background, respectively. (Figure C redrawn from Françon, 1968.)

Phase contrast microscopy imparts contrast to unstained biological material by transforming phase differences of light caused by differences in refractive index between cellular components into differences in amplitude of light, i.e., light and dark areas, which can be observed. As light rays pass through areas within the tissue of different optical path (refractive index and geometric path length) they may be retarded in phase by up to ¼Ü but will remain unchanged in amplitude. Since the eye cannot discern phase differences, a mechanism for transforming phase changes into amplitude changes is required.

In the early 1950s Zernike[13] discovered the method by which phase differences can be transformed into amplitude differences. Zernike invented what is now known as positive or dark phase contrast. An alternate method, negative or bright phase contrast, was subsequently developed and has supplanted Zernike's original approach. In positive phase contrast the object (e.g., cell component) appears darker than the surrounding background. In negative phase contrast the object appears brighter than the background.

A compound microscope equipped for negative phase contrast has two additional components: a "phase plate" that retards light exactly ¼ wavelengthin a centered, ring-shaped area located at the back focal plane of the objective lens and a matching "phase annulus" in the condenser consisting of a clear ring on a black field (Figure 2-3B). The presence of the annulus and matching phase plate causes the direct (unmodified background) light to pass only through the phase ring and thus be retarded ¼Ü. Because the light intensity of the diffracted light will be slightly diminished by absorption within the specimen, a neutral density coating on top of the phase ring attenuates undiffracted, background light to balance total illumination.

The light rays interacting with the specimen, on the other hand, diffract away from the sample as spherical waves that do not impinge on the phasing areas of the phase plate to any appreciable degree, but are focused by the objective onto the image plane. Due to a difference in optical path between the specimen and the surrounding medium, the refracted waves will be retarded in phase up to 1/4 wavelength.

Thus, in the correctly adjusted phase contrast microscope, there are two possible light paths. Light that does not interact with the specimen is collected by the objective, passes through the phase plate ring, and is retarded exactly ¼ wavelength. The phase shift is not detectable by the eye so the resulting image on the image plane in the microscope appears as a normal bright background.

Conversely, light that passes through the specimen may be diffracted by edges and local irregularities within the tissue. This refracted light will be retarded in phase by up to ¼Ü. The diffracted light will diverge from the object, fill the back focal plane of the objective, and be resolved on the image plane within the microscope. Since the background light is restricted to, and attenuated by, the relatively small annular phase plate, the light diffracted by the specimen can assume a significant role in image construction at the image plane.

Light from both possible paths (background path and specimen path) will interact at the image plane resulting in wave interference where light from the specimen interacts with light from the objective phase plate. In negative phase contrast, constructive interference occurs at the image plane. The results of this interference are bright areas in the specimen image that correspond to refractive index differences in the specimen itself (organelles, cell walls, etc.) set against a background of "normal" intensity derived from nonrefracted light.

Optical path and the "phase halo"

Phase contrast objects always have a "halo" of light (either bright around dark objects or dark surrounding bright objects), which is the result of diffracted light passing through the phase ring as well as the nonphase areas and interacting at the image plane. This halo, also referred to as shading-off, is representative only of light diffraction and interference and not of the optical path of the sample itself. That is, the halo adds artificial structure to the specimen. Intensities seen at the image plane are the result of optical path difference (refractive index plus geometric distance) within the specimen and may not necessarily represent the actual structure of the specimen.

Ans. B. 3 Classification of virus

7 class of Baltimore classification

Class	Description of genome and replication strategy	Example of bacterial virus	Example of animal virus
I	Double stranded DNA genome	Lamda,T4	Herpesvirus, poxvirus
II	Single stranded DNA genome	ØX174	Chicken anemia virus
III	Double stranded RNA genome	Ø6	Reovirus
IV	Single stranded RNA genome plus sense	MS2	Poliovirus
V	Single stranded RNA genome minus sense		Influenza virus,Rabies virus
VI	Single stranded RNA genome that replicated with DNA intermediate		Retrovirus
VII	Double stranded DNA genome that replicates with RNA intermediate		Hepatitis B virus

Ans. B. 4 Nutritional diversity of Bacteria

Nutritional type	**Energy source**	**Carbon source**	**Examples**
Photoautotroph	Light	Inorganic carbon, i.e. CO_2	Some purple and green bacteria (*Chromatium)*
Photoheterotroph	Light	Organic compounds	Some purple and green bacteria (*Rhodospirillum)*
Chemoautotroph (Lithotroph, Lithoautotroph)	Inorganic compounds; H_2, H_2S, NH_3	CO_2	Many Archaea and few bacteria (*Nitrosomanas)*
Chemoheterotroph (Heterotroph)	Organic compounds	Organic compounds	Few Arcaea and many bacteria *(Pseudomonas)*

Type	**Minimum (^{0}C)**	**Optimum (^{0}C)**	**Maximum (^{0}C)**	
Psychrophile	Below 0	10-15	Below 20	Contain **unsaturated** fatty acids in plasma membrane to tolerate.
psychrotroph	0	15-30	Above 25	Able to grow at low T but prefer moderate T
Mesophile	10-15	30-40	Below 45	Most bacteria especially the ones associated with warm-blooded animals.
Thermophile	45	45-70	Above 100	Contain **Saturated** fatty acids in plasma membrane. High glucose and carbon content as well as high melting point for DNA.
Hyperthermophile	80	80-115	Above 115	Contain phytane and modified proteins in plasma membrane. High glucose and carbon content as well as high melting point for DNA.

Type	**Aerobic condition**	**Anaerobic condition**
Obligate aerobe	Growth	No growth
Microaerophiles	Growth; when the O_2 is at very low level	No growth

Obligate anaerobe	No growth; O_2 is toxic	Growth
Facultative anaerobe/ facultative aerobe	Growth; Not essential to grow but utilized when available	Growth
Aerotolerant anaerobe	Growth; neither essential nor utilized	Growth

Type	**Speciality**
Halophile	Require NaCl for growth
Halo tolerant	Able to grow at moderate salt concentrations but grow best in the absence of NaCl
Osmophile	Able to grow in high levels of sugar
Xerophile	Able to grow in dry conditions

Ans **B.** **5**

Bacteria reproduce by **Vegetative, asexual and sexual** methods.

Vegetative reproduction includes **Budding, Fragmentation** and **Binary fission**

Budding:

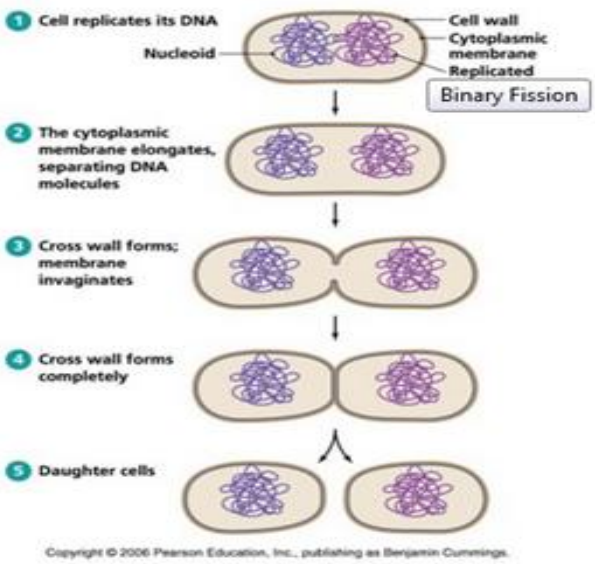
- In this case, a small protuberance, called bud, develops at one end of the cell. Genome replication follows, and one copy of the genome gets into the bud. Then the bud enlarges, eventually become a daughter cell and finally gets separated from the parent cell.

Fragmentation:

- Mostly during unfavorable conditions, bacterial protoplasm undergoes compartmentalization and subsequent fragmentation, forming minute bodies called **gonidia.** Under favorable conditions, each gonidium grows to a new bacterium. It becomes apparent that prior to fragmentation the bacterial genome has to undergo repeated replication so that each fragment gets a copy of it.

Binary fission.

- It is the commonest type of reproduction under favorable conditions in which cell divides into two similar daughter cells. During the process, the bacterial chromosomes get attached to the cell membrane and replicates to the bacterial chromosomes. As the cell enlarges the daughter chromosomes gets separated. A cross wall is formed between the separating daughter chromosomes. It divides the cell into two daughter cells. The daughter cells soon grow to maturity within 20-30 minutes. Under favorable conditions many bacteria divide once in 20-30 minutes.

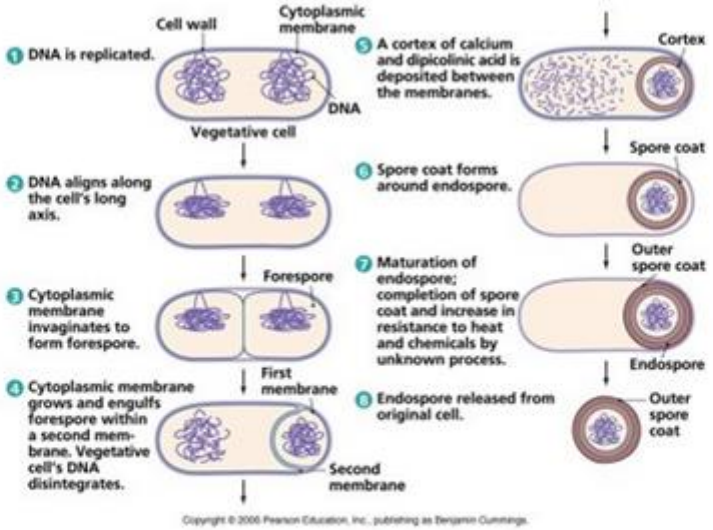


Binary Fission

Asexual reproduction

takes place by endospore formation, conidia and zoo spores.

- **Endospore formation**: Endospore are resting spores formed in some gram positive bacteria (Bacillus and Clostridium) during unfavourable conditions. They are formed within the cells. During this process a part of the protoplast becomes concentrated around the chromosome. A hard resistant wall is secreted around it. The rest of the bacterial cell degenerates; Endospore are very resistant to extreme physical conditions and chemicals. During favourable conditions the spore wall gets ruptured and the protoplasmic mass gives rise to a new bacterium.



Endospore Formation

Sexual reproduction occurs in the form of **genetic recombination.** There are three main methods of Genetic Recombination: Transformation, Transduction and Conjugation.

- **Transformation:** Here genetic material of one bacterial cell goes into another bacterial cell by some unknown mechanism and it converts one type of bacterium into another type (non capsulated to capsulated form). This was first studied by Griffith (1928) in *Diplococcus pneumonia*.
- **Transduction:** In this method, genetic material of one bacterial cell goes to other bacterial cell by agency of bacteriophages or phages (viruses, infecting bacteria). It was first of all reported in Salmonella typhimeurium by Zinder and Lederberg (1952).
- **Conjugation:** It was first reported by Lederberg and Tatum (1946) in E.coli bacteria. Cell to cell union occurs between two bacterial cells and genetic material (DNA) of one bacterial cell goes to another cell lengthwise through conjugation tube which is formed by sex pili.

Ans B. 6

1. **Fungi** are one of the most important groups of organisms on the planet. This is easy to overlook, given their largely hidden, unseen actions and growth. They are important in an enormous variety of ways.

Recycling

Fungi, together with bacteria, are responsible for most of the recycling which returns dead material to the soil in a form in which it can be reused. Without fungi, these recycling activities would be seriously reduced. We would effectively be lost under piles many metres thick, of dead plant and animal remains.

Mycorrhizae and plant growth

Fungi are vitally important for the good growth of most plants, including crops, through the development of mycorrhizal associations. As plants are at the base of most food chains, if their growth was limited, all animal life, including human, would be seriously reduced through starvation.

Food

Fungi are also important directly as food for humans. Many mushrooms are edible and different species are cultivated for sale worldwide. While this is a very small proportion of the actual food that we eat, fungi are also widely used in the production of many foods and drinks. These include cheeses, beer and wine, bread, some cakes, and some soya bean products.

While a great many wild fungi are edible, it can be difficult to correctly identify them. Some mushrooms are deadly if they are eaten. Fungi with names such as 'Destroying Angel' and 'Death Cap' give us some indication that it would not be a terribly good idea to eat them! In some countries, collecting wild mushrooms to eat is a popular activity. It is always wise to be totally sure that what you have collected is edible and not a poisonous look-a-like.

Medicines

Penicillin, perhaps the most famous of all antibiotic drugs, is derived from a common fungus calledPenicillium. Many other fungi also produce antibiotic substances, which are now widely used to control diseases in human and animal populations. The discovery of antibiotics revolutionized health care worldwide.

Some fungi which parasitise caterpillars have also been traditionally used as medicines. The Chinese have used a particular caterpillar fungus as a tonic for hundreds of years. Certain chemical compounds isolated from the fungus may prove to be useful treatments for certain types of cancer.

A fungus which parasitises Rye crops causes a disease known as Ergot. The fungus can occur on a variety of grasses. It produces small hard structures, known as sclerotia. These sclerotia can cause poisoning in humans

and animals which have eaten infected material. However, these same sclerotia are also the source of a powerful and important drug which has uses in childbirth.

Biocontrol

Fungi such as the Chinese caterpillar fungus, which parasitise insects, can be extremely useful for controlling insect pests of crops. The spores of the fungi are sprayed on the crop pests. Fungi have been used to control Colorado potato beetles, which can devastate potato crops. Spittlebugs, leaf hoppers and citrus rust mites are some of the other insect pests which have been controlled using fungi. This method is generally cheaper and less damaging to the environment than using chemical pesticides.

Crop Diseases

Fungal parasites may be useful in biocontrol, but they can also have enormous negative consequences for crop production. Some fungi are parasites of plants. Most of our common crop plants are susceptible to fungal attack of one kind or another. Spore production and dispersal is enormously efficient in fungi and plants of the same species crowded together in fields are ripe for attack. Fungal diseases can on occasion result in the loss of entire crops if they are not treated with antifungal agents.

Animal Disease

Fungi can also parasitise domestic animals causing diseases, but this is not usually a major economic problem. A wide range of fungi also live on and in humans, but most coexist harmlessly. Athletes foot and Candida infections are examples of human fungal infections.

Food Spoilage

It has already been noted that fungi play a major role in recycling organic material. The fungi which make our bread and jam go mouldy are only recycling organic matter, even though in this case, we would prefer that it didn't happen! Fungal damage can be responsible for large losses of stored food, particularly food which contains any moisture. Dry grains can usually be stored successfully, but the minute they become damp, moulds are likely to render them inedible. This is obviously a problem where large quantities of food are being produced seasonally and then require storage until they are needed.

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Ans B. 8. A thermophile is an organism — a type of extremophile — that thrives at relatively high temperatures, between 45 and 122 °C (113 and 252 °F).[1][2] Many thermophiles are archaea. Thermophilic eubacteria are suggested to have been among the earliest bacteria.[3]

Thermophiles are found in various geothermally heated regions of the Earth, such as hot springs like those in Yellowstone National Park (see image) and deep sea hydrothermal vents, as well as decaying plant matter, such as peat bogs and compost.

Unlike other types of bacteria, thermophiles can survive at much hotter temperatures, whereas other bacteria would be damaged and sometimes killed if exposed to the same temperatures.

As a prerequisite for their survival, thermophiles contain enzymes that can function at high temperatures. Some of these enzymes are used in molecular biology (for example, heat-stable DNA polymerases for PCR), and in washing agents.

"Thermophile" is derived from the Greek: θερμότητα (thermotita), meaning heat, and Greek: φίλια (philia), love.

One of the early successful commercialised examples was analytical use of a thermostable enzyme, *Taq*-polymerase, in polymerase chain reactions (PCR) for amplification of DNA, and a number of other DNA modifying enzymes from thermophilic sources have, since then, been commercialised in this area.

Another area of interest has been the prospecting for industrial enzymes for use in technical products and processes, often in a very large scale. Enzymes can be advantageous as industrial catalysts as they rarely require toxic metal ions for functionality, hence creating the possibility to use more environmentally friendly processing .

Thermostable enzymes offer robust catalyst alternatives, able to withstand the often relatively harsh conditions of industrial processing.

Conversion of biomass into sugars for *e.g*. energy utilization was a topic of concern about 30 years ago. Renewed interest in biocatalytic conversions has recently emerged, with the growing concern on the instability and possible depletion of fossil oil resources as well as growing environmental concern, and focus is again put on biorefining, and the biorefinery concept. In biorefining, renewable resources such as agricultural crops or wood are utilized for extraction of intermediates or for direct bioconversion into chemicals, commodities and fuels.

Thermostable enzymes have an obvious advantage as catalysts in these processes, as high temperatures often promote better enzyme penetration and cell-wall disorganisation of the raw materials.

By the parallel development in molecular biology, novel and developed stable enzymes also have a good chance to be produced at suitable levels. This review will discuss the potential and possibilities of thermostable enzymes, developed or isolated from thermophiles, including examples where whole cells are considered, in bioconversions of renewable raw materials with a biorefining perspective. Examples of commercial thermostable enzymes acting on renewable raw materials will be illustrated.