IQRA NATIONAL UNIVERSITY PESHAWAR

MID-TERM EXAMINATION-SPRING 2020

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Q1. Explain Structure of bacteria in detail ? also Explain some cell organelle of bacterial cell and its function.

Bacteria (singular: bacterium) are classified as prokaryotes, which are single-celled organisms with a simple internal structure that lacks a nucleus, and contains DNA that either floats freely in a twisted, thread-like mass called the nucleoid, or in separate, circular pieces called plasmids. Ribosome's are the spherical units in the bacterial cell where proteins are assembled from individual amino acids using the information encoded in ribosomal RNA.

Bacterial cells are generally surrounded by two protective coverings: an outer cell wall and an inner cell membrane. Certain bacteria, like the mycoplasmas, do not have a cell wall at all. Some bacteria may even have a third, outermost protective layer called the capsule. Whip-like extensions often cover the surfaces of bacteria — long ones called flagella or short ones called pili — that help bacteria to move around and attach to a host.

Cell organelles:

Cell Envelope:

The cell envelope is made up of two to three layers: the interior cytoplasmic membrane, the cell wall, and -- in some species of bacteria -- an outer capsule.

Cell Wall:

Each bacterium is enclosed by a rigid cell wall composed of peptidoglycan, a protein-sugar (polysaccharide) molecule. The wall gives the cell its shape and surrounds the cytoplasmic membrane, protecting it from the environment. It also helps to anchor appendages like the pili and flagella, which originate in the cytoplasm membrane and protrude through the wall to the outside. The strength of the wall is responsible for keeping the cell from bursting when there are large differences in osmotic pressure between the cytoplasm and the environment.

Cell wall composition varies widely amongst bacteria and is one of the most important factors in bacterial species analysis and differentiation. For example, a relatively thick, meshlike structure that makes it possible to distinguish two basic types of bacteria. A technique devised by Danish physician Hans Christian Gram in 1884, uses a staining and washing technique to differentiate between the two forms. When exposed to a gram stain, gram-positive bacteria retain the purple color of the stain because the structure of their cell walls traps the dye. In gram-negative bacteria, the cell wall is thin and releases the dye readily when washed with an alcohol or acetone solution.

Cytoplasm :

The cytoplasm, or protoplasm, of bacterial cells is where the functions for cell growth, metabolism, and replication are carried out. It is a gel-like matrix composed of water, enzymes, nutrients, wastes, and gases and contains cell structures such as ribosomes, a chromosome, and plasmids. The cell envelope encases the cytoplasm and all its components. Unlike the eukaryotic (true) cells, bacteria do not have a membrane enclosed nucleus. The chromosome, a single, continuous strand of DNA, is localized, but not contained, in a region of the cell called the nucleoid. All the other cellular components are scattered throughout the cytoplasm.

One of those components, plasmids, are small, extra chromosomal genetic structures carried by many strains of bacteria. Like the chromosome, plasmids are made of a circular piece of DNA. Unlike the chromosome, they are not involved in reproduction. Only the chromosome has the genetic instructions for initiating and carrying out cell division, or binary fission, the primary means of reproduction in bacteria. Plasmids replicate independently of the chromosome and, while not essential for survival, appear to give bacteria a selective advantage.

Plasmids are passed on to other bacteria through two means. For most plasmid types, copies in the cytoplasm are passed on to daughter cells during binary fission. Other types of plasmids, however, form a tube like structure at the surface called a pilus that passes copies of the plasmid to other bacteria during conjugation, a process by which bacteria exchange genetic information. Plasmids have been shown to be instrumental in the transmission of special properties, such as antibiotic drug resistance, resistance to heavy metals, and virulence factors necessary for infection of animal or plant hosts. The ability to insert specific genes into plasmids have made them extremely useful tools in the fields of molecular biology and genetics, specifically in the area of genetic engineering.

Cytoplasmic Membrane :

A layer of phospholipids and proteins, called the cytoplasmic membrane, encloses the interior of the bacterium, regulating the flow of materials in and out of the cell. This is a structural trait bacteria share with all other living cells; a barrier that allows them to selectively interact with their environment. Membranes are highly organized and asymmetric having two sides, each side with a different surface and different functions. Membranes are also dynamic, constantly adapting to different conditions.

Flagella:

Flagella (singular, flagellum) are hairlike structures that provide a means of locomotion for those bacteria that have them. They can be found at either or both ends of a bacterium or all over its

surface. The flagella beat in a propeller-like motion to help the bacterium move toward nutrients; away from toxic chemicals; or, in the case of the photosynthetic cyanobacteria; toward the light.

Nucleoid:

The nucleoid is a region of cytoplasm where the chromosomal DNA is located. It is not a membrane bound nucleus, but simply an area of the cytoplasm where the strands of DNA are found. Most bacteria have a single, circular chromosome that is responsible for replication, although a few species do have two or more. Smaller circular auxiliary DNA strands, called plasmids, are also found in the cytoplasm.

Pili:

Many species of bacteria have pili (singular, pilus), small hairlike projections emerging from the outside cell surface. These outgrowths assist the bacteria in attaching to other cells and surfaces, such as teeth, intestines, and rocks. Without pili, many disease-causing bacteria lose their ability to infect because they're unable to attach to host tissue. Specialized pili are used for conjugation, during which two bacteria exchange fragments of plasmid DNA.

Ribosome:

Ribosome are microscopic "factories" found in all cells, including bacteria. They translate the genetic code from the molecular language of nucleic acid to that of amino acids—the building blocks of proteins. Proteins are the molecules that perform all the functions of cells and living organisms. Bacterial ribosomes are similar to those of eukaryotes, but are smaller and have a slightly different composition and molecular structure. Bacterial ribosomes are never bound to other organelles as they sometimes are (bound to the endoplasmic reticulum) in eukaryotes, but are free-standing structures distributed throughout the cytoplasm. There are sufficient differences between bacterial ribosomes and eukaryotic ribosomes that some antibiotics will inhibit the functioning of bacterial ribosomes, but not a eukaryote's, thus killing bacteria but not the eukaryotic organisms they are infecting.

Q2. What is Bacterial culture media ? write down some types of bacterial culture media in detail.?

Culture media:

Culture media contain nutrients and physical growth parameters necessary for microbial growth. All microorganisms cannot grow in a single culture medium and in fact many can’t grow in any known culture medium.

Organisms that cannot grow in artificial culture medium are known as obligate parasites. Mycobacterium leprae, rickettsias, Chlamydias, and Treponema pallidum are obligate parasites. Bacterial culture media can be classified on the basis of composition, consistency and purpose.

Types:

1. General purpose media/ Basic media

Basal media are basically simple media that supports most non-fastidious bacteria. Peptone water, nutrient broth and nutrient agar (NA) are considered as basal medium. These media are generally used for the primary isolation of microorganisms.

2. Enriched medium (Added growth factors):

Addition of extra nutrients in the form of blood, serum, egg yolk etc, to basal medium makes enriched media. Enriched media are used to grow nutritionally exacting (fastidious) bacteria. Blood agar, chocolate agar, Loeffler's serum slope etc are few of the enriched media. Blood agar is prepared by adding 5-10% (by volume) blood to a blood agar base. Chocolate agar is also known as heated blood agar or lysed blood agar.

3. Selective and enrichment media:

Are designed to inhibit unwanted commensal or contaminating bacteria and help to recover pathogen from a mixture of bacteria. While selective media are agar based, enrichment media are liquid in consistency. Both these media serve the same purpose. Any agar media can be made selective by addition of certain inhibitory agents that don't affect the pathogen of interest. Various approaches to make a medium selective include addition of antibiotics, dyes, chemicals, alteration of pH or a combination of these.

4. Differential/ indicator medium: differential appearance:

Certain media are designed in such a way that different bacteria can be recognized on the basis of their colony colour. Various approaches include incorporation of dyes, metabolic substrates etc, so that those bacteria that utilize them appear as differently coloured colonies. Such media are called differential media or indicator media. Differential media allow the growth of more than one microorganism of interest but with morphologically distinguishable colonies.

5. Transport media:

Clinical specimens must be transported to the laboratory immediately after collection to prevent overgrowth of contaminating organisms or commensals. This can be achieved by using transport media. Such media prevent drying (desiccation) of specimen, maintain the pathogen to commensal ratio and inhibit overgrowth of unwanted bacteria. Some of these media (Stuart's & Amie's) are semi-solid in consistency. Addition of charcoal serves to neutralize inhibitory factors.

6. Anaerobic media:

Anaerobic bacteria need special media for growth because they need low oxygen content, reduced oxidation –reduction potential and extra nutrients.

Media for anaerobes may have to be supplemented with nutrients like hemin and vitamin K. Such media may also have to be reduced by physical or chemical means. Boiling the medium serves to expel any dissolved oxygen. Addition of 1% glucose, 0.1% thioglycollate, 0.1% ascorbic acid, 0.05% cysteine or red hot iron filings can render a medium reduced. Before use the medium must be boiled in water bath to expel any dissolved oxygen and then sealed with sterile liquid paraffin.

Q3.What is the difference between Sterilization and disinfection ? write down some methods used for sterilization ?

Sterilization:

Definition:

To sterilize means to kill all microbes whether harmful or not and their spores present on a surface or object.

Methods:

Heat, chemicals, irradiation, high pressure, and filtration.

Types:

Steam, heating, chemical sterilization, radiation sterilization, sterile filtration**.**

Application:

Sterilization is used for food, medicine and surgical instruments.

Disinfect:

To disinfect means to eliminate most harmful microorganisms (not including their spores) from surfaces or objects; inactivate viruses.

Methods:

Phenolic disinfectants, heavy metals, halogens (e.g. chlorine), bleach, alcohols, hydrogen peroxide, detergents, heating and pasteurization.

Types:

Air disinfectants, alcohols, aldehydes, oxidizing agents, phenolics.

Application:

Disinfection is used mostly to decontaminate surfaces and air.

Methods use for sterilization:

Steam:

Used in machines called autoclaves. Autoclaves use steam heated to 121–134 °C (250–273 °F). To achieve sterility, a holding time of at least 15 minutes at 121 °C (250 °F) or 3 minutes at 134 °C (273 °F) is required. Autoclave treatment inactivates all fungi, bacteria, viruses and also bacterial spores. Pressure cooking food is also steam sterilization though it is not that thorough.

Heating:

Under heating flaming, incineration, boiling in water, tindalization, dry heat. These methods inactivate and kill microorganisms in objects like glass, metals. Boiling in water for 15min inactivates viruses and kills most vegetative bacteria. However it has no effect on the spores. Tindilization means boiling for 20 minutes and then cooling, again re-boiling and cooling for three times. This method is more effective on sporulating bacteria than just boiling. Dry heat method can be used on powders and items that bear very high them of heat.

Chemical sterilization:

Chemicals like Ethylene oxide, Ozone, Bleach, Glutaraldehyde and Formaldehyde, Phthalaldehyde, Hydrogen Peroxide, Dry sterilization process, Peracetic acid and Silver are used in varying degrees. Products that can get damaged due to heat are subjected to chemical sterilization for e.g. biological materials, fiber optics, electronics, and plastics. Ethylene oxide gas and Ozone gas oxidize most organic matter. Though bleach and Glutaraldehyde and formaldehyde solutions is used as a disinfectant, it’s a much more concentrated in sterilization also infected item is left immersed for long duration for effective sterilization. Dry sterilization process with chemicals is useful for sterilizing plastic bottles medical and pharmaceutical applications.

Radiation sterilization:

Electron beams, X-rays, gamma rays, or subatomic particles are used for sterilizing disposable medical equipment, such as syringes, needles, cannulas, IV sets and biological safety cabinets between uses.

Sterile filtration:

Clear liquids that would be damaged by heat, irradiation or chemical sterilization can be sterilized by mechanical filtration. Fileration is done through pores that are smaller in size than the organism in question and this has to be done very slowly.

Q4. Write a note on Structure of fungi in detail ?

Except for yeasts, which grow as single cells, most fungi grow as thread-like filaments. The filaments are called hyphae (singular, hypha). Each hypha consists of one or more cells surrounded by a tubular cell wall. A mass of hyphae make up the body of a fungus, which is called a mycelium (plural, mycelia).

The hyphae of most fungi are divided into cells by internal walls called septa (singular, septum). Septa usually have little pores that are large enough to allow ribosomes, mitochondria and sometimes nuclei to flow among cells. Hyphae that are divided into cells are called septate hyphae. However, the hyphae of some fungi are not separated by septa. Hyphae without septae are called coenocytic hyphae. Coenocytic hyphae are big, multinucleated cells.

A mycelium may range in size from microscopic to very large. In fact, one of the largest living organisms on Earth is the mycelium of a single fungus. The giant fungus covers 8.9 square kilometers (3.4 square miles) in an Oregon forest. That's about the size of a small city. The fungus didn't grow that large overnight. It's estimated to be 2,400 years old, and it's still growing.

Q5. What are few Hospital based infections that can be transfer to others due to un hygienic condition ? Explain with an example ?

Health care facilities whether hospitals, nursing homes, or outpatient facilities can be dangerous places for the acquisition of infections. The most common type of nosocomial infections are surgical wound infections, respiratory infections, genitourinary infections, as well as gastrointestinal infections.

These infections are often caused by breaches of infection control practices and procedures, unclean and non-sterile environmental surfaces, and/or ill employees. We have the experience to understand and ascertain the facts behind these hospital acquired nosocomial infections.

With nearly 100 million procedures performed at hospitals each year, litigation arising from nosocomial infections is increasing nationwide. These infections can be acquired in the hospital, nursing home, rehabilitation centers, as well as extended care facilities. Immunocompromised patients, the elderly and young children are usually more susceptible than others. These infections are transmitted through direct contact from the hospital staff, inadequately sterilized instruments, aerosol droplets from other ill patients or even the food or water provided at hospitals. EHA provides litigation expertise in the areas of infections contracted in the healthcare environment.

Example:

According to the CDC, the most common pathogens that cause nosocomial infections are:

Staphylococcus aureus, Pseudomonas aeruginosa, and E. coli. Some of the common nosocomial infections are urinary tract infections, respiratory pneumonia, surgical site wound infections, bacteremia, gastrointestinal and skin infections.

Nosocomial infections are not just limited to bacteria; certain fungi such as Candida albicans and aspergillus, as well as, viruses such as Respiratory Syncytial Virus and influenza have also been implicated in a number of hospital acquired infections.