Benha university **Microbial ecology**
Faculty of Science **Final examination**
Botany Department **Micro.and entomology students**
2012-2013 **Fourth year**

Answer the following questions

1- A particular sites of human body are microenvironments for different species of bacteria refered as normal flora. Discuss ten species of this flora.

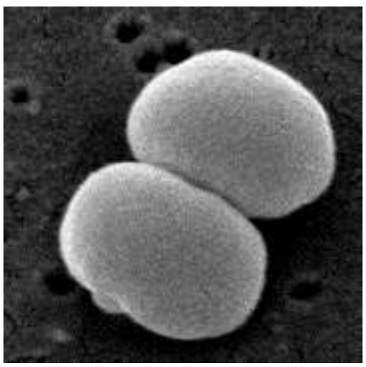
2- Explain the following
a-Basic environments and alkalophiles
b-Hyper saline environments and halophiles
c- Deep sea and barophiles
d-Mechanism of extremophiles behavior

3- Give an account on microbial interaction with plants. Write with drawing.

With best my wishes

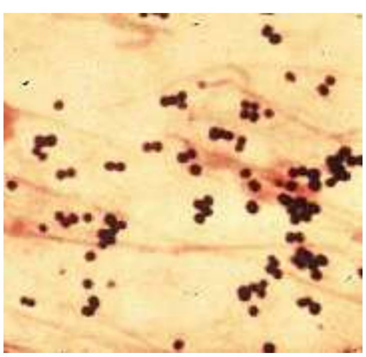
اجابة السؤال الاول

(1) The staphylococci and corynebacteria occur at every site listed. *Staphylococcus epidermidis* is highly adapted to the diverse environments of its human host. *S. aureus* is a potential pathogen. It is a leading cause of bacterial disease in humans. It can be transmitted from the nasal membranes of an asymptomatic carrier to a susceptible host.



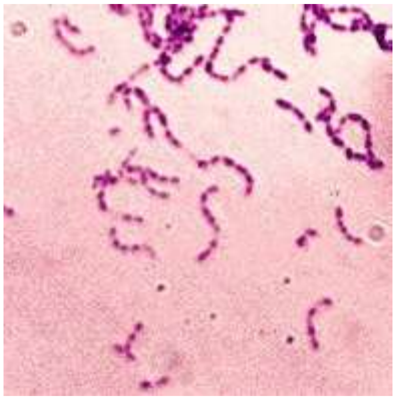
S. epidermidis. Scanning EM. CDC.

(2) Many of the normal flora are either pathogens or opportunistic pathogens, The asterisks indicate members of the normal flora a that may be considered major pathogens of humans.



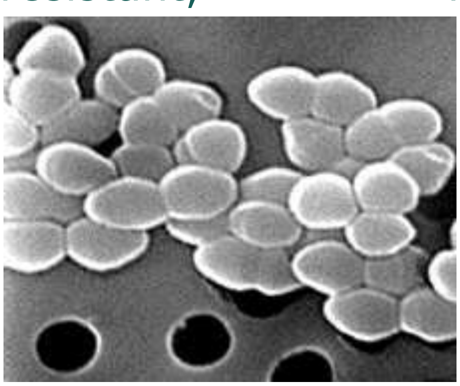
S. aureus. Gram stain.

(3) *Streptococcus mutans* is the primary bacterium involved in plaque formation and initiation of dental caries. Viewed as an opportunistic infection, dental disease is one of the most prevalent and costly infectious diseases in the United States.



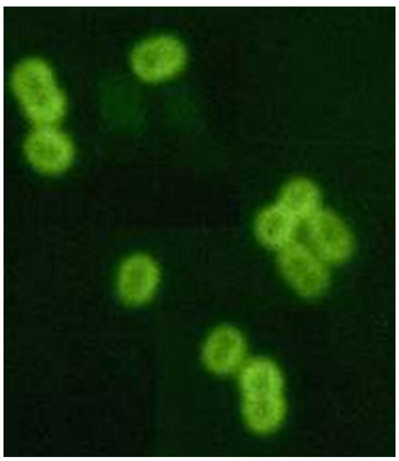
Streptococcus mutans. Gram stain. CDC

(4) *Enterococcus faecalis* was formerly classified as *Streptococcus faecalis.* The bacterium is such a regular a component of the intestinal flora, that many European countries use it as the standard indicator of fecal pollution, in the same way we use *E. coli* in the U.S. In recent years, *Enterococcus faecalis* has emerged as a significant, antibiotic-resistant, nosocomial pathogen.



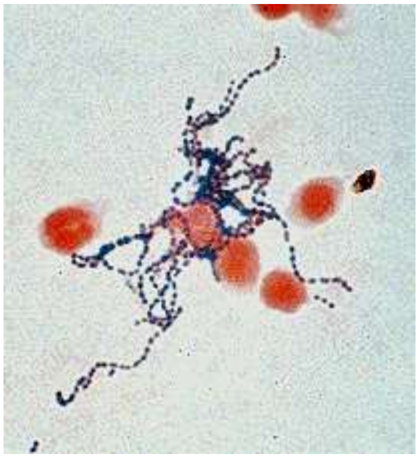
Vancomycin Resistant *Enterococcus faecalis.* Scanning E.M. CDC

(5) *Streptococcus pneumoniae* is present in the upper respiratory tract of about half the population. If it invades the lower respiratory tract it can cause pneumonia. *Streptococcus pneumoniae* causes 95 percent of all bacterial pneumonia.



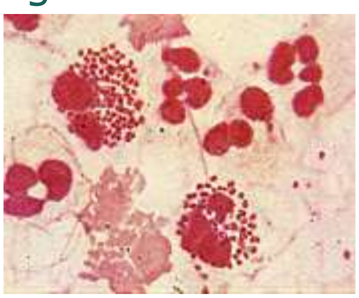
Streptococcus pneumoniae. Direct fluorescent antibody stain. CDC.

(6) *Streptococcus pyogenes* refers to the Group A, Beta-hemolytic streptococci. Streptococci cause tonsillitis (strep throat), pneumonia, endocarditis. Some streptococcal diseases can lead to rheumatic fever or nephritis which can damage the heart and kidney.



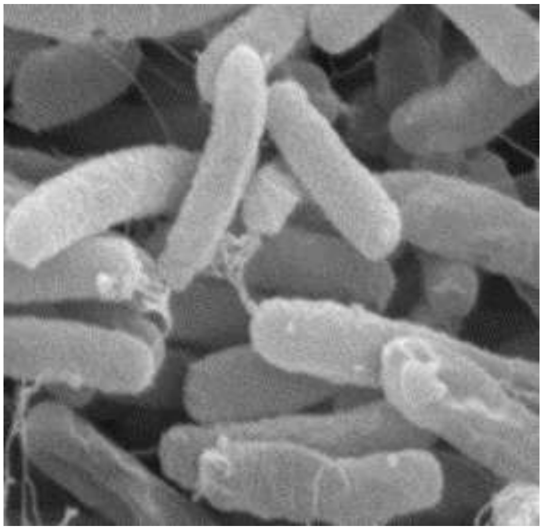
Streptococcus pyogenes. Gram stain.

(7) *Neisseria* and other Gram-negative cocci are frequent inhabitants of the upper respiratory tract, mainly the pharynx. *Neisseria meningitidis,* an important cause of bacterial meningitis, can colonize as well, until the host can develop active immunity against the pathogen.



Neisseria meningitidis. Gram stain.

(8) While *E. coli* is a consistent resident of the small intestine, many other enteric bacteria may reside here as well, including *Klebsiella, Enterobacter* and *Citrobacter.* Some strains of *E. coli* are pathogens that cause intestinal infections, urinary tract infections and neonatal meningitis.



E. coli. Scanning E.M. Shirley Owens. Center for Electron Optics. Michigan State University.

(9) *Pseudomonas aeruginosa* is the quintessential opportunistic pathogen of humans that can invade virtually any tissue. It is a leading cause of hospital-acquired (nosocomial) Gram-negative infections, but its source is often exogenous (from outside the host).

Colonies of *Pseudomonas aeruginosa* growing on an agar plate. The most virulent Pseudomonas species produce mucoid colonies and green pigments such as this isolate.

(10) *Haemophilus influenzae* is a frequent secondary invader to viral influenza, and was named accordingly. The bacterium was the leading cause of meningitis in infants and children until the recent development of the Hflu type B vaccine.

Haemophilus influenzae. Gram stain.

اجابة السؤال الثانى

Basic Environments and Alkaliphiles

Alkaliphilic microorganisms are widespread in nature. Bacteria (e.g. species of the genus Bacillus) that grow at pH 9–10 while being unable to grow at neutral pH can easily be isolated from soils. High pH conditions are common in the surface layers of productive freshwater lakes when CO2 becomes depleted during daytime photosynthesis, and many planktonic eukaryotic algae as well as cyanobacteria are adapted to an existence at high pH, at least temporarily. However, most true alkaliphiles known have been isolated from permanently alkaline lakes (‘‘soda lakes’’) in which the high pH is caused by geological-geochemical rather than by biological processes. Such lakes are found on all continents, examples being Mono Lake, California, Lake Magadi and other East African soda lakes, theWadi Natrun lakes in Egypt, and soda lakes in China and Tibet. Many of these alkaline lakes are characterized by high salt concentrations as well, so that their inhabitants should have alkaliphilic as well as halophilic properties. Part of the described members of the Halobacteriaceae, the family of halophiles par excellence, are obligatory alkaliphilic as well (Oren, 2002). Alkaliphiles are thus found in each of the three domains of life: Archaea, Bacteria, and Eukarya. Alkaliphilic behavior is also not limited to cells with a particular mode of life, so that even the most extremely alkaline lakes appear to support complete cycling of carbon, nitrogen and sulfur (Zavarzin and Zhilina, 2000; Zhilina and Zavarzin, 1994).

Hypersaline Environments and Halophiles

The seas that cover nearly 70% of the surface of planet Earth contain about 35 g l_1 dissolved salt. Hypersaline environments are easily formed when seawater dries up in coastal lagoons and salt marshes, as well as in manmade evaporation ponds of saltern systems built to produce common salt by evaporation of seawater. There are also inland saline lakes in which the salt concentration can reach values close to saturation. Well-known

examples are the Great Salt Lake, Utah – a lake in which the ionic composition of the salts resembles that of seawater, and the Dead Sea on the border between Israel and Jordan – a lake dominated by magnesium rather than by sodium as the most abundant cation. Furthermore, there are extensive underground deposits of rock salt that originated by the drying up of closed marine basins. All of these environments, as well as others such as saline soils, provide a habitat for salt-adapted microorganisms, obligate halophiles as well as halotolerant types, that can adjust to life over a wide range of salt concentrations.

The pink to red-purple color that characterizes brines approaching NaCl saturation provides direct evidence of the massive numbers of pigmented halophilic microorganisms often found in such environments. Halophiles adapted to life at salt concentrations up to saturation are found in all three domains of life. Dunaliella, a genus of unicellular green algae, is found worldwide in salt lakes and salterns. The archaeal order Halobacteriales is entirely composed of highly salt-requiring species, most of which are colored red due to carotenoid pigments as well as by retinal pigments (bacteriorhodopsin, halorhodopsin) (Oren, 2000, 2002). In addition, many phylogenetic branches of the domain Bacteria contain halophilic or highly halotolerant representatives, and these inhabit a variety of hypersaline environments. Physiologically the halophilic world is highly diverse, as it encompasses aerobic and anaerobic

heterotrophs, fermentative organisms, sulfate reducers, cyanobacteria, as well as anoxygenic photosynthetic sulfur bacteria (Halochromatium, Halorhodospira) (Oren, 2002). The recent discovery of Salinibacter, a red bacterium affiliated with the Cytophaga/Flavobacterium/Bacteroides branch of the Bacteria (Anto´n et al., 2002), shows that adaptation to the highest salt concentrations is not limited to the archaeal domain

The Deep Sea and Barophiles/Piezophiles

The hydrostatic pressure in the sea increases by about 1 atmosphere (0.1 MPa) every 10 meters. The mean depth of the oceans is about 4 km, equivalent to a pressure of 400 atmospheres or 40 MPa, and the deepest parts of the oceans are more than 10 km deep. Microorganisms living in such environments have to withstand pressures of over a thousand atmospheres.

Some microbial isolates from the deep sea (obligate barophiles or piezophiles) cannot live at normal atmospheric pressure, and these require a pressurized environment for growth. Bacterial isolates related to Shewanella and Moritella (g-Proteobacteria), recovered from a depth of up to 11 km in the Mariana Trench, the Japan Trench and the Philippine Trench, require pressures of 70–80 MPa for optimal growth and do not grow below 50 MPa (Kato and Bartlett, 1997; Kato et al., 1998). Strain MT41, isolated by Yayanos et al. (1981) from the Mariana Trench, is probably the most barophilic of all isolates characterized to date: it does not grow at pressures lower than 50 MPa, has its optimum at 70 MPa, and tolerates pressures of at least 100 MPa (see also Yayanos, 2000).

As the deep sea is also a cold environment, barophiles generally exhibit psychrophilic properties as well. Thermophilic barophilic or barotolerant bacteria are associated with deep-sea hot vents. Thermococcus barophilus, a hyperthermophile isolated from a Mid-Atlantic Ridge hydrothermal vent, requires high pressure when grown at the highest temperatures, and can then grow up to 100_C and higher (Marteinsson et al., 1999).

THE MECHANISMS EXTREMOPHILE BEHAVIOR

An in-depth discussion of the mechanisms that enable the different categories of extremophilic microorganisms to survive and to grow in environments apparently hostile to life is outside the scope of this book; extensive information about this topic can be found in monographs, multi-author review books and review articles cited above. One general aspect, however, deserves to be discussed here, and that is the issue of the nature of the intracellular environment of the cell. In some cases, the intracellular environment is not at all exposed to the stress factor that makes the environment outside the cell so extreme. This is due to the properties of the cell membrane and the activity of energy-dependent processes that allow homeostasis of the intracellular milieu at a level that is far less stressful than the medium outside the cell. In other cases, the cells are unable to exclude the stressful factor from their cytoplasm, and all intracellular components have therefore to be functional at the environmental extremes. Which of these two strategies is used depends on the nature of the stress factor involved and sometimes on the type of organism as well. In the case of temperature (low as well as high) and hydrostatic pressure, no microorganism is able to regulate the intracellular values at a level different from that of their environment. All enzymes of a hyperthermophile should be active at the ambient temperature to enable growth of the cell, and the same is true for a psychrophile that grows in the cold deep sea or in Arctic or Antarctic environment. On the other hand, none of the known acidophiles and alkaliphiles allows the intracellular pH to equilibrate with the low or high extracellular pH, respectively, and intracellular pH is always maintained at a value close to neutrality. A very low permeability of the membrane to protons and absence of uncontrolled proton movement are first prerequisites for any acidophile or alkaliphile. In fact these properties are not at all unusual: a low proton permeability of the membrane is a basic property for any cell, as its bioenergetic processes are based on the generation and exploitation of transmembrane proton gradients. The only components of the cell that thus require special adaptation to the environmental stress are the cytoplasmic membrane, periplasmic proteins, and (if present) exoenzymes excreted from the cell.

The halophiles present us with an interesting case of two fundamentally different strategies that microorganisms have developed to allow life at the extremely high osmotic pressures exerted by salt-saturated brines. No halophilic microorganism can maintain a dilute cytoplasm because of the high permeability of the cytoplasmic membrane to water: a cell with a cytoplasm of high water activity would immediately lose water and dry out. One strategy is based on the accumulation of salts inside the cells at concentrations no less than those in the outside medium, the other on exclusion of salts from the cytoplasm to a large extent and production or accumulation of organic solutes to balance the osmotic pressure of the medium.

A similar case in which resistance to environmental stress can be achieved in multiple ways is ultraviolet radiation. Some cyanobacteria are highly UV radiation-resistant thanks to the presence of the UVabsorbing pigment scytonemin in the sheath that surrounds the cell. Others accumulate UV-absorbing pigments (mycosporine-like amino acids) within their cytoplasm, and thus protect their DNA from radiation damage (Castenholz and Garcia-Pichel, 2000). The prodigious resistance of Deinococcus radiodurans to radioactive and other forms of ionizing radiation cannot be explained by such protective compounds, and here the explanation must rather be sought in effective repair mechanisms as well as special ways of packaging of the DNA (Englander et al., 2004). In addition, resistance to desiccation is probably key to the survival of ionizing radiation resistant life forms. Many of these organisms also produce extracellular polysaccharides as a protection against extreme dryness.

اجابةالسؤال الرابع

Microbial interaction with plants

- Lichen: Symbiosis between algae and fungi

- Mycorrhizae: Symbiosis between plants and fungi

- Root nodule: Symbiosis between legumes and nitrogen-fixing bacteria

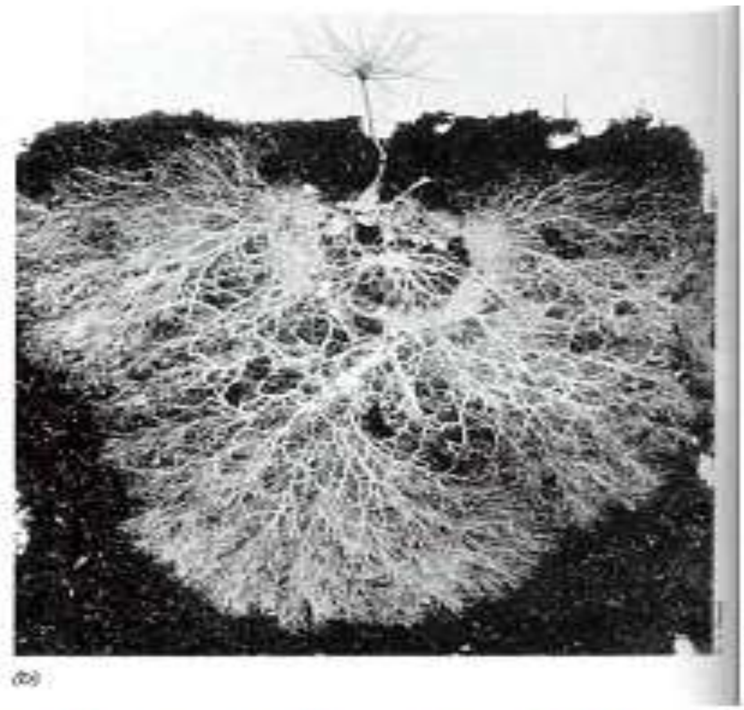
Mycorrhizae

- Symbiosis of plant roots and fungi

- Hypothesis: All terrestrial plants live mycorrhizal

- Ectomycorrhizae - Endomycorrhizae

- Fungi benefit from release of organic substances by the plant
    - Plant benefit from increased surface and protection again other plants



Typical ectomycorrhizal root of a pine with *Thelophora terrestris* (fungi)

Root nodules: Symbiosis between plants and N_2-fixing bacteria

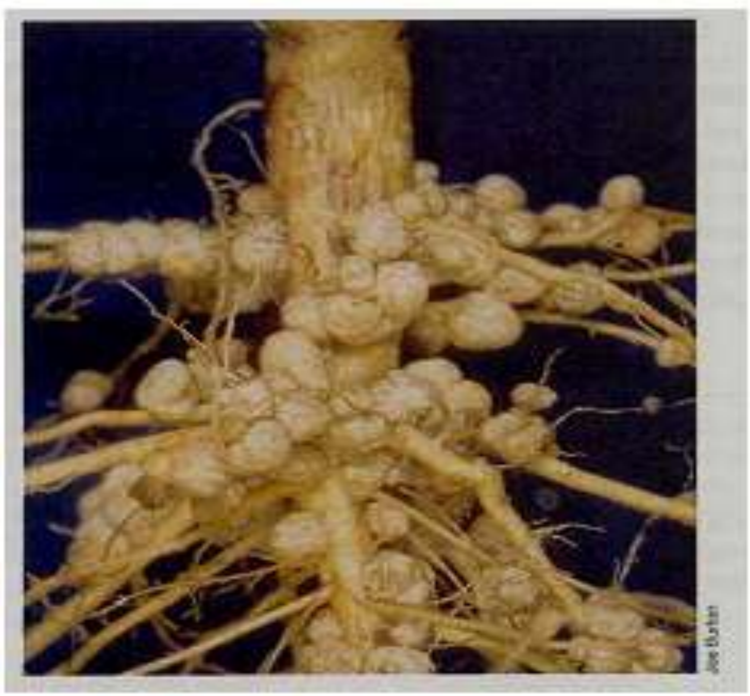
- Symbiosis between legumes and certain, mainly Gram-negative bacteria

- Happen in many agricultural important plant like soybean, bean und pea

- Fixation of N_2 in special nodules connected to the roots

- Selcetion advantage by growth on nutrient poor soil

- Hots plants and bacteria synthesize together O_2-binding leghemoglobin (red colour)



Root nodules of a soybean
(*Bradyrhizobium japonicum*)

Fixation of elemental nitrogen gas (N_2)

Only performed by prokaryotes

Occurs in free-living or symbiotic-associated microorganisms

Reduction of N_2 to ammonium (utilized for anabolism)

Requires high amount of energy (Cleavage of a triple-bonds)

Catalyzed by the enzyme-complex **Nitrogenase**

Oxygen changes(irreversible) function of Dinitrogenase-Reductase

Different protection mechanisms to prevent O_2-Inactivation:

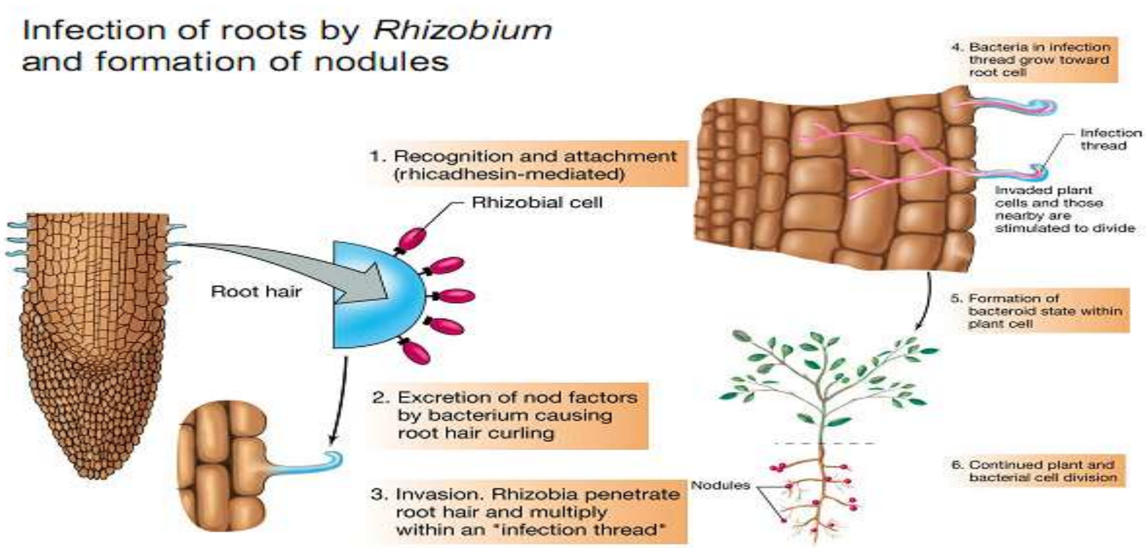
a) Rapid removing of O_2 by high respiration rates

b) Slimeformation (eg. Azotobacter)

c) Specific cells/compartements (eg. Cyanobakterien)

Ecological advantage: Growth in environments with low or no nitrogen source

Infection of roots by Rhizobium and formation of nodules



Symbiosis between plants and N_2-fixing bacteria

Legumes	Bacteria
Soy bean, bean	*Rhizobium, Bradyrhizobium*
Pea	*Rhizobium*
Clover	*Rhizobium*
Other plants	
Alder (*Alnus*)	*Frankia*
Sugarcane	*Acetobacter*