Benha University
Faculty of Science
Botany Department

Credit hour students
Summer Term 2016
Time: 2 hrs.

General Microbiology (291B) Exam

Answer the following questions:

1. **12 mark**

a) What is the function of the following in the bacterial cell structure:

i. Cell wall. ii. Flagella. iii. Capsule.

b) Complete the following:

i. *Bacilli* are ……shaped bacteria.

ii. The groups of bacteria which require free of oxygen for growth are called……..

iii. Antibodies are…….

iv. Microbial pathogenicity is a character of the ……..of microbe.

v. The Hcl acid of stomach is…….....and considered one of the chemical barriers of ……..defense mechanisms.

vi. The common types of antigens are……..,……and……

2. **12 mark**

a) Explain one only of the following:

i. Mode of nutrition of bacteria.

ii. Economic importance of fungi.

b) Write briefly on two only of the following:

i. Microbial virulence.

ii. Staphylococcal infections.

iii. Diagnosis of *Streptococcus pyogenes.*

"With my best wishes"

Dr. Mohamed Atef

The model answer for General Microbiology (291B) Exam (2/9/2016).

1. **12 mark**

a) What is the function of the following in the bacterial cell structure:

i. Cell wall. ii. Flagella. iii. Capsule.

i. Cell wall protects the, delicate, cytoplasm membrane and maintains the characteristic shape of the micro-organism. It also plays an essential role in cell division.
ii. Flagella are considered to be organs of locomotion.
iii. Capsule affords the cell some protection against drying; it may also protect the bacteria from the normal body defenses against invasion.

b) Complete the following

i. Rod
ii. Aerobes
iii. Antibodies are specialized serum protein capable to react specifically with antigens that stimulated their production. These antibodies called immunoglobulin.
iv. Species
v. Bactericidal , nonspecific host
vi. Microbial antigens, tissue antigen , some drugs and food antigens

2. **12 mark**

a) Explain one only of the following:

i. Mode of nutrition of bacteria.
ii. Economic importance of fungi.

i. The mode of nutrition of bacteria can divide into two categories:

1- Autotrophs :

Such organisms can build up complex substances (proteins and carbohydrates) from simple inorganic source such as CO_2

a) Photosynthetic bacteria:

Special groups of bacteria example, the purple bacteria contain red and green pigments (bacteriochlorophyll and carotenoids) and with the help of these, they can synthesis carbohydrates from the atmospheric carbon dioxide in the presence of sunlight (usually they utilize infrared waves).

All bacterial photosynthesis differs from photosynthesis of higher plants in that oxygen is not liberated.

b) Chemosynthetic bacteria:

These bacteria bring about the oxidation of certain inorganic or simple organic substances. These oxidation reactions are all exothermic and the energy thus liberated is

used in the synthesis of carbohydrates from the atmospheric carbon dioxide. The energy required for the assimilation of carbon comes from chemical reactions, hence the process here is known as chemosynthesis. These are several types of chemosynthetic bacteria according to the oxidation reactions brought about by them.

The most important chemosynthetic bacteria are:

1) The nitrifying bacteria.
2) The iron bacteria
3) Sulphur oxidizing bacteria.
4) Hydrogen oxidizing bacteria.

2- Heterptrophs :

These are the bacteria which cannot make up their own food from simple inorganic sources. These microorganisms derive their energy primarily through the chemical breakdown of more complex food materials especially in the form of organic matter.

• Heterotrophs which obtain their food from dead organic matter are called saprophytes.
• Others which obtain their food from living organisms are called, parasites.

Some heterotrophs live in a symbiotic relation sharing benefits with other living organisms such as Rhizobium (nodule bacteria) which lives in symbiosis with the root of legumes forming root nodules.

ii. Economic importance of fungi.

- **As 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.

- **Medical uses**

  Penicillin, perhaps the most famous of all antibiotic drugs, is derived from a common fungus called *Penicillium.* 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.

- **Biocontrol**

  Fungi such as the Chinese caterpillar fungus, which parasitise insects, can be extremely useful for controlling insect pests of crops.

- **Soil fertility**

  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.

b) Write briefly on <u>two</u> only of the following:

i. Microbial virulence.
ii. Staphylococcal infections.

iii. Diagnosis of *Streptococcus pyogenes*

i. Microbial virulence is a character of individual strains with species.
So a certain strain of diphtheria bacillus is capable of producing severe disease if it is highly virulent, while another strain produces a mild disease if it is of low virulence.
Virulence factors help bacteria to (1) invade the host, (2) cause disease, and (3) evade host defenses. The following are some types of virulence factors:
Adherence Factors: Many pathogenic bacteria colonize mucosal sites by using *pili* (fimbriae) to adhere to cells.
Capsules: Many bacteria are surrounded by capsules that protect them from opsonization and phagocytosis.
Endotoxins: The lipopolysaccharide endotoxins on Gram-negative bacteria cause fever, changes in blood pressure, inflammation, lethal shock, and many other toxic events.

ii. Staphylococcal infections.

Staph infections may cause disease due to direct infection or due to the production of toxins by the bacteria.

1. Production of toxin:
   - Toxic shock syndrome
   - Staphylococcal food poisoning
2. Direct invasion: localized skin infection as abscesses and boils

iii. Diagnosis of *Streptococcus pyogenes*

- Specimens: swabs from throat or other lesions, pus.
- Direct smear: stained by gram is G + ve.
- Culture: done on blood agar show colonies producing complete haemolysis.

Dr. Mohamed Atef

Part B

2. **Penicillin** (PCN or pen) is a group of antibiotics which include penicillin G (intravenous use), penicillin V (oral use), procaine penicillin, and benzathine penicillin (intramuscular use). Penicillin antibiotics were among the first medications to be effective against many bacterial infections caused by staphylococci and streptococci. Penicillins are still widely used today, though many types of bacteria have developed resistance following extensive use.

About 10% of people report that they are allergic to penicillin; however, up to 90% of this group may not actually be allergic. Serious allergies only occur in about 0.03%. All penicillins are β-lactam antibiotics.

Penicillin was discovered in 1928 by Scottish scientist Alexander Fleming. People began using it to treat infections in 1942. There are several enhanced penicillin families which are effective against additional bacteria; these include the antistaphylococcal penicillins, aminopenicillins and the antipseudomonal penicillins. They are derived from *Penicillium* fungi.

The term "penicillin" is often used generically to refer to benzylpenicillin (penicillin G, the original penicillin found in 1928), procaine benzylpenicillin (procaine penicillin), benzathine benzylpenicillin (benzathine penicillin), and phenoxymethylpenicillin (penicillin V). Procaine penicillin and benzathine penicillin have the same antibacterial activity as benzylpenicillin but act for a longer period of time. Phenoxymethylpenicillin is less active against gram-negative bacteria than benzylpenicillin. Benzylpenicillin, procaine penicillin and benzathine penicillin are given by injection (parenterally), but phenoxymethylpenicillin is given orally.

The term "penam" is used to describe the common core skeleton of a member of the penicillins. This core has the molecular formula R-$C_9H_{11}N_2O_4S$, where R is the variable side chain that differentiates the penicillins from one another. The penam core has a molecular weight of 243 g/mol, with larger penicillins having molecular weights near 450—for example, cloxacillin has a molecular weight of 436 g/mol. The key structural feature of the penicillins is the four-membered β-lactam ring; this structural moiety is essential for penicillin's antibacterial activity. The β-lactam ring is itself fused to a five-membered thiazolidine ring. The fusion of these two rings causes the β-lactam ring to be more reactive than monocyclic β-lactams because the two fused rings distort the β-lactam

amide bond and therefore remove the resonance stabilisation normally found in these chemical bonds.

Penicillin is a secondary metabolite of certain species of *Penicillium* and is produced when growth of the fungus is inhibited by stress. It is not produced during active growth. Production is also limited by feedback in the synthesis pathway of penicillin.

> α-ketoglutarate + AcCoA → homocitrate → L-α-aminoadipic acid → L-lysine + β-lactam

The by-product, L-lysine, inhibits the production of homocitrate, so the presence of exogenous lysine should be avoided in penicillin production.

The *Penicillium* cells are grown using a technique called fed-batch culture, in which the cells are constantly subject to stress, which is required for induction of penicillin production. The available carbon sources are also important: Glucose inhibits penicillin production, whereas lactose does not. The pH and the levels of nitrogen, lysine, phosphate, and oxygen of the batches must also be carefully controlled.

The biotechnological method of directed evolution has been applied to produce by mutation a large number of *Penicillium* strains. These techniques include error-prone PCR, DNA shuffling, ITCHY, and strand-overlap PCR. Semisynthetic penicillins are prepared starting from the penicillin nucleus 6-APA.

Overall, there are three main and important steps to the biosynthesis of penicillin G (benzylpenicillin).

- The first step is the condensation of three amino acids—L-α-aminoadipic acid, L-cysteine, L-valine into a tripeptide. Before condensing into the tripeptide, the amino acid L-valine must undergo epimerization to become D-valine. The condensed tripeptide is named δ-(L-α-aminoadipyl)-L-cysteine-D-valine (ACV). The condensation reaction and epimerization are both catalyzed by the enzyme δ-(L-α-aminoadipyl)-L-cysteine-D-valine synthetase (ACVS), a nonribosomal peptide synthetase or NRPS.
- The second step in the biosynthesis of penicillin G is the oxidative conversion of linear ACV into the bicyclic intermediate isopenicillin N by isopenicillin N synthase (IPNS), which is encoded by the gene *pcbC*. Isopenicillin N is a very weak intermediate, because it does not show strong antibiotic activity.[57]
- The final step is a transamidation by isopenicillin N N-acyltransferase, in which the α-aminoadipyl side-chain of isopenicillin N is removed and exchanged for a phenylacetyl side-chain. This reaction is encoded by the gene *penDE*, which is unique in the process of obtaining penicillins.

3. Fermentation is a metabolic process that converts sugar to acids, gases, or alcohol. It occurs in yeast and bacteria, and also in oxygen-starved muscle cells, as in the case of lactic acid fermentation. Fermentation is also used more broadly to refer to the bulk growth of microorganisms on a growth medium, often with the goal of producing a specific chemical product. French microbiologist Louis Pasteur is often remembered for his insights into fermentation and its microbial causes. The science of fermentation is known as zymology.

Fermentation takes place when the electron transport chain is unusable (often due to lack of a final electron receptor, such as oxygen), and becomes the cell's primary means of ATP (energy) production. It turns NADH and pyruvate produced in glycolysis into NAD^+ and an organic molecule (which varies depending on the type of fermentation; see examples below). In the presence of O_2, NADH and pyruvate are used to generate ATP in respiration. This is called oxidative phosphorylation, and it generates much more ATP than glycolysis alone. For that reason, cells generally benefit from avoiding fermentation when oxygen is available, the exception being obligate anaerobes which cannot tolerate oxygen.

The first step, glycolysis, is common to all fermentation pathways:

$$C_6H_{12}O_6 + 2\ NAD^+ + 2\ ADP + 2\ P_i \rightarrow 2\ CH_3COCOO^- + 2\ NADH + 2\ ATP + 2\ H_2O + 2H^+$$

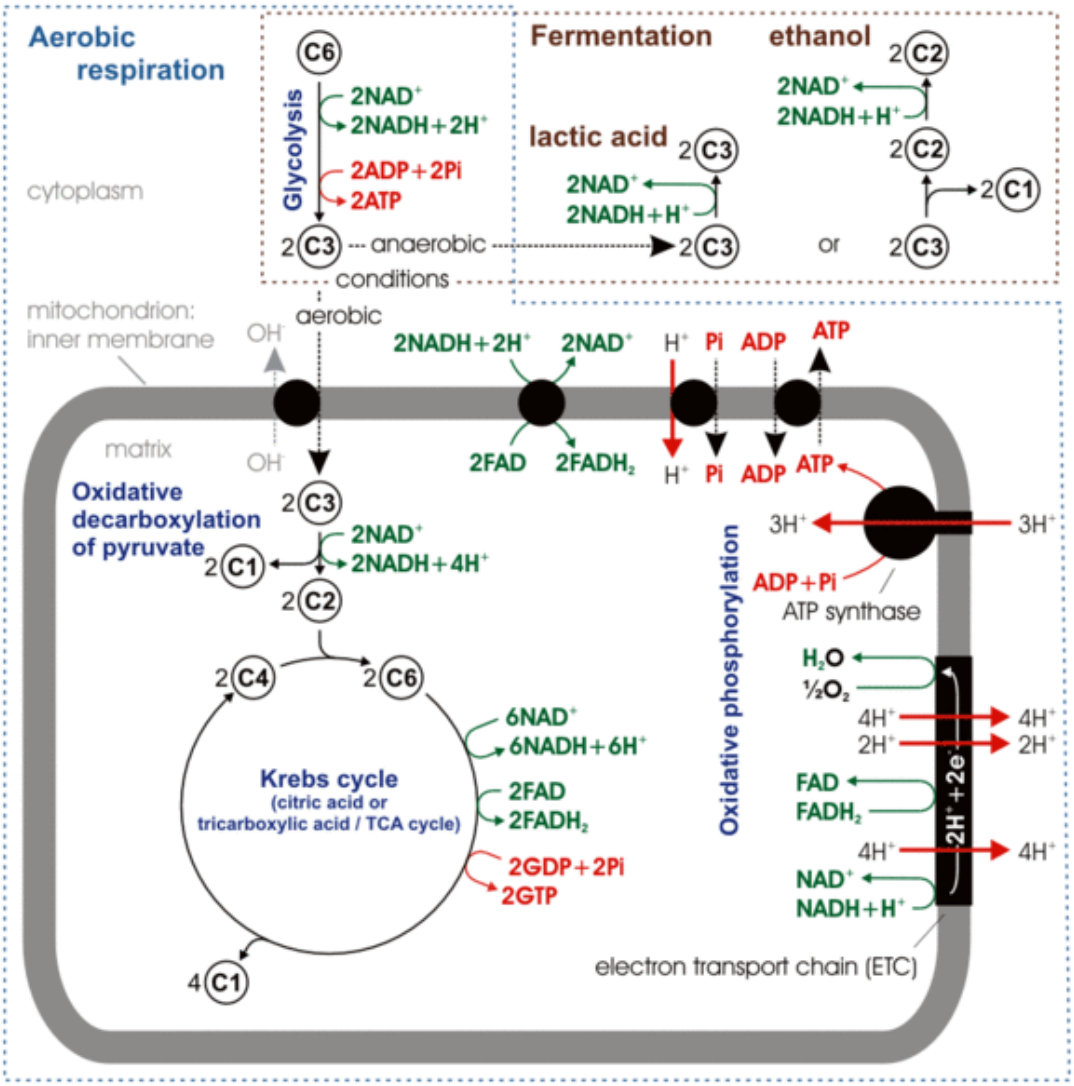
Pyruvate is CH_3COCOO^-. P_i is inorganic phosphate. Two ADP molecules and two P_i are converted to two ATP and two water molecules via substrate-level phosphorylation. Two molecules of NAD^+ are also reduced to NADH.

In oxidative phosphorylation the energy for ATP formation is derived from an electrochemical proton gradient generated across the inner mitochondrial membrane (or, in the case of bacteria, the plasma membrane) via the electron transport chain. Glycolysis has substrate-level phosphorylation (ATP generated directly at the point of reaction).

Humans have used fermentation to produce food and beverages since the Neolithic age. For example, fermentation is used for preservation in a process that produces lactic acid as found in such sour foods as pickled cucumbers, kimchi and yogurt (see fermentation in food processing), as well as for producing alcoholic beverages such as wine (see fermentation in winemaking) and beer. Fermentation can even occur within the stomachs of animals, such as humans.

To many people, fermentation simply means the production of alcohol: grains and fruits are fermented to produce beer and wine. If a food soured, one might say it was 'off' or fermented. Here are some definitions of fermentation. They range from informal, general usage to more scientific definitions.

1. Preservation methods for food via microorganisms (general use).
2. Any process that produces alcoholic beverages or acidic dairy products (general use).
3. Any large-scale microbial process occurring with or without air (common definition used in industry).
4. Any energy-releasing metabolic process that takes place only under anaerobic conditions (becoming more scientific).
5. Any metabolic process that releases energy from a sugar or other organic molecules, does not require oxygen or an electron transport system, and uses an organic molecule as the final electron acceptor (most scientific).



4. Single-cell protein (**SCP**) refers to edible unicellular microorganisms. The biomass or protein extract from pure or mixed cultures of algae, yeasts, fungi or bacteria may be

used as an ingredient or a substitute for protein-rich foods, and is suitable for human consumption or as animal feeds.

Whereas industrial agriculture is marked by a high water footprint, high land use, biodiversity destruction, general environmental degradation and contributes to climate change by emission of a third of all greenhouse gases, production of SCP does not necessarily exhibit any of these serious drawbacks. As of today, SCP is commonly grown on agricultural waste products, and as such inherits the ecological footprint and water footprint of industrial agriculture. However, SCP may also be produced entirely independent of agricultural waste products through autotrophic growth.[4] Thanks to the high diversity of microbial metabolism, autotrophic SCP provides several different modes of growth, versatile options of nutrients recycling, and a substantially increased efficiency compared to crops.

With the world population reaching 9 billion by 2050, there is strong evidence that agriculture will not be able to meet demand and that there is serious risk of food shortage. Autotrophic SCP represents options of fail-safe mass food-production which can produce food reliably even under harsh climate conditions.

Single-cell proteins develop when microbes ferment waste materials (including wood, straw, cannery, and food-processing wastes, residues from alcohol production, hydrocarbons, or human and animal excreta). The problem with extracting single-cell proteins from the wastes is the dilution and cost. They are found in very low concentrations, usually less than 5%. Engineers have developed ways to increase the concentrations including centrifugation, flotation, precipitation, coagulation, and filtration, or the use of semi-permeable membranes.

The single-cell protein must be dehydrated to approximately 10% moisture content and/or acidified to aid in storage and prevent spoilage. The methods to increase the concentrations to adequate levels and the de-watering process require equipment that is expensive and not always suitable for small-scale operations. It is economically prudent to feed the product locally and soon after it is produced

Advantages

Large-scale production of microbial biomass has many advantages over the traditional methods for producing proteins for food or feed.

1. Microorganisms have a much higher growth rate (algae: 2–6 hours, yeast: 1–3 hours, bacteria: 0.5–2 hours). This also allows to select for strains with high yield and good nutritional composition quickly and easily compared to breeding.
2. Whereas large parts of the crop, such as stems, leaves and roots are not edible, single-cell microorganisms can be used entirely. Whereas parts of the edible

fraction of crops contains is undigestible, many microorganisms are digestible at a much higher fraction.

3. Microorganisms usually have a much higher protein content of 30–70% in the dry mass than vegetables or grains. The amino acid profiles of many SCP microorganisms often have excellent nutritional quality, comparable to a hen's egg.
4. Some microorganisms can build vitamins and nutrients which eukaryotic organisms such as plants cannot produce or not produce in significant amounts, including vitamin B12.
5. Microorganisms can utilize a broad spectrum of raw materials as carbon sources including alkanes, methanol, methane, ethanol and sugars. What was considered "waste product" often can be reclaimed as nutrients and support growth of edible microorganisms.
6. Like plants, autotrophic microorganisms are capable to grow on CO_2 . Some of them, such as bacteria with the Wood-Ljungdahl-Pathway or the reductive TCA can fix CO_2 between 2-3, up to 10 times more efficiently than plant. when also considering the effects of photo-inhibition.
7. Some bacteria, such as several homoacetogenic clostridia are capable to perform syngas fermentation. This means they can metabolize synthesis gas, a gas mixture of CO, H_2 and CO_2 that can be made by gasification of residual intractable biowastes such as lignocellulose.
8. Some bacteria are diazotrophic, i.e. they can fix N_2 from the air and are thus independent of chemical N-fertilizer, whose production, utilization and degradation causes tremendous harm to the environment, deteriorates public health, and fosters climate change.
9. Many bacteria can utilize H_2 for energy supply, using enzymes called hydrogenases. Whereas hydrogenases are normally highly O_2-sensitive, some bacteria are capable of performing O_2-dependent respiration of H_2. This feature allows autotrophic bacteria to grow on CO_2 without light at a fast growth rate. Since H_2 can be made efficiently by water electrolysis, in a manner of speaking, those bacteria can be "powered by electricity".
10. Microbial biomass production is independent of seasonal and climatic variations, and can be easily shielded from extreme weather events that are expected to cause crop failures with the ongoing climate-change. Light-independent microorganisms such as yeasts can continue to grow at night.
11. Cultivation of microorganisms generally has a much lower water footprint than agricultural food production. Whereas the global average blue-green water footprint (irrigation, surface, ground and rain water) of crops reaches about 1800 liters per kg crop due to evaporation, transpiration, drainage and runoff, closed bioreactors producing SCP exhibits none of these causes.
12. Cultivation of microorganisms does not require fertile soil and therefore does not compete with agriculture. Thanks to the low water requirements, SCP cultivation

can even be done in dry climates with infertile soil and may provide a means of fail-safe food supply in arid countries.

13. Photosynthetic microorganisms can reach a higher solar-energy-conversion efficiency than plants, because in photobioreactors supply of water, CO_2 and a balanced light distribution can be tightly controlled.
14. Unlike agricultural products which are processed towards a desired quality, it is easier with microorganisms to direct production towards a desired quality. Instead of extracting amino acids from soy beans and throwing away half of the plant body in the process, microorganisms can be genetically modified to overproduce or even secrete a particular amino acid. However, in order to keep a good consumer acceptance, it is usually easier to obtain similar results by screening for microorganisms which already have the desired trait or train them via selective adaptation.

Disadvantages

Although SCP shows very attractive features as a nutrient for humans, however there are some problems that deter its adoption on global basis:

- Fast growing microorganisms such as bacteria and yeast tend to have a high concentration of nucleic acid, notably RNA. Levels of must be limited in the diets of monogastric animals to <50 g per day. Ingestion of purine compounds arising from RNA breakdown leads to increased plasma levels of uric acid, which can cause gout and kidney stones. Uric acid can be converted to allantoin, which is excreted in urine. Nucleic acid removal is not necessary from animal feeds but is from human foods. A temperature hold at 64 °C inactivates fungal proteases and allows. However, this problem can be remediated. One common method consists in a heat treatment which kills the cells, inactivates proteases and allows endogenous RNases to hydrolyse RNA with release of nucleotides from cell to culture broth.
- Similar to plant cells, the cell wall of some microorganisms such as algae and yeast contain non-digestible components, such as cellulose. The cells of some kind of SCP should be broken up in order to liberate the cell interior and allow complete digestion.
- Some kind of SCP exhibits unpleasant color and flavors.
- Depending on the kind of SCP and the cultivation conditions, care must be taken to prevent and control contamination by other microorganisms because contaminants may produce toxins such as mycotoxins or cyanotoxins. An interesting approach to address this problem was proposed with the fungus *Scytalidium acidophilum* which grows at a pH as low as 1. This allows to hydrolyse paper wastes to a sugar medium and creates aseptic conditions at low-cost.
- Some yeast and fungal proteins tend to be deficient in methionine.