Montogue

Quiz BI105

MICROBIOLOGY and PROKARYOTES

Lucas Monteiro Nogueira

PROBLEMS

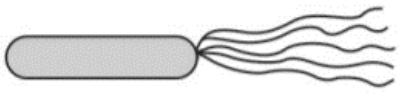
Problem 1

Which of the following is *not* a distinguishing feature of prokaryotic cells?

A) They lack a plasma membrane.
B) They lack membrane-enclosed organelles.
C) They have cell walls containing peptidoglycan.
D) They usually have a single circular chromosome.

Problem 2

A bacterium with a tuft of flagella present at one end, as illustrated below, is said to be:



A) Monotrichous.
B) Amphitrichous.
C) Lophotrichous.
D) Peritrichous.

Problem 3

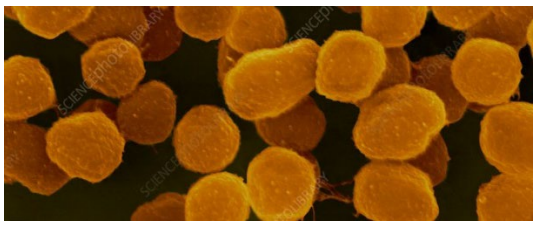
Regarding aspects of prokaryotic cells and their biology, true or false?

1.( ) Peptidoglycan is a sugar that constitutes the cell wall of most bacteria. Its basic structure is a dimer of *N*-acetylglucosamine (NAG) and *N*-acetylmuramic acid (NAM). Although peptidoglycan is found in the walls of most prokaryotes, some groups of bacteria exhibit cell walls with no peptidoglycan.

2.( ) A peptidoglycan cell wall is observed in most bacteria, including most archaea. However, no known species of archaea is enveloped by a capsule.

3.( ) Another prominent difference between bacteria and archaea is that protein synthesis in the former begins with *N*-formylmethionine, whereas in archaea the first amino acid in protein synthesis is methionine. This latter characteristic is one of the traits that archaea share with many types of eukaryotic cells.

4.( ) Carbonate respiration is performed by archeans such as *Methanococcus* (e.g., *M. jannaschii*, shown below) and *Methanobacterium*, which inhabit anoxic environments low in nitrate and sulfate. They are obligate anaerobes that reduce CO_2 to methane using hydrogen gas. Some species are capable of reducing carbon monoxide as well.



5.( ) The periplasm is the region between the plasma membrane and the outer membrane of gram-negative bacteria. This area is not just empty space and exhibits substantial activity, including, for instance, action of specific electron transport proteins and solute transport mediated by periplasmic binding proteins.

6.( ) Forms of oxygen and certain of its metabolites are toxic to many microorganisms. For instance, the hydrogen peroxide produced in some reaction pathways involving oxygen is highly toxic. Many prokaryotes possess enzymes that afford protection against toxic O_2 products; one example is peroxidase, which converts H_2O_2 into water and oxygen, as in the reaction

$$2\,H_2O_2 \xrightarrow{\text{Peroxidase}} 2H_2O + O_2$$

7.( ) The OmpA protein has been observed in *E. coli* and some comparable proteins have been observed in many other Gram-negative bacteria. One of its documented functions is a role in plasmid conjugation proceeding via *F*-pili. Indeed, strains lacking OmpA show a drastic decrease in plasmid transfer.

8.( ) Another important feature of OmpA proteins in Gram-negative bacteria is their role in stabilizing the outer membrane and the cell wall. This property has not been verified with the Braun lipoprotein, another molecule encountered in the wall of Gram-negative bacteria.

9.( ) The vegetative cell cycle of prokaryote reproduction consists of three successive phases, in the following order: the B period, the C period, and the D period. In some well-nourished cell cultures at high growth rates, the C period is often absent.

10.( ) The first morphological sign of cell division is the centripetal synthesis of a septum at midcell, which forms by inward growth of the cell membrane and peptidoglycan layers. In *E. coli,* this process begins soon after chromosome replication is complete. In many Gram-negative bacteria, including *E. coli,* the outer envelope also invaginates at this time. Invagination is manifested as a constriction, which becomes narrower as septation proceeds. Initially there is a double layer of peptidoglycan in the septum, but this is split in half as constriction proceeds and the cells separate. Septation and peptidoglycan synthesis are independent processes.

11.( ) In the multiplication of *E. coli,* there are at least 10 proteins known to be associated with the formation of the septal ring, an essential step of cell division. They are FtsA, FtsZ, ZipA, FtsK, FtsQ, FtsL, FtsB (also called YgbQ), FtsW, FtsI (also called PBP3), and FtsN. Among these, FtsA and FtsK are the only cytoplasmic proteins, while the remaining eight are inner membrane proteins associated with the constriction site or the septum. Importantly, FtsK forms a ring in the cell center, which is made before the invagination and is believed to contract, thereby contributing to cell constriction.

12.( ) Plasmids are molecules of genetic material not linked to the main chromosome of prokaryotes. A number of biochemical processes can be plasmid-mediated, including antibiotic resistance and production of enterotoxins and bacteriocins.

13.( ) The process of transfer of DNA from a donor bacterium to a recipient bacterium via intercellular bridges formed by sex pili is known as transduction.

14.( ) Some bacteria have resistance or R factors, plasmids that confer resistance to antibiotics after being transferred from one bacterium to another. When assimilated in the main chromosome of bacteria, R factors can encode for resistance against multiple drugs at once. What's more, transfer of R factors between bacteria of different genera has been reported in the literature.

15.( ) Several strains of coliform bacteria produce colicins, antibiotic-like substances which are specifically and selectively lethal to other enterobacteria. Synthesis of these substances is regulated by the main chromosome; indeed, extrachromosomal determination factors such as plasmids have not been observed to play a role in the production of colicins.

16.( ) The amount of guanine and cytosine as compared to the sum of all DNA bases is sometimes known as the GC ratio. Its range of values is substantially higher for prokaryotes than for plant and animal cells.

17.( ) Bacterial communication between cells assumes many avenues, and one of these unique systems is the response of cells to chemicals produced by organisms of the same species. The process of autoinduction, or *quorum sensing*, was first reported by K. Nealson and J. Hastings as a result of studies involving the role of *Streptomyces* in antibiotic production. The signal molecules in their experiments were homoserine lactones, but other classes of such molecules have been observed since then, e.g., γ-butyrolactone in bioluminescent bacteria (e.g., *Vibrio fischeri*) and a variety of peptides in cocci such as *Streptococcus* and *Myxococcus*.

→ Problem 4.1

A fast-growing strain of a bacterial species with an initial population of 10,000 cells/mL increases to one million cells/mL in 120 minutes. The generation time of this strain is most nearly:

A) G = 9.43 min
B) G = 18.1 min
C) G = 24.4 min
D) G = 30.1 min

→ Problem 4.2

The specific growth rate (in reciprocal hours) of the strain described above is most nearly:

A) $\mu = 1.18\ h^{-1}$
B) $\mu = 2.30\ h^{-1}$
C) $\mu = 3.16\ h^{-1}$
D) $\mu = 4.08\ h^{-1}$

▶ Problem 5

Suppose you have an exponentially growing culture at a density of 10^9 cells/mL and you would like to subculture it so that 24 hr later the cell density would also be 10^9 cells/mL. If the generation time is 3 hr, what should be the volume of inoculum required to grow 1.2 liters of cells within the specified time?

A) V_0 = 2.9 mL
B) V_0 = 4.7 mL
C) V_0 = 6.6 mL
D) V_0 = 8.4 mL

▶ Problem 6

In an exponentially-growing bacterial population, the lengths of the C and D periods are 40 and 20 min, respectively. The generation time is 30 min. For binary fission, the average number of copies of a gene with relative distance from the origin *oriC* of 0.32 is, most nearly:

A) F = 1 copy
B) F = 2 copies
C) F = 3 copies
D) F = 4 copies

▶ Problem 7

Regarding aspects of prokaryotic cell growth, counting, and control, true or false?

1.( ) The growth of microorganisms in culture is conventionally divided in four stages: the lag phase, the exponential phase, the stationary phase, and the death phase. Microbial growth is concentrated in the exponential phase, where, as the name implies, the number of individuals increases in geometric progression. Other growth patterns have not been observed; for instance, the microbial population cannot increase linearly with time.

2.( ) Like many biochemical processes, growth rate at the log phase of bacterial growth exhibits first-order reaction kinetics.

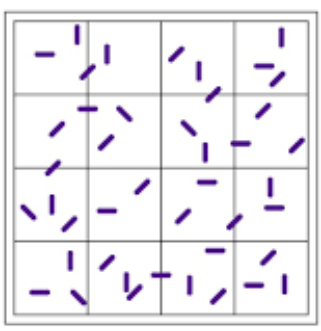
3.( ) Microorganisms that can metabolize two or more nutrients in a substrate, one preferred over the other and present in limiting concentrations, can show growth in two stages separated by a lag phase. This phenomenon is known as *diauxic growth*.

4.( ) Another important pattern of microbial growth is *cooperative growth*. In this case, each type of microbe is capable of growing independently and producing some metabolites at lower rates. When the types are allowed to grow in a mixed population, both the growth rate and the level of by-product formation greatly increase. Indeed, the increase is often greater than the

additive of the amounts produced by growing the two microorganisms separately.

5.( ) A plate count is done by either the pour plate method or the spread plate method. The pour plate method is preferred for heat-sensitive microorganisms.

6.( ) In one cell count experiment carried out with a Petroff-Hauser counting chamber, 150 bacterial cells with a dilution factor of 10^{-2} (0.01) were counted in 16 squares. Accordingly, the original cell density (OCD) of this sample is greater than 18 billion cells per mL. In your analysis of this statement, bear in mind that the volume of one small square is 5×10^{-8} mL.

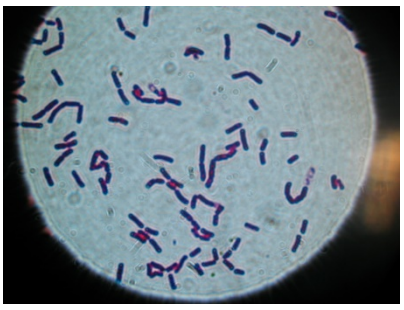


7.( ) Microbes can also be counted by indirect means. One device that provides indirect counts is the spectrophotometer. The amount of light that passes through a suspension of cells in a spectrophotometer is inversely proportional to the concentration of organisms, provided that particle size does not change.

8.( ) In preservation of bacterial cultures by lyophilization, a suspension of microbes is quickly frozen at temperatures ranging from -54°C to -72°C, and the water is removed by a high vacuum (sublimation). While under vacuum, the container is sealed by melting the glass with a high-temperature torch. The remaining powderlike residue that contains the surviving microbes can be stored for years on end, and the microorganisms can be readily revived when necessary.

9.( ) An exposure of a medium to steam at 100°C for 20 minutes on three successive days is known as *pasteurization*.

10.( ) Spores of *Bacillus cereus* (shown below) are often used for biological sterilization control in autoclaves.



11.( ) Psychrotrophs are bacteria that grow well at 0°C and have an optimum temperature close to 15°C or lower. Few species constitute this category; however, one set of such species, which can grow at 0 to 7°C even though they have optima between 20°C and 30°C, and maxima at 35°C, has gained interest because of its role in the spoilage of refrigerated foods. A new designation, that of *psychrophiles* or *facultative psychrotrophs*, was adopted to accommodate these species.

12.( ) In first-order kinetics of microbial heat inactivation, the reciprocal of the rate constant is known as the *D value*, which is the time, usually expressed in minutes, required to reduce the size of a given microbial population by a factor of 2.

13.( ) Another definition of interest, especially in the canning industry, is the *Z* value, which is the change in temperature, usually in °C, required to give a 10-fold change in the *D* value, or to produce a unit change in the base 10 logarithm of the *D* value.

14.( ) A third important parameter is the Q_{10} value, which may be defined as the change in reaction rate constant that corresponds to a temperature variation of 10°C. The Q_{10} can be related to the *Z* value. For example, if $Q_{10} = 3$, the *Z* value will be greater than 20°C.

15.( ) A thermal death time (TDT) curve is a plot of *log D* (where *D* is the *D* value) versus time.

▶ Problem 8

The dynamic change of the decline and survival of microorganisms during frozen storage can be represented by the equation

$$N = \left(N_0 - \Delta N_m\right) + \Delta N_m e^{-kt}$$

where N_0 (log cfu/g) is the concentration of microorganisms before freezing, N (log cfu/g) is the concentration of microorganisms at storage time t (days), ΔN_m (log cfu/g) is the maximum reduction in the microbial population during frozen storage, and k (day^{-1}) is the rate of microbial inactivation. The following table lists the kinetic parameters for use of the foregoing equation with *Salmonella* spp. With reference to these data, which of the following serovars is most sensitive to freezing?

Serovar	k (day^{-1})	N_0 (log cfu/g)	ΔN_m (log cfu/g)
S. Typhi	5.20×10^{-2}	6.91	1.72
S. Typhimurium	1.48×10^{-2}	7.39	0.743
S. Anatum	9.68×10^{-3}	6.85	1.29
S. Paratyphi B	1.47×10^{-2}	6.95	0.671

A) *S. Typhi*
B) *S. Typhimurium*
C) *S. Anatum*
D) *S. Paratyphi* B

▶ Problem 9

A sample of pooled raw milk contains a bacterial population of 5×10^5 individuals per mL. The average D value at 70°C for the bacterial population was verified to be 9 min, and the Z value was determined as 8°C. The milk will be processed at 86°C for 27 seconds. How many bacteria will remain after pasteurization and what time would be required at 70°C to achieve the same degree of lethality?

A) Rem. bacteria = 5 individuals, time for same degree of lethality = 36 min
B) Rem. bacteria = 5 individuals, time for same degree of lethality = 45 min
C) Rem. bacteria = 50 individuals, time for same degree of lethality = 36 min
D) Rem. bacteria = 50 individuals, time for same degree of lethality = 45 min

▶ Problem 10

The following statements are characteristics of chemostats or turbidostats, two types of continuous microbial culture.

1. The regulating factor is the turbidity of the medium as measured by an electronic cell.
2. The dilution rate is constant.
3. A limiting nutrient is present.
4. The device operates best at high dilution rates.

Which of the following associations is correct?

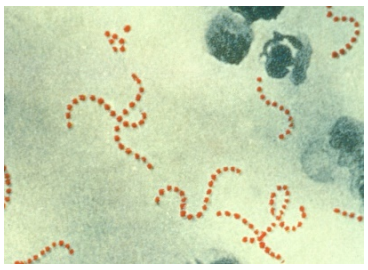
A) 1. Chemostat; 2. Chemostat; 3. Turbidostat; 4. Turbidostat
B) 1. Turbidostat; 2. Chemostat; 3. Turbidostat; 4. Chemostat
C) 1. Turbidostat; 2. Turbidostat; 3. Chemostat; 4. Chemostat
D) 1. Turbidostat; 2. Chemostat; 3. Chemostat; 4. Turbidostat

▶ Problem 11

Regarding aspects of medical microbiology, true or false?

1.( ) *Neisseria gonorrheae* is the pathogen that causes gonorrhea. One of its main virulence factors is the presence of pili or fimbriae, hair-like appendages found on the surface of some microbes. In spite of the importance of pili in the human infection process, *Neisseria* are capable of infecting a host and multiplying to pathological levels even when they are devoid of these appendages.

2.( ) Another noteworthy virulence factor is the M protein, an anti-phagocytic agent encountered in some species of *Streptococcus*, including *S. pyogenes* (shown below), a highly infectious gram-positive pathogen.



3.( ) Lipopolysaccharide (LPS), sometimes known simply as *endotoxin*, is part of the outer membrane of gram-positive bacteria and one of their main virulence factors. The toxicity of the molecule is mainly attributed to its lipid component, known as lipid A.

4.( ) Assume the average serum level of gentamicin is 6 to 8 mcg/mL. A *Klebsiella* isolate with minimal inhibitory concentration (MIC) of 0.8 mcg/mL for this agent can be said to be susceptible to gentamicin.

5.( ) Penicillin-type antibiotics such as doxycycline act by binding penicillin binding proteins (PBPs) on the cell wall. Without the action of PBPs, bacteria upregulate autolytic enzymes and are unable to build and repair the cell wall, leading to the bactericidal action of these antibiotics.

6.( ) Tetracycline-class antibiotics diffuse passively through porin channels in the bacterial membrane and bind to ribosomal proteins, thereby preventing binding of tRNA to the mRNA-ribosome complex and ultimately inhibiting protein synthesis.

7.( ) Cephalosporin-class antibiotics such as cephalexin share the mechanism of action of tetracyclines. First-generation cephalosporins, available since the 1960s, are more effective against Gram-positive bacteria than against Gram-negative bacteria.

8.( ) Quinolones are another important class of commercial antibiotics. Their mode of action on growing bacteria is primarily prevention of glycine cross-linking.

9.( ) In humans, the most common portal of entry for *C. tetani*, the cause of tetanus, is the gastrointestinal tract.

10.( ) *M. tuberculosis* is a pathogen that causes tuberculosis. Its main portal of entry in humans is the respiratory tract.

11.( ) Shigellosis, a bacterial infection caused by Gram-negative *Shigella* bacteria, is mainly caused by ingestion of contaminated food or water.

12.( ) *Neisseria gonorrhoeae,* the prokaryote mentioned in statement 1, is a fastidious pathogen and can be found in sites often contaminated with normal flora. The best medium for isolation of this microbe is Löwenstein-Jensen medium.

13.( ) *Vibrio cholerae*, the causative agent of cholera, is best isolated using Löffler's medium.

▶ Problem 12

Symptoms of *C. botulinum* (shown below) food poisoning include double vision, inability to speak, and respiratory paralysis. These symptoms are consistent with:

A) Invasion of the gut epithelium by *C. botulinum*.
B) Activation of cyclic AMP.
C) Endotoxin shock.
D) Ingestion of a neurotoxin.

SOLUTIONS

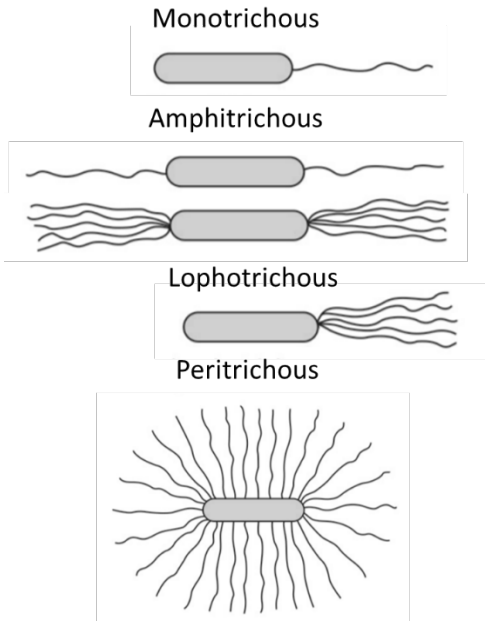
P.1 ➡ *Solution*

Some bacteria lack capsules or cell walls (e.g., *Mycoplasma*), but every prokaryotic cell, however primitive, is encircled by a plasma membrane.

♦ The correct answer is **A**.

P.2 ➡ *Solution*

A bacterium may have four types of arrangement of flagella, namely (1) *monotrichous*, a single flagellum on either pole of the cell (e.g., *Vibrio cholerae*); (2) *amphitrichous*, two flagella or two tuft of flagella, one on each end of the cell (e.g., *Alkaligenes faecalis*); (3) *lophotrichous*, a tuft of flagella at one end of the cell (e.g., *Spirillum volutans*); and (4) *peritrichous*, a set of flagella stemming from many directions (e.g., *E. coli*). Each type is illustrated below. (Bear in mind that some authors consider bacterium with two tufts of flagella at opposite ends to be lophotrichous, not amphitrichous.)



♦ The correct answer is **C**.

P.3 ➡ *Solution*

1. True. Indeed, some groups of bacteria have cell walls devoid of peptidoglycan. One example is the obligately intracellular *Chlamydia*, which stains Gram-negative, has an outer membrane, but does not exhibit peptidoglycan. Since *Chlamydia* grow only inside animal cells and are not exposed to dilute growth environments, there is less of a requirement to prevent cell lysis because the osmotic differences between the host cell and *Chlamydia* are not great. *Isosphera*, *Planctomyces*, and *Pirella* are genera of bacteria that lack muramic acid and therefore do not have a true peptidoglycan layer.

2. False. The statement errs twice, firstly by mentioning that most archaea have peptidoglycan cell walls (most do not have typical PG walls, although they might exhibit different types of cross-linked polymers) and secondly by stating that capsule do not occur in archaea (they do, albeit rarely).

3. True. There are other fundamental metabolic differences between the two types of prokaryote. For one, ribosomal-mediated protein synthesis is sufficiently distinct in that streptomycin and tetracycline serve as inhibitors in archaea but not in eubacteria. A single DNA-dependent RNA polymerase occurs in bacteria while several of these enzymes exist in archaea. Indeed, the fact that archaea have multiple DNA-dependent RNA polymerases with 8 to 12 subunits is another trait that these organisms share with many eukaryotic cells.

4. True. Indeed, some carbonate-respiring archaeans are capable of producing methane at the expense of carbon monoxide.

5. True. As an example of the first function mentioned in the statement, Barton says that, in *Paracoccus denitrificans*, NO reductase and N_2O reductase, which function in the denitrification process, and dehydrogenases for the oxidation of methanol or methylamine have been shown to involve *c*-type cytochromes in the periplasm.

6. False. The reaction mentioned in the statement is mediated by catalase, another enzyme used in the neutralization of hydrogen peroxide. The reaction catalyzed by peroxidase does not produce oxygen:

$$H_2O_2 + 2H^+ \xrightarrow{\text{Peroxidase}} 2H_2O$$

7. True. Indeed, OmpA seem to be quite important in plasmid conjugation via F-pili, as a severe decrease in transfer ensues when strains are devoid of this protein. If donor strains conjugating via F-pili are incubated with purified OmpA, a decrease in the transfer frequency hardly occurs. This takes place only after the addition of lipopolysaccharide. This is not to say that OmpA is the real receptor even in the presence of LPS. Rather, in its absence, the formation of the stable aggregates between donor and recipient strain indispensable for the conjugation process does not occur.

8. False. One of the earliest findings conveyed by studies of the Braun Lpp since it was discovered in the late 1960s is its role in wall stability. In *E. coli,* the C-terminal lysine residue of the Lpp is covalently attached to the peptidoglycan, providing the only covalent connection between the outer membrane and the cell wall. The role of these proteins in cell wall stability has been verified from studies using a Lpp OmpA double mutant of *E. coli* lacking Braun lipoprotein and OmpA. These cells grow in an almost spherical form instead of normal rod-like form, require high concentrations of divalent cations for growth, and show frequent blebbing. These properties were ascribed to the observed defect whereby peptidoglycan was no longer connected with the outer membrane.

9. False. The C period is the phase of the reproductive cycle where DNA is synthesized, and cannot be omitted in normal binary fission of a prokaryotic cell. It is the B period, where cells grow in mass before replicating their genetic material, that may not be verified at moderate and high growth rates.

10. False. The entire paragraph offers an accurate description of septum formation and the initial steps of cell division, but errs at the end by stating that septation and peptidoglycan synthesis are independent processes. Septation and peptidoglycan synthesis are coupled, which is why inhibitors of septal peptidoglycan synthesis, such as agents that disrupt the PBP3 enzyme in *E. coli*, also prevent septation.

11. False. The 10 proteins mentioned in the paragraph possibly do participate in the formation of the septum, and only FtsA and FtsZ (not FtsK) are cytoplasmic; the others are inner (cell) membrane proteins associated at the constriction site or the septum. The ring in question is actually formed by FtsZ, not FtsK. Interestingly, FtsZ is related to tubulin, which forms the eukaryotic cytoskeletal microtubules, and it has been suggested that FtsZ and tubulin have evolved from the same protein.

12. True. Plasmid-mediated antibiotic resistance is a well-documented process. Plasmids have also been shown to be involved in the production of bacteriocins (e.g., in some strains of *Carnobacterium piscicola* in meat) and enterotoxins (e.g., in enterotoxin B production in *S. aureus*).

13. False. "Traditional" transfer of DNA from one bacterium to another is called conjugation. Transduction, in actuality, is the carrying of genetic material from a donor to a number of recipients by phage.

14. True. Indeed, R factors may induce resistance to as many as eight drugs at once. In addition, they can be profusely transmitted to bacterial offspring; in *Proteus mirabilis,* as many as 30 copies of resistance genes have been verified in a single cell. Transfer of resistance plasmids among bacteria of different genera has been observed, for instance, between *Klebsiella* and *Pseudomonas* over the course of prolonged antibiotic treatment of burn patients.

15. False. Colicin production is in fact determined by extrachromosomal genetic material, namely by a class of plasmids known as colicinogenic (Col) factors.

16. True. The GC ratios of prokaryotes range from 20 to 80%, while those of plants and animals range from 30 to 50%. If a large protein were configured to have the lowest mol% G + C and also configured to have the highest mol% G + C, the range of mol% G + C would be comparable to the range seen in prokaryotic cells. Thus, prokaryotes represent the biological limits that G + C are found in nature.

17. False. The experiments carried out by Nealson and Hastings indeed revealed homoserine lactones to be signal molecules, but actually involved *Vibrio* bioluminescent rods, not *Streptomyces*. The latter also display quorum sensing when cultured for antibiotic production, but the signaling molecules are γ-butyrolactones, not homoserine lactones.

♦ The correct answer is **A**.

P.4 ➡ *Solution*

Part 1: The generation time is given by the formula

$$G = \frac{0.301\Delta t}{\log_{10} N - \log_{10} N_0} = \frac{0.301\times 120}{\log_{10} 10^6 - \log_{10} 10^4} = \boxed{18.1\ \text{min}}$$

♦ The correct answer is **B**.

Part 2: The specific growth rate is given by the formula

$$\mu = \frac{2.3\left(\log_{10} N - \log_{10} N_0\right)}{\Delta t} = \frac{2.3\left(\log_{10} 10^6 - \log_{10} 10^4\right)}{2} = \boxed{2.3\,\text{h}^{-1}}$$

♦ The correct answer is **B**.

P.5 ➡ *Solution*

The concentration x of cells obtained from an original concentration x_0 is given by $x = x_0 2^Y$, where Y is the number of generations elapsed; in the case at hand, $Y = 24/3 = 8$. Substituting and solving for x_0 brings to

$$x = x_0 2^Y \rightarrow 10^9 = x_0 \times 2^8$$

$$\therefore x_0 = 3.91\times 10^6\ \text{cells/mL}$$

The initial culture should have an initial cell concentration close to 4 million cells per milliliter. Since, however, the cell density of the inoculum is 10^9 cells/mL, the inoculum must be diluted $10^9/(3.91\times 10^6) = 256$ times. Accordingly, to grow 1.2 liter of cells the inoculum size would have to be $1{,}200/256 \approx 4.7$ mL.

♦ The correct answer is **B**.

P.6 ➡ *Solution*

The average number of copies of a gene per cell in an exponentially growing population such as the present one is given by

$$F = 2^{\left[C(1-x)+D\right]/\tau}$$

Here, $C = 40$ min is the length of the C period, $D = 20$ min is the length of the D period, $\tau = 30$ min is the generation time, and $x = 0.32$ is the relative distance from the gene to *oriC*. Thus,

$$F = 2^{\left[40\times(1-0.32)+20\right]/30} = 2.98 \approx \boxed{3\ \text{copies}}$$

♦ The correct answer is **C.**

P.7 ➡ *Solution*

1. False. Under adverse physiological or metabolic conditions, the growth of some prokaryotes can evolve linearly with time. Srivastava reports that one such situation may occur when cells are grown in the presence of an antimetabolite. For example, if *E. coli* cells are grown in the presence of ethionine, an analogue of methionine, the analogue is incorporated into their metabolic pathways and leads to the formation of faulty proteins. As a result, the cell will depend on the proteins produced before the analogue was added and its growth curve will be linear with time.

2. False. Growth rate at the log phase is exponential in nature and as such follows first-order chemical kinetics.

3. True. In diauxic growth, a microbe population first grows at the expense of a preferred nutrient until that nutrient is depleted. Then, a short lag phase follows until the organisms begin taking up another type of nutrient available in the substrate and start to grow in number again. This kind of log phase → lag phase → log phase behavior is called diauxic growth. The most well-documented cases of diauxic growth pertain to bacteria populations in substrates containing glucose and lactose; the bacteria preferably grow by

consuming glucose, but can also assimilate lactose once they run out of the former nutrient.

4. False. The statement offers a definition of *synergistic growth*; there is no such term as *cooperative growth* in circulation. It is true that the by-product amount achieved with a combination of two organisms can be greater than the additive of their yields when grown individually. Ray offers one example: both *Str. thermophilus* and *Lab. delbrueckii* subsp. *bulgaricus*, when growing in milk independently, produce about 8 to 10 ppm of acetaldehyde, a desirable flavor component of yogurt. However, when growing together in milk, 30 ppm or more of acetaldehyde is produced, a concentration substantially greater than those achieved by the two microbes when cultured independently.

5. False. In the pour plate method, a dilution of bacterial suspension is introduced into a Petri dish. The nutrient medium, in which the agar is kept liquid by holding it in a water bath at about 50°C, is poured over the sample, which is then mixed into the medium by gentle agitation of the plate. When the agar solidifies, the plate is incubated. With the pour plate technique, colonies will grow within the nutrient agar (from cells suspended in the nutrient medium as the agar solidifies) as well as on the surface of the agar plate. One immediate drawback of the pour plate method is that heat-sensitive organisms may be damaged by the melted agar and hence do not form colonies. This limitation can be circumvented by the spread plate technique, which does not involve contact between molten agar and the cells to be counted.

6. True. The OCD is given by

$$OCD = \frac{\text{Cells counted}}{\text{Squares} \times \text{Dilution} \times \text{Volume}}$$

Noting that the volume of one small square is 5×10^{-8} mL and substituting, we get

$$OCD = \frac{\text{Cells counted}}{\text{Squares} \times \text{Dilution} \times \text{Volume}} = \frac{150}{16\times10^{-2}\times\left(5\times10^{-8}\right)} = \boxed{18.75\times10^{9}\ \text{cells/mL}}$$

7. True. It is somewhat intuitive that the greater the concentration of microbes, the lower will be the amount of light passing through the suspension. This change of light may be registered in a spectrophotometer's scale as the *percentage of transmission*. Also printed on the instrument's scale is a logarithmic expression called the *absorbance* or *optical density*. The absorbance is used to plot bacterial growth; when the bacteria are in logarithmic growth or decline, a graph of absorbance versus time will form an approximately straight line.

8. True. The lyophilized organisms can be revived at any time by hydration with a suitable liquid nutrient medium.

9. False. Exposure to steam at 100°C for 20 minutes on three successive days constitutes a process known as *tyndallization* or *intermittent sterilization.* The principle that underpins this method is that vegetative cells and spores not eliminated in an initial heating procedure will likely be inactivated on a second or third run. This method is useful to sterilize heat-sensitive culture media that may not withstand the higher temperature of an autoclave.

10. False. One way to establish sterilization control in an autoclave is to introduce an envelope containing a filter paper strip impregnated with 10^6 spores of *Bacillus stearothermophilus.* No growth of *B. stearothermophilus* after the heating process indicates proper sterilization. One reason why this bacterium is often used in sterilization control is that its spores withstand a temperature of 121°C for up to 12 minutes, a time period just below the holding period of 15 min used in many autoclave runs.

11. False. The terms have been swapped. Bacteria that withstand exceptionally low temperatures are *psychrophiles*, and the new phrases created for cold-loving bacteria that spoil refrigerated foods are *psychrotrophs* or *facultative psychrophiles*.

12. False. The *D* value is the time required to reduce the size of a microbial population by a factor of 10. (Hence the letter *D*, which stands for *decimal*; indeed, an older term for *D* value is *decimal reduction time*). It is something of a measure of the microbe's heat resistance in the particular medium in which inactivation has been monitored.

13. True. The Z value is the temperature required to produce a 10-fold change in *D* or the rate constant *K*. A reasonable value for a spore-forming bacterium is 10°C.

14. True. Q_{10} and the *Z* value are related by the simple expression

$$Z\left[^{\circ}\mathrm{C}\right]=\frac{10}{\log_{10} Q_{10}}$$

For $Q_{10} = 3$, we obtain $Z = 20.96$°C. Values of Q_{10} range from 2.2 to 4.6 for dry heat processes and 6.8 to as much as 100 for moist heat processes.

15. False. The TDT is a plot of the logarithm of the *F* value versus temperature. This plot should not be confused with the thermal resistance (TR) curve, which is a graph of *log D* versus time. The TR curve differs from the TDT curve in that the TR curve is, by definition, the time (*D* value) at a range of temperatures necessary to destroy 90% of the microbes in a homogeneous microbial population, whereas the TDT curve is the time (*F* value) at a range of temperatures necessary to produce some given or specified level of microbial destruction ($F_T = D_T \times SLR$, where *SLR* is the spore-log reduction). For the same homogeneous microbial population under the same conditions, the TDT and TR curves are parallel.

P.8 ➡ *Solution*

With reference to the equation we were given, the most sensitive microbe should have a high inactivation rate (i.e., a high *k*) and a high reduction in microbial population during refrigerated storage (that is, a high ΔN_m). Among the serovars listed in the table, *S. Typhi* has the greatest values of *k* and ΔN_m; thus, we conclude that *S. Typhi* is the most sensitive to freezing.

♦ The correct answer is **A.**

P.9 ➡ *Solution*

The *Z* value is 8°C, which means that two log cycles will have passed in a transition of temperature from 70 to 86 degrees. The *D* value is now only 0.01 × 9 = 0.09 min. The milk is processed during 27/60 = 0.45 min, which amounts to 0.45/0.09 = 5 cycles of reduction. The final bacterial population is then $(5\times10^5)/10^5 = 5$ individuals. The time required to produce the same reduction at a temperature of 70°C is 9 × 5 = 45 minutes. As can be seen, the time required to process the milk at 70°C is 100-fold greater than that required at a temperature only 16 degrees higher.

♦ The correct answer is **B.**

P.10 ➡ *Solution*

The following table provides a comparison of characteristics of chemostats and turbidostats.

Chemostat	Turbidostat
Regulating factor is a nutrient present in limiting quantities	Regulating factor is the turbidity of the medium as monitored by a photoelectric cell
Dilution rate remains constant	Dilution rate varies
Limiting nutrient is present	Limiting nutrient absent
Operates best at low dilution rates	Operates best at high dilution rates

♦ The correct answer is **D.**

P.11 ➡ *Solution*

1. False. The virulence factor in question is indispensable for *Neisseria* to achieve infection.

2. True. The M protein is indeed the main virulence factor of *S. pyogenes*. Other important virulence factors that disrupt phagocytosis are the A protein of *Staphylococcus aureus* and, of course, the pili of *Neisseria*.

3. False. The statement provides an accurate definition of LPS and the role of lipid A in the toxicity of the molecule, but errs by attributing it to gram-positive bacteria (which happen to not even have an outer membrane); LPS is part of the outer membrane of gram-*negative* bacteria. LPS is generally not released until the death of the cell, although there is at least one important exception, *N. meningitides*, which over-produces outer membrane fragments.

4.True. The interpretation of quantitative antimicrobial susceptibility tests is based on both the minimum inhibitory concentration (MIC) and the achievable blood level of a given antibiotic. A MIC greater than the achievable

concentration of an antibiotic suggests resistance. A MIC at or near the achievable level is equivocal. Finally, a MIC significantly lower than the achievable level – say, by 75% - suggests susceptibility to the antibiotic being tested. Accordingly, a *Klebsiella* colony with MIC of 0.8 mcg/mL for gentamicin, an agent with serum level greater than 6 mcg/mL or so, can certainly be considered susceptible.

5. False. The paragraph accurately defines the action mechanism of penicillin-type antibiotics, but the keen reader will notice that doxycycline, offered as an example of this class of medications, is in fact a tetracycline antibiotic.

6. True. Specifically, tetracyclines bind to the 30S ribosomal subunit, preventing binding of aminoacyl-tRNA to the mRNA translation complex. As a result, protein synthesis is disrupted.

7. False. The cephalosporins actually share the mechanism of action of β-lactam antibiotics such as penicillins, and have no relationship with the molecular biology of tetracyclines. Nonetheless, it is true that the first tetracyclines are particularly effective against Gram-positive bacteria such as *Streptococcus*.

8. False. Quinolones act by preventing DNA from unwinding and duplicating. Specifically, they inhibit DNA gyrase. Examples of quinolones include norfloxacin, ofloxacin, the widely popular ciprofloxacin, and fluorinated quinolones such as lomefloxacin.

9. False. The skin is the portal of entry for *C. tetani*. Breaches of the skin such as wounds, burns, or the like predispose patients to a variety of infections, including by spores of *C. tetani* and direct infection by *Staphylococcus*, *Streptococcus*, and Gram-negative rods such as *Serratia* or *Pseudomonas*.

10. True. The respiratory tract is a common portal of entry for airborne pathogens such as *M. tuberculosis*, which causes tuberculosis, *Neisseria meningitidis*, an agent that causes a form of bacterial meningitis, and a countless number of viruses.

11. True. Shigellosis is a gastrointestinal infection, and as such usually stems from ingestion of contaminated food or water. *Shigella* are gram-negative, facultative anaerobic, and genetically related to *E. coli*.

12. False. Gonococci (*N. gonorrhoeae*) are normally isolated with a selective medium known as Thayer-Martin agar. TM agar is a selective, enriched medium containing hemoglobin, the supplement Isovitalex, and the antibiotics vancomycin, colistin, nystatin, and trimethoprim.

13. False. *V. cholera* and other vibrios, including *V. parahaemolyticus* and *V. alginolyticus*, are isolated best on thiosulfate citrate bile salts sucrose medium, although media such as mannitol salt agar also support the growth of vibrios. Maximal growth occurs at a pH of 8.5 to 9.5 and at 37°C incubation.

P.12 ➡ *Solution*

Clostridium botulinum growing in food produces a potent neurotoxin that causes diplopia, dysphagia, respiratory paralysis, and speech difficulties when ingested by humans. The toxin is thought to act by blocking the action of acetylcholine at neuromuscular junctions. Botulism is associated with high mortality, but *C. botulinum* infection in humans is rare.

♦ The correct answer is **D.**

ANSWER SUMMARY

Problem 1		A
Problem 2		C
Problem 3		T/F
Problem 4	4.1	B
	4.2	B
Problem 5		B
Problem 6		C
Problem 7		T/F
Problem 8		A
Problem 9		B
Problem 10		D
Problem 11		T/F
Problem 12		D

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