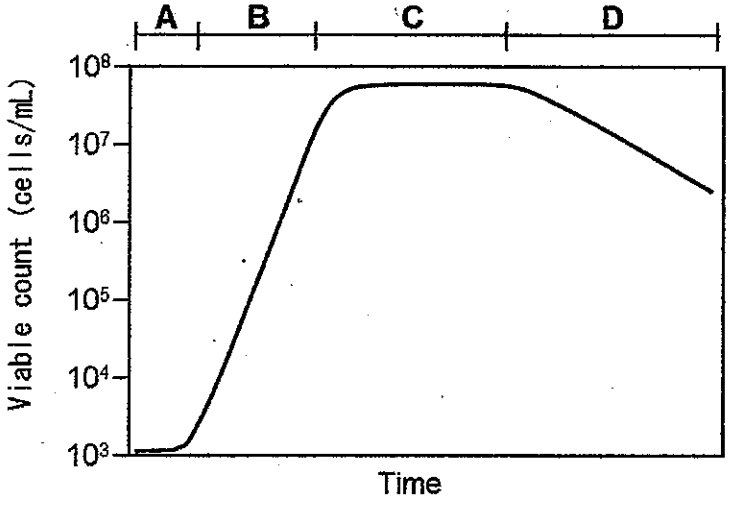
AY2026 Entrance Examination for the Master's Program, Graduate School of Bioagricultural Sciences, Nagoya University

Subject chosen	Microbiology	For this subject Total pages (3) Page number (1)

There are three major questions for microbiology (from I to III). Please be sure to use a separate answer sheet for each question.

Question I.

The figure shows the typical growth curve of bacteria in a closed culture system. Answer the following questions.



1. Give the name of each growth stage (from phase A to D).

2. Answer the reason why the bacterial growth in phase A is slower than that in phase B, and give a way to shorten the period of phase A without changing the cultivation conditions.

3. Calculate the time required for bacteria in growth phase B to increase from 1.0×10^4 cells/mL to 1.0×10^7 cells/mL when growing at a specific growth rate (μ) of 1.5 h^{-1}. Use ln10 = 2.303 and show the calculation process as well.

4. List two reasons why the viable count is kept constant in phase C.

5. List three methods for measuring bacterial growth and briefly explain their principles.

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Question II

1. Offer a brief explanation for microbial terms (a) through (c). In doing so, choose one suitable term from the list and incorporate it into the explanation.

(a) phosphoroclastic reaction
(b) syntrophy
(c) coenzyme F_{420}

List: blue-green fluorescence, chlorophyll, heterolactic fermentation, homolactic fermentation, interspecies hydrogen transfer, pyruvate

2. Identify three microorganisms that can conserve energy through respiration in the absence of oxygen from the list provided. For each microorganism, specify (a) the ***initial*** electron donor and (b) the ***final*** electron acceptor in the respiratory chain. Additionally, describe (c) the ***product released*** in the final step of the respiratory chain. The answers for (c) should identify the substances whose oxidation numbers are altered during electron transport.

List: *Brocadia anammoxidans*, *Escherichia coli*, *Lactobacillus delbrueckii*, *Micrococcus luteus*, *Pseudomonas stutzeri*, *Saccharomyces cerevisiae*

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Question III

Explain the following terms in molecular biology of *Escherichia coli*. Choose multiple terms from the list below and use them in your explanations (You may use the same term multiple times).

(1) F plasmid
(2) SOS response
(3) Replisome
(4) Polysome (Polyribosome)
(5) Riboswitch

List:

aptamer, cAMP-CRP, conjugation, DNA damage, DNA helicase (DnaB), DNA polymerase III, horizontal transfer, LacI, LexA, ORF, post-transcriptional regulation, post-translational regulation, primase (DnaG), protospacer, RecA, regulation of transcriptional initiation, ribosomes, sex pili, topoisomerase, transcription-translation coupling, transduction, 5' -UTR, 3' -UTR

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Question I

1.

A. Lag phase (inducible phase)
B. Log phase (logarithmic phase, exponential phase)
C. Stationary phase
D. Death phase

2. During growth phase A (lag phase), the culture is in a preparatory stage, preparing enzymes necessary for adapting to new culture conditions, such as changes in medium composition. To shorten the induction phase, inoculate and culture bacteria that have already grown under the same conditions during the log phase.

3.

Approximately 4.6 hours.

The cell growth rate (dX/dt) is expressed as the specific growth rate (μ) multiplied by the cell number (X):
$dX/dt = \mu X$

Integrating this yields $\ln X - \ln Xo = \mu (t - to)$.
Converting to common logarithms gives $\log X - \log Xo = \mu (t - to) / \ln 10$.
$\log 10^7 - \log 10^4 = 3 = \mu \times (t - to) / \ln 10$
$(t - to) = 3 / 1.5 \times \ln 10 = 4.606$

4.

・Because the culture medium ran out of sufficient nutrients.
・Because metabolites that inhibit growth accumulated in the culture medium.

5.

・Dilution plate method. A small amount of culture medium is taken from the growing culture, diluted to an appropriate density, and a fixed volume of this diluted medium is spread onto agar plates. After incubation, the number of colonies is counted, and the bacterial count is determined based on the dilution factor.
・Method using measurement of the culture medium's absorbance. This exploits the fact that as a cell density increases, light scattering increases and transmittance decreases (= absorbance increases). The measurement wavelength uses red light around 600 nm.
・Method using microscopic observation. Using a grid on a special slide glass (Petroff-Hausser counting chamber) with a fixed volume, cells are counted directly under a microscope to measure the cell concentration per unit volume.

Each method measures bacterial growth by tracking increases over time.
(Note: The above is an example answer. Other methods such as dry weight measurement, cell component measurement, quantitative PCR, and flow cytometry are also considered correct answers.)

Purpose of the Question

To test basic knowledge of terminology, equations, physiology, and analytical methods in microbial growth.

Question II

1.

(a)phosphoroclastic reaction

Some bacteria can maintain redox balance through the production of molecular hydrogen. They oxidatively decarboxylate **pyruvate** to acetyl-CoA, which is then transformed into acetate via acetyl-phosphate, with the energy released being conserved as ATP (equation 1).

pyruvate + ADP + Pi ↔ acetate + H_2 + CO_2 + ATP (equation 1)

In this process, the reversible conversion of acetyl phosphate from pyruvate is known as the phosphoroclastic reaction, which includes an oxidative decarboxylation of pyruvate to form acetyl-CoA, carbon dioxide, and hydrogen gas, known as “clostridial type” (equation 2) and a cleavage of pyruvate into acetyl-CoA and formate, followed by oxidation of formate to carbon dioxide with the release of hydrogen gas, known as “coliform type” (equation 3).

pyruvate + HS-CoA ↔ acetyl-CoA + H_2 + CO_2 (equation 2)

pyruvate + HS-CoA ↔ acetyl-CoA + formate (equation 3)

formate ⟶ H_2 + CO_2

(b)syntrophy

Syntrophy refers to a metabolic process where two distinct microorganisms work together to break down a substance that neither can decompose on its own. In many cases, the core of syntrophic interactions is the **interspecies hydrogen transfer**, where one microorganism produces H_2 and the other consumes it. A syntroph performs a reaction with a positive standard free-energy change. However, the H_2 generated by the syntroph can serve as an electron donor for another syntroph in an exergonic reaction. When these two reactions are combined, the overall process becomes exergonic, and the released free energy is shared by both microorganisms.

(c)coenzyme F_{420}

Coenzyme F_{420} functions as an electron donor in the process of methanogenesis and is involved in multiple stages of CO_2 reduction. It is a flavin derivative that structurally resembles FMN and mediates the transfer of electrons from H_2. The oxidized form of F_{420} absorbs light at a wavelength of 420 nm and emits a **blue-green fluorescence**, which aids in the microscopic identification of methanogens.

2.

Brocadia anammoxidans

(a)ammonium ion, NH_4^+
(b)nitric oxide, NO
(c)nitrogen gas, N_2

Escherichia coli
(a)NADH
(b)nitrate, NO_3^-
(c)nitrite, NO_2^-

Pseudomonas stutzeri
(a)NADH
(b)nitrous oxide, N_2O
(c)nitrogen gas, N_2

Purpose of the Question

To test fundamental knowledge concerning the catabolic processes of microorganisms.

Question III

(1) F plasmid

The F plasmid is a fertility plasmid in *Escherichia coli* that enables conjugation, one of the major mechanisms of horizontal transfer in bacteria. Cells carrying the F plasmid (F^+ cells) produce sex pili, which establishes contact with F^- recipient cells. Through this contact, the F^+ cell transfers the plasmid DNA unidirectionally to the F^- cell. This process allows rapid dissemination of genetic information across bacterial populations.

(2) SOS response

The SOS response is a global regulatory mechanism activated by severe DNA damage in bacteria. Under normal conditions, the repressor LexA inhibits the transcription of SOS genes. When DNA damage produces stretches of single-stranded DNA, the RecA protein becomes activated and promotes autocleavage of LexA. Derepression of SOS genes induces a variety of DNA repair enzymes, allowing cell survival.

(3) Replisome

The replisome is a multi-enzyme complex that carries out DNA replication at the replication fork. Major components of this complex include the DNA helicase (DnaB), which unwinds duplex DNA near the origin of replication; the primase (DnaG), which synthesizes RNA primers required for replication initiation; and DNA polymerase III, which replicates both the leading and lagging strands. Replication of the lagging strand proceeds through the repeated synthesis of short Okazaki fragments, a process that requires the coordinated action of multiple enzymes. This orchestration ensures high-efficiency and high-fidelity DNA replication.

(4) Polysome (Polyribosome)

A polysome, or polyribosome, is a structure in which multiple ribosomes simultaneously bind to a single mRNA molecule to carry out translation. This arrangement allows the cell to synthesize a large number of protein molecules from a single mRNA in a short period, thereby enhancing translational efficiency. In *Escherichia coli*, transcription and translation occur concurrently, so ribosomes can bind to mRNA immediately upon its synthesis, forming polysomes. This feature facilitates transcription-translation coupling and ensures that proteins are rapidly produced before the mRNA diffuses within the cytoplasm.

(5) Riboswitch

A riboswitch is an RNA regulatory element commonly located in the 5'-UTR of bacterial mRNAs. It contains an aptamer domain that binds specific metabolites directly. Ligand binding induces secondary structure changes that modulate post-transcriptional regulation, such as controlling transcription termination or blocking the ribosome-binding site. Riboswitches allow RNA molecules themselves to function as sensors and regulators without requiring proteins.

Purpose of the Question

To test fundamental knowledge concerning molecular biology of microorganisms and its applications in biotechnology.