Module Code: BIO00075H

Examination Candidate Number: __________

BA, BSc, MSc Degree Examinations 2019-20

Department:
BIOLOGY

Title of Exam:
Advanced topics in Microbiology

Time Allowed:
24 Hours (PLEASE NOTE: Late papers will not be marked)

Time Recommended:
2 Hours and 30 Minutes

Word limit: Please answer the questions within the line limit stated. Content beyond the line limit will not be marked.

Allocation of Marks:
Total marks available for this paper: 100
The marks available for each question are indicated on the paper.

Instructions for Candidates:
All questions should be answered on this question paper using minimum font size Arial 11.
Each question should be answered within the stated line limit.
Do not adjust the margin width.
A note on Academic Integrity

We are treating this online examination as a time-limited open assessment, and you are therefore permitted to refer to written and online materials to aid you in your answers.

However, you must ensure that the work you submit is entirely your own, and for the whole time the assessment is live you must not:

- communicate with departmental staff on the topic of the assessment
- communicate with other students on the topic of this assessment.
- seek assistance with the assignment from the academic and/or disability support services, such as the Writing and Language Skills Centre, Maths Skills Centre and/or Disability Services. (The only exception to this will be for those students who have been recommended an exam support worker in a Student Support Plan. If this applies to you, you are advised to contact Disability Services as soon as possible to discuss the necessary arrangements.)
- seek advice or contribution from any third party, including proofreaders, friends, or family members.

We expect, and trust, that all our students will seek to maintain the integrity of the assessment, and of their award, through ensuring that these instructions are strictly followed. Failure to adhere to these requirements will be considered a breach of the Academic Misconduct regulations, where the offences of plagiarism, breach/cheating, collusion and commissioning are relevant - see AM.1.2.1” (Note this supersedes section 7.3 of the Guide to Assessment).
1. Replication assays similar to those in Guy et al. have been carried out using the plasmid template shown in panel C. The *E. coli* replisome is assembled at oriC before replication is initiated. RNA polymerase can be stalled at multiple specific locations on this plasmid: $P_{\text{lacUV5}}$ and sites within *ColE1* called $P_{\text{RNA1}}$ and $P_{\text{RNA2}}$. Rep and UvrD helicases were added to the replication assay reactions to test their function and the results are shown in panel A. A version of Rep helicase missing the 2B domain was used in panel B (CW = clockwise, CCW = counter-clockwise). Panel C shows the predicted replication products: i = CW, ii = CCW.

(a) What effect does the addition of Rep or UvrD have on the replication products produced? (2 marks; 3 lines)

The amount of full-length replication products are increased (1) and the intermediate products formed due to replication blockage are reduced (1).
(b) What does losing the 2B domain do to Rep function and how do you know that from this data? (3 marks; 4 lines)

Rep function is abolished (1) because the shorter replication products are still prominent (1) and the amount of full-length replication products is not increased (1).

(c) What does lane 5 in panel A tell you about Rep and UvrD helicase function? (2 marks; 5 lines)

Addition of both Rep and UvrD does not result in more efficient RNAP block clearance than addition of a single helicase i.e. lane five = lane three/four (1). Therefore, Rep and UvrD do not function in an additive or cooperative manner (1).

(d) Why is the ability of Rep/UvrD demonstrated here useful in vivo? (4 marks; 7 lines)

Transcription and DNA replication occur simultaneously in bacteria (1) so RNAP is a common block to DNA replication forks (1). To avoid genome instability problems caused by replication fork stalling (1), helicases capable of resolving these replication-transcription conflicts are vital (1).

(e) In vitro results might not apply to an E. coli cell. Briefly describe one method that would allow you to test whether RepΔ2B can perform the function of Rep in vivo? (4 marks; 7 lines)

Plasmid loss assay (1): Construct a ΔuvrD repΔ2B strain with rep on a covering plasmid (1). If RepΔ2B is not capable of performing the Rep accessory helicase function (1) then all plated colonies will still contain the plasmid after non-selective growth (1). Marks also given for explaining the results and conclusion as if RepΔ2B can function as an accessory helicase. Credit given for a complementation approach or another sensible method.

LO1. Explain molecular mechanisms underpinning microbial function in, for example, DNA replication, prokaryotic cell biology, regulation of gene expression.

LO3. Explain the basis and application of techniques for analysis of microbiology at a molecular, cellular and community levels.

LO5. Critically analyse research papers in microbiology.
2. A group of researchers have succeeded in fusing yeast chromosomes using CRISPR–Cas9. Their strategy was broadly similar to that employed by Shao et al., (core paper five). They produced strains that contain eight, four and two chromosomes compared to the usual sixteen for *S. cerevisiae*. This figure shows a comparison of selected genes from the transcriptome analysis of the sixteen and two chromosome strains.

Comparison of transcriptomes of *n* = 2 and *n* = 16 strains. Genes located within 20 kb of remaining telomeres are shown in blue; those within 20 kb of fused telomeres are shown in red; others are shown in grey.

Red = VBA3, COS4, COS1, YIR042C, PHO11, COS1, IMD2, AAD10, HXT9  
BLUE = COS10, DLD3, YLR460C, RMD6, YEL077C, AIF1

(a) Why might distance to telomeres be relevant when examining expression in these strains?  
(3 marks; 5 lines)

The telomere position effect (TPE) silences genes that are close to telomeres (1). In the strain with two chromosomes many telomeres will have been deleted (1) so the TPE no longer represses their expression (1).
What is the relationship between chromosome number and sporulation efficiency when fusion strains are crossed with a wild type strain? (1 mark; 2 lines)

Sporulation efficiency goes down as the number of chromosomes decreases (1).

How do the two different spore germination/viability assays used in panels c and d differ? (2 marks; 4 lines)

Panel c is testing how well fused chromosome strains can mate with a wild type strain (1) whereas panel d tests how well strains with fused chromosomes can mate with themselves (1).
(d) Using information contained within this figure, what long term predictions would you make about the ability of the four chromosome strain to survive in the wild if released into the environment? Briefly justify your answer. (4 marks; 7 lines)

Since the sporulation efficiency is much lower than wild type yeast (1), the fused strain will be less likely to survive harsh conditions via the sporulation response (1). The reduced chromosome strain mates with itself much more efficiently than with the wild type (1), therefore it is likely to become reproductively isolated and may evolve into a new species (1). Credit given for other sensible predictions.

LO1. Explain molecular mechanisms underpinning microbial function in, for example, DNA replication, prokaryotic cell biology, regulation of gene expression.

LO2. Address a question in contemporary microbiology from a variety of perspectives.

LO5. Critically analyse research papers in microbiology.

3. CBASS is an anti-phage defence system encoded by a four-gene operon and is widespread in bacteria. Cohen et al. introduced the operon from *Vibrio cholerae* El Tor and *Escherichia coli* TW11681 into a lab *E. coli* strain that does not harbour this defence system to test if this operon confers protection against phages.

(a) The authors performed an efficiency of plating assay (also known as plaque forming assay) after infecting the constructed *E. coli* strains with different phages. What conclusions can be drawn from the result plot? Describe the plot and interpret the results. (6 marks; 11 - 12 lines)

The plot shows the results obtained by infecting an *E. coli* strain containing no CBASS system that acts as a negative control (gray bars)(1 mark), an *E. coli* strain containing the CBASS operon from *E. coli* TW11681 (red bars) (0.5 mark) and the *V. cholerae* El Tor strain (yellow bars)(0.5 mark) with different phages. The results indicate that:
- both the *V. cholerae* and *E. coli* derived operons grant protection against multiple phages (1 mark);
- the *E. coli* derived system confers stronger protection (less plaques formed, shorter red bars) as compared to the system from *V. cholerae* (1 mark); this is probably due to a better compatibility, i.e. operon from *E. coli* in an *E. coli* host (1 mark);
- the four-gene system from either *E. coli* or *V. cholerae* does not protect against some phages such as T7, SECphi17, SECphi18 and SECphi27 (1 mark).

(b) To assess the importance of each gene of the operon derived from *E. coli* TW11681, operons harbouring either deletions of individual genes or mutations were tested using the plaque forming assay to check if they provide protection against phage P1.

![Operon Diagram](image)

What conclusion can you draw from the plot shown below? (3 marks; 7-8 lines)

The results indicate that:
- the operon does not provide protection against phage P1, when either gene A or gene B are deleted or contain mutations in specific residues (1 mark), showing that these two genes encoding the phospholipase and the cyclic GMP-AMP synthase (cGAS) are essential for this defence system to work (1 mark);
- the other two genes, gene C and D, do not seem to be crucial as the number of plaques formed in the absence of these genes is the same as when the whole system is present (1 mark).

(c) With the aid of the diagram below, discuss how the CBASS system works.
(7 marks; 8 - 10 lines)

- Infection of the bacterium by a phage is sensed by the cyclic GMP-AMP synthase (cGAS) that becomes active and produces cyclic GMP-AMP (cGAMP) (1 mark).
- These cyclic molecules bind to the CapV phospholipase activating it (2 marks). The active phospholipase cleaves the membrane (1 mark) resulting in cell lysis and death before the phage can complete its replication cycle (1 mark).
- This phenomenon is known as abortive infection (1 mark) and involves few infected bacterial cells committing suicide in order to avoid phage propagation through the population (1 mark).

LO1. Explain molecular mechanisms underpinning microbial function in, for example, DNA replication, prokaryotic cell biology, regulation of gene expression.

LO2. Address a question in contemporary microbiology from a variety of perspectives.

LO5. Critically analyse research papers in microbiology.
4. Recent studies have shown that some phages assemble a nucleus-like structure in bacteria during infection. Phage DNA is replicated within this compartment, before being packaged into the capsid. Chaikeeratisak et al. performed fluorescence microscopy experiments to investigate phage particle assembly in bacteria.

(a) Outline the setup used for the experiment and discuss the results shown in the figure below. 

This is a fluorescence microscopy time-lapse experiment in which the phage PhuZ tubulin-like protein is fused to magenta mCherry and a phage capsid protein is fused to green GFP (2 marks). The images show that the capsids migrate rapidly from the cell membrane to the phage nucleus by moving along PhuZ filaments (1 mark). After 20 seconds, most of the capsids are docked on the surface of the phage nucleus (1 mark).

(b) The authors introduced a mutation in the gene encoding the PhuZ protein that results in the replacement of D190 with alanine. What conclusions can be drawn by comparing wild type and mutant PhuZ in the experiments below?

In the cells containing wild type PhuZ protein, all the phage capsids have been transported along the PhuZ filaments (1 mark) and are docked on the nucleus by 50 minutes post infection (1 mark). In contrast, by the same time point and even
after 70 minutes, capsids are still stuck on the mutant PhuZ filament (1 mark). The mutant PhuZ is defective in GTP hydrolysis (1 mark) and this impairs capsid migration along the filaments (1 mark).

**LO1.** Explain molecular mechanisms underpinning microbial function in, for example, DNA replication, prokaryotic cell biology, regulation of gene expression.

**LO2.** Address a question in contemporary microbiology from a variety of perspectives.

**LO5.** Critically analyse research papers in microbiology.

5. Flux balance analysis is now widely used in studies of microorganisms to better understand and construct modifications to manipulate metabolism. Shown below is a depiction of the central metabolism of *E. coli* from Yim et al. Nature Chemical Biology volume 7, pages 445–452(2011).

(a) Explain what the nodes and the links represent. Explain why a link might not represent the activity of a single gene

(3 marks, 4 lines)
Nodes represent metabolites.
Links represent possible biochemical reactions that can carry flux (1 for both).
There is not necessarily a simple relationship between a Gene, the protein it produces, and the enzymatic reaction it permits. The GPR relationship can be 1:1 but it can be many to one (where multiple genes are required to facilitate a single reaction, e.g. an ABC transporter) (1) or one to many (where the same protein is encoded elsewhere on the genome or same reaction can be facilitated by multiple proteins, or both. FBA does not distinguish between reactions with different kinetics. e.g. common reaction such as acetate kinase) (1). Examples not needed
Having successfully added genes from alternative organisms to produce the novel product, the authors constructed a series of interventions to increase flux into the BDO pathway.

<table>
<thead>
<tr>
<th>Strain</th>
<th>MG1655 ΔldhA lacQ</th>
<th>MG1655 ΔldhA lacQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB3</td>
<td>MG1655 ΔldhA lacQ</td>
<td>AB3 ΔlpdA::K.p.lpdD354K</td>
</tr>
<tr>
<td>ECKh-138</td>
<td>MG1655 lacQ ΔadhE ΔldhA ΔpflB</td>
<td>ECKh-138 Δmdh ΔarcA</td>
</tr>
<tr>
<td>ECKh-401</td>
<td>ECKh-138 Δmdh ΔarcA</td>
<td>ECKh-401 gltAR163L, StrR</td>
</tr>
</tbody>
</table>

(b) Briefly explain how the four new strains cumulatively differ from the parental strain and outline the impact of these sequential differences in each case.

(8 marks, 10 lines)

AB3 contains two new knockouts which reduce the overflow to ethanol (adhE) and modify the flux from pyruvate (pflB). 3 of the computationally predicted 4 knockouts are now implemented. This removes the flux to succinate (2).

ECKh-138 then encodes a new version of the lpdA gene (from Klebsiella pneumoniae) to improve the anaerobic functionality (NADH sensitivity also acceptable) (2)

ECKh-401 then completes the optKnock predictions. It increases the oxidative TCA cycle by knocking out mdh, stopping backflow and accumulation of succinate and by removing arcA, a repressor of a number of oxidative TCA genes (2).
ECKh-422 encodes a point change in a citrate synthase change, reducing inhibition by NADH, further increasing TCA flow (2).

(c) In the discussion the paper states “Systems Biology approaches can...” “...address bottlenecks that are obstacles to commercialization”. Comment on the key bottlenecks addressed in this paper and what is further required.

4 lines
This demands closer reading of the discussion. Removal of the competing products. Removal of a key regulator (ArcA). Removal of mdh which keeps the reductive activity of TCA cycle to a minimum (1 for at least one of these). The paper comments that transfer more economically suitable feedstocks is also achieved (xylose) but a 3-5 fold increase in yield is also required (1 for at least one of these).

6. Consider the following plot from Elowitz et al. 2002

![Plot from Elowitz et al. 2002](image)

(a) Explain what is meant by extrinsic and intrinsic noise and describe an experiment in a bacterial system to distinguish between them

(4 marks, 5 lines)

Extrinsic noise is the noise environmental effects on the gene. This can be both intra and extra cellular effects (1). Intrinsic noise is the noise integral to the gene expression, i.e. from the processes of transcription and translation (1). Following Elowitz et al, two identical fluorescent genes, barring their colour, were placed in symmetric places on the genome under the control of the identical constitutive promoters (1). Extrinsic noise is then “in phase” noise, and the colour mixture is created. Intrinsic noise is then “out of phase” noise and a spread of different colours between cells is created (1).

(b) Strain D22 is noted as being a “noisy” strain relative to M22. Explain a possible mechanism by which a strain could be “noisier”.

(2 marks, 3 lines)
The mostly likely source is the quality of the DNA. Elowitz et al found that knocking out the recA gene, responsible for DNA repair, had the effect of increasing intrinsic noise, so dramatically it was lethal in isolation but could be mitigated by addition of IPTG.

Consider the following two motifs for a gene $X$.

(c) Identify and explain in both cases how the presence of noise modifies their behaviour. (3 marks, 4 lines)

Case 1 is positively autoregulated (0.5). Noise here can create switching behaviour between competing stable steady states (1). Case 2 is negatively autoregulated (0.5). This motif is routinely found in biological systems to buffer the gene expression against noise by decoupling the expected steady state from the production rate (which is affected by intrinsic noise). (1)

(d) Discuss, with an example, how noise is used to provide function in biological systems rather than simply a feature that needs to be “engineered away”. (3 marks, 5 lines)

Microbes can use noise-suppression gene motifs (such as negative-autoregulation and incoherent feed forward loops) to reduce noise to key genes but many genes are left unregulated and the noise accepted or even exploited (1). In many systems noise is utilised to create a phenotypically heterogeneous population from a genetically identical one (1). This is used in bacteria most prominently in the quorum sensing systems and is mechanistically created by the presence of thresholds that are in hit at varying times. It is also used in some metabolic uptake motifs so that the population can exploit a variety of metabolic inputs differentially (1 for an example).

(a) The paper focuses on the hypothesis that the human gut microbiome influences mental health. What methods (experimental and analytical) are used to explore this idea?  

8 marks; 12 lines

DNA was extracted from faecal samples from human volunteers and 16S rRNA sequencing was used to assess the phylogenetic microbial diversity.

Additionally DNA was extracted for full metagenomic sequencing from each faecal sample in order to gain insight into the metabolic capabilities of the microbiome. The data from this were used bioinformatically to generate “gut-brain modules” as inferences about the “neuroactive potential” of microbial metabolism.

DNA samples were obtained from a large cohort (> 1000 individuals), and for all these individuals quality of life (QoL) data were gathered, including general practitioner-reported depression, age, sex, BMI (Body Mass Index) and BSS (Bristol Stool Scale).

The correlation of mental and physical well-being to microbial composition at the level of taxa, enterotypes and gene functions were assessed.

[b] Based on the information in Fig. 1 (shown) what can you conclude, regarding the link between the microbiome and mental health?  

4 marks; 5 lines

![Diagram](image)

- Anthropometrics
- Gastrointestinal
- QoL
- Depression

- Faecalbacterium
- Coprocoruscus
- Dialister
- Butyrivibrio
- Gemmiger
- Fusobacterium
- Prevotella
- Butyricoccus
- Parabacteroides
- Streptococcus
- Flavonifractor
Figure 1. a, Combined explanatory power of FGFP covariates pooled in predefined categories on microbiome community variation (stepwise dbRDA on Aitchison distance; n = 1,054). Anthropometrics: age, sex, BMI; gastrointestinal parameters: BSS and gastrointestinal disease; depression: diagnosis of depression and use of antidepressants. b, Cumulative effect sizes of FGFP covariates on microbiome community variation (left bars; stepwise dbRDA on Aitchison distance; grey, variables not entering the dbRDA model; n = 1,054) compared to individual effect sizes assuming covariate independence (right bars). c, Associations between QoL scores or depression and bacterial genera after partialling out the effect of the main microbiota covariates (anthropometric and gastrointestinal parameters; GLMs, FDR < 0.1) in the FGFP cohort (n = 1,054). Validation of QoL associations in the LLD data set (n = 1,063; tick marks indicate successful validation) and validation of depression associations in the non-medicated subset of FGFP patients (depressed non-medicated) and in published case–control depression studies6,7,8,9 (literature). Positive correlations (or taxa elevated in the non-depressed group) are displayed in blue and negative correlations in red. Colour intensity is proportional to standardized GLM β coefficients. MCS, mental component summary; MH, emotional well-being; SF, social functioning; VT, vitality; RE, role limitations caused by emotional health problems; GH, general health perception; PF, physical functioning; RP, role limitations caused by physical health; BP, body pain; PCS, physical component summary.

The authors were able to conclude that particular taxa are correlated with mental health, both positively and negatively. The magnitude of the influence of mental health status on microbiome (or vice versa) was small (explaining less than 1 % of the variation), was typically shared between impact on physical and mental well-being.

(c) This is Figure 2 from the paper:

Figure 2. a, Mean QoL (RAND) scores in the four DMM enterotypes (B1, Bacteroides enterotype 1; B2, Bacteroides enterotype 2; P, Prevotella; R, Ruminococcaceae); Kruskal–Wallis test (FDR < 0.1 for all scores;
Supplementary Table 9) with post hoc Dunn’s test (FDR < 0.1 for associations between Bacteroides enterotype 2 and other enterotypes; Supplementary Table 9) in the FGFP cohort (n = 1,054). MH, emotional well-being; SF, social functioning; VT, vitality; RE, role limitations caused by emotional health problems; GH, general health perception; PF, physical functioning; RP, role limitations caused by physical health; BP, body pain. b, Enterotype distribution in FGFP individuals with general practitioner-reported depression (n = 151) versus controls (n = 933) (χ² test, χ² = 16.77, P = 7.87 × 10⁻⁴). Bacteroides enterotype 2 was the only one with a different distribution in cases versus controls (pairwise χ² tests; χ² = 5.21, FDR = 8.99 × 10⁻²;).

(i) What is meant by “enterotype”, and identify the paper where this concept was originally developed. (3 marks; 4 lines + a reference citation)

The microbial communities from the gut can be grouped into various types which share similarity in their composition. These are named for a major component - in the case of Bacteroides, there are two different distinct community types.

First paper to introduce enterotypes:
Linking long-term dietary patterns with gut microbial enterotypes.

(ii) Generate a hypothesis about what might be giving rise to the observations in the figure, and how might you test this? (4 marks; 8 lines)

This is a little open ended. An obvious hypothesis would be that a member of the typical Bacteroides type 2 community generates a metabolite that is deleterious to mental (and physical well-being) [2 marks]. This might be tested in an observational experiment using data from metagenomics, and measuring metabolites. Alternatively, an experimental approach would be to alter the microbial community in some experimental animal system. [2 marks]

(d) With respect to microbiome studies, what do you see as the value of observational versus experimental studies, and assess this in relation to this particular area of work. (4 marks; 8 lines)

Observational studies allow us to measure correlations between large numbers of individuals and to work with humans. The downside is that it is difficult to determine causation. Experimental studies typically involve using a model system so are not as representative of the situation in human populations, but do allow some dissection of mechanism.
(e) Suggest how these findings could be exploited in a healthcare setting.

(2 marks; 6 lines)

Again, open ended. Some suggestions would be: identification of an organism that might be used in faecal transplant therapy, or some chemical components that could be used as dietary supplements. Some sort of diagnostic for microbial well-being might be developed.

End of Exam