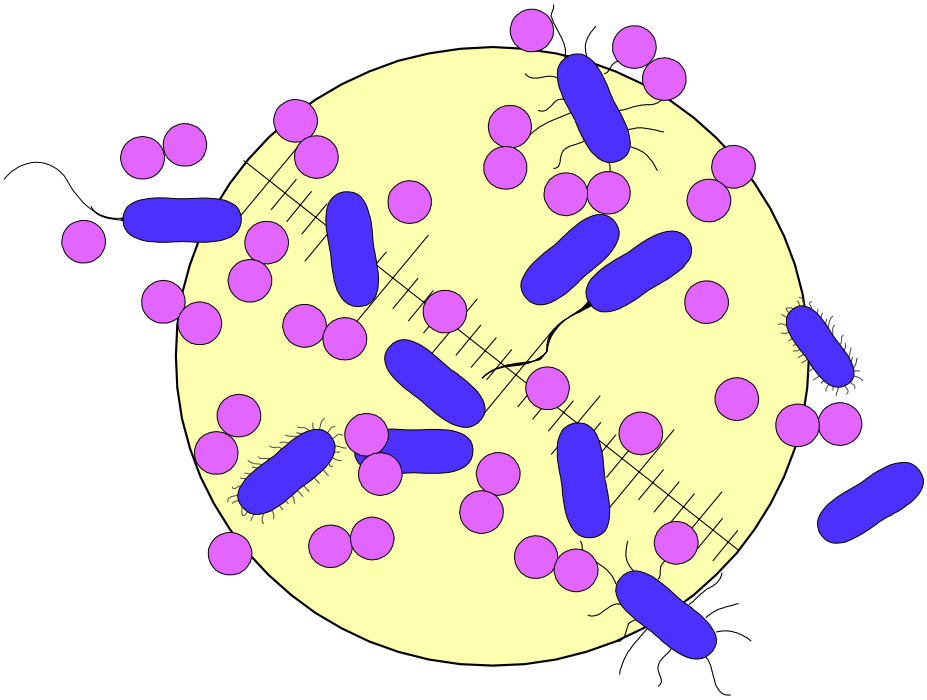
Dept. of Science & Technology
Grande Prairie Regional College

MI 2650
General Microbiology (3-0-4)
3 credits

Course Outline
2005-2006



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Schedule: Classes - Monday 1130-1250 and Friday 1000-1120 (J229)
Labs - Wednesday 1430-1820 (J130)

Description: This course covers aspects of bacterial physiology such as nutrient uptake, metabolism, extracellular proteins, chemotaxis and differentiation. Symbiotic associations and interaction of microbes with the environment are major topics. Basic principles of industrial microbiology and the use of biotechnology for the production of economically and medically important substances will be covered. Laboratory exercises are designed to compliment the material included in the classes.

Transferability:

Athabasca University	BIOL 3xx
Augustana University College	BIO 274
Concordia University College	BIO 2xx
University of Alberta	MICRB 265
University of Lethbridge	BIOL 3200
University of Calgary	Junior BIOL

(will not get credit for BIOL 231, but MI 2650 acts as pre-req. for CMMB 343)

Text-book: Brock - Biology of Microorganisms (11th edition)
MADIGAN & MARTINKO (2006)
Prentice-Hall Publishers

This text-book is recommended for the course - it is not compulsory. 9th or 10th editions are also very good, however the page numbers and figures will differ. For extra help with the text, Prentice-Hall Publishers have made available a companion web page for the text containing Chapter summaries, self-tests, and other information that you may find useful. The URL address for this web-page is:

http://www.prenhall.com/bookbind/pubbooks/brock2/

The web page for the 10th edition is: ***http://www.prenhall.com/brock/***

Relevant articles and other materials will occassionally be recommended to students. It is strongly recommended that they be read since the information may appear in exams.
A number of alternative textbooks may also be placed on reserve in the GPRC Library, and students are advised to take advantage of their availability.

Text-books on World Wide Web:

Medical Microbiology (4th Edition) Editor: Samuel Baron
http://129.109.136.65/microbook/toc.html

Microbiology 101 Internet Text (Washington State University)
http://www.wsu.edu/~hurlbert/pages/101hmpg.html

Requirements:

Lab. Reports 15%
Quizzes 5%
Mid-term Exam 20%
Final Lab Exam 20%
Final Exam 40%
TOTAL.....100%

In order to successfully complete MI 2650, students must attend ALL laboratory sessions and achieve a mean score of 50% on the Lab Reports, Lab Quizzes and Final Lab Exam.All assignments MUST be handed in by the time and date specified.

Late reports will not be marked!

Many of the Laboratory exercises require that students perform some of the procedures at times other than the scheduled lab period. To do this, prior arrangements must be made with **Mr. Rick Scott**, the Biology Lab. Technologist. In case of injury, it is preferable that students work with at least one partner when coming into the laboratory outside of scheduled times.

Quizzes in both class and laboratory sessions may be given **without any advanced notice** to students.

Since participation in lectures, and completion of assignments are important components of this course, students will serve their best interests by regular attendance. Those who chose not to attend must assume whatever risks are involved. In this regard, your attention is directed to the Academic Guidelines of Grande Prairie Regional College.

MI 2650
TOPIC DESCRIPTIONS

It is strongly recommended that students thoroughly review their BI 1070 notes especially in regards to Procaryotic Structure and Metabolism

Approx. # of Hours	TOPIC
1	Introduction to the course
6	**Functional Morphology of bacteria:** Definitions and descriptions of microbes. Correlation of cell structure and function. Differentiation of bacteria by cell wall type and key metabolic characteristics. Structural features important in both beneficial and harmful (pathogenic) interactions, motility. Pathogenesis
14	**Microbial diversity and environments:** Growth patterns in relation to oxygen (its use and toxicity). The major nutritional types with an emphasis on energy and carbon sources. Practical examples of diverse nutritional types: methanogenesis, autotrophs, photosynthetic microbes, extremophiles. Bioremediation, food microbiology, normal flora, symbioses.
1	**MID-TERM EXAM:** during lecture period (80 minutes)
9	**Sensory systems and intercellular communication:** Review of transcriptional control systems in bacteria. Role of sigma factors as transcriptional activators. Global regulation. Nitrogen cycling and regulation, symbiotic nitrogen fixation. Chemotaxis. Microbe-microbe signalling (quorum sensing) and plant-microbe interactions (eg. *Rhizobium* and *Agrobacterium* spp.).
7	**Bacterial growth and control of growth:** Effects of temperature, nutrient levels and growth conditions. Analysis of the exponential growth curve, using the growth equation to predict growth rate and cell yield. Control of growth using heat and chemicals (heavy metals, antibiotics). Resistance of bacteria to chemical agents (especially antibiotics)

MI 2650
Detailed Topic Outline

TOPIC	READINGS		
	11th Edition	10th Edition	9th Edition
Introduction and Review			
Overview of Microbial Life	Chapter 2	Chapter 2	Chapter 1
Procaryotes vs Eucaryotes	314-318, Tbl 11.3	338-340, Tbl 11.3	445, Tbl 12.3
Microbial sizes & shapes	63-66	64-65	58-59
Functional Morphology			
Cell Membrane	66-74	66-74	60-64
Cell walls	74-82	74-81	68-77
Mycobacterium tuberculosis	388-390	414-416	517-518
Pseudopeptidoglycan / S-layer	82-83	79	72
Wall-less bacteria	383-386	409-411	513-515
Pili and Fimbriae	82	90-91	85-86
Sex pili	278	291	319
Fimbriae	91, 711	738-739	784-785
Capsules	83, 617-619, 704-706	91-92; 636-638; 731-733	86-87; 646-647; 776-780
Appendaged bacteria	363-364	391-392	
Motility			
Flagella	92-95	82-87	79-82
Taxes	97-100	87-90	83-85
Gliding	95-97	86	497; 526
Spirochaetes	407-411	434-435	537-538
Pathogens and Normal Flora	703-710	730-748	-796

Microbial Diversity			
Oxygen Requirements	160-164	161-165	158-161
ETC and chemiosmosis	123-126	125-128	123-126
Nutritional Diversity			
Carbon and Energy sources	28-29, 127-130	28-29; 130-131	
Chemoheterotrophs			
Bioremediation	577-579, 651-653	674-676; 597-598	696-698; 632-634
Industrial Fermentations	375-379	400-404	504-507
Sulphate-reducing bacteria	371-373	396-399	498-502
Chemoautotrophs			
Sulphur-oxidizing bacteria	337-340, 550-553	360-363; 568-571	595-598; 670-675
Hydrothermal vents	628-631	647-651	670-675
Acidic habitats	157-158		
Iron (Fe^{2+})-oxidizing bacteria	337-340, 553-555	571-573	462-464
Acid mine drainage	644-647	666-669	598-601
Bioleaching	647-649	669-672	689-694
Methanogens	426-430, 564-568, 634-637	453-455; 583-587; 654-658	553-556; 613-617; 677-681
The Rumen	637-640	658-662	681-685
Environmental Extremes			
Salinity	158-160, 422-426	159-161; 448-452	156-158; 548-552
Temperature	150-157	151-157	147-154
Applications			
Environmental Sensing and Response			

Bacterial Gene Regulation			
Promoters and Sigma factors	188-191	187-192	191-194
Positive and Negative Control	210-221	211-220	213-223
Quorum Sensing	221-222	224	228
Bioluminescense	355-357	379-381	482-484
Agrobacterium tumefasciens	659-660	683-685	706-709
Signal transduction	224-226	224-226	230-231
Nitrogen Fixation			
Nitrogen Cycle	641-642	662-664	685-686
Nitrogen fixation	586-591	606-611	634-639
Rhizobium-legume symbiosis	661-667	685-691	709-717
Population Growth Kinetics			136-145
Growth	136-137	139	
Calculating exponential growth	140-142	142-144	
Population Growth Curve	142-144	144-145	
Control of Microbial Growth		Chapter 20	Chapter 18
Heat	671-673		
Irradiation	673-675		
Chemical methods	677-688		
Bacterial endospores	87-91	95-100	91-95
Microbial Resistance to Antibiotics	692-698	719-723	765-769

Laboratory Exercises
Fall Semester 2005-06

Exercise 1: Microscopy

a) Use of a light microscope including oil immersion
b) Observation of various microorganisms (prepared slides) to observe Gram stain reaction, cell shape, cell arrangement:
    Staphylococcus aureus
    Streptococcus sp.
    Bacillus anthracis
    Branhamella catarrhalis
    Spirillum serpens
    Escherichia coli
    Streptomyces sp.
    Saccharomyces cerevisiae
c) Demonstrations of:
    Acid-fast stain (*Mycobacterium* sp.)
    Endospore stain (*Bacillus subtilis*)
    Flagella stain (*Pseudomonas aeruginosa*)
    Capsule stain (*Streptococcus mutans*)
d) Demonstrations of:
    Phase Contrast microscopy
    Dark Field microscopy
    Differential Interference Contrast Microscopy
    Transmission Electron micrographs
    Scanning Electron micrographs

Exercise 2: Isolation of bacteria from a mixture

a) Preparation of Streak Plates from mixture of three bacterial species.
b) Preparation of Streak Plates from pure cultures provided.
c) Preparation of Gram Stains of samples from mixture.
d) Distinguish Gram positive and Gram negative organisms.
e) Prepare pure cultures from mixture plate after 48 hours incubation.
f) Describe characteristics of colonies
g) Prepare Gram stain on prepared pure cultures.
h) Perform Catalase Test and Oxidase Test.

Exercise 3: Identification of Gram positive and Gram negative bacteria isolated in Ex. 2

a) Perform following test on the Gram positive organism:
    Catalase test
    Bile-esculin test
    Blood haemolysis
b) Perform following tests on the Gram negative organism:
    Oxidase test
    ONPG test
    Urease test

DMCA test

c) Prepare streak plates of Gram negative species on following agar:
   MacConkey's medium
   Eosin Methylene Blue agar
d) Prepare a Rapid Multiple Test System (API 20E System) on Gram negative organism

Exercise 4: Collecting and Gram staining of an environmental biofilm

a) Sample and Gram stain dental plaque from own teeth as follows
   Prior to rinsing with mouthwash
   Immediately after rinsing with mouthwash
   20 minutes after rinsing with mouthwash
b) Compare samples of dental plaques:
   Gram stain, cell morphology, cell arrangement of dominant organism
   Relative cell densities

Exercise 5: Microbiological Analysis of Food

a) Calculate bacterial population of 'clean' and 'contaminated' samples of ground beef using Plate Count technique.
b) Isolate and identify *Escherichia coli* using:
   Gram stain
   Eosin Methylene Blue agar
   Double strength lactose broth with Durham tube
c) Identify *Enterococcus faecalis* using:
   Double strength Azide Dextrose broth
   KF agar
   BHI broth
   Catalase test
d) Identify *Staphylococcus aureus* using:
   Double strength TCS broth
   Blood agar
   Coagulase test
e) Determine if meat samples are considered to be safe for consumption.

Exercise 6: Microbial activity in milk and yogurt

a) Assess bacterial content of following milks:
   UHT milk
   Pasteurized milk
   Pasteurized milk left overnight at $30^{o}C$
   Buttermilk
   using:
   Dye reduction assay
   Methylene blue staining and microscopic examination
b) Assess bacterial population changes during yogurt fermentation;
   Perform Gram stain and determine pH immediately after preparation and after 24 hours incubation

Exercise 7: Activity of a Microbial Consortium – Methanogenesis

a) Determine methane production in sewage sludge using:
   Various incubation temperatures
   Gas chromatography to assess %age methane production

Exercise 8: Determination of Cellulase Activity

a) Preparation of cellulase enzyme from culture of *Trichoderma viride*
b) Spectrophotometric Assay of enzyme activity using Carboxymethyl cellulose as substrate and Dinitrosalicylic acid as indicator.

Exercise 9: Extracellular Enzymes of *Erwinia carotovora*

a) Inoculate whole potatoes with *Erwinia carotovora* and *Escherichia coli*
b) After one week prepare following plates from each potato:
   Starch agar (determination of amylase)
   Skim milk agar (determination of proteases)
   Spirit Blue agar (determination of lipid hydrolysis)
   Pectin medium (determination of pectinase)

Exercise 10: Antibacterial Agents

a) Assess the activity of various chemical agents and household products against common species of bacteria.
b) Assess the activity of various antibiotics against common species of bacteria using the Kirby Bauer Assay.

Exercise 11: Quorum Sensing

a) Plate Detection of Homo-serine Lactones using three strains of *Agrobacterium tumefaciens*:
   A. tumefaciens NTL4 – used as HSL negative control
   A. tumefaciens pTiC58 – contains Ti plasmid, produces HSL
   A. tumefaciens pZLR4 – responds to HSL by producing β-galactosidase
b) Assess rate of HSL production in batch culture over 24 hrs. (Demonstration)

Exercise 12: *Agrobacterium*-mediated Plant Transformation

Assay of transgenic *Arabidopsis* seedling tissue for expression of the reporter gene (*gusA*) for β-glucuronidase cloned from *Escherichia coli*.

a) Extract β-glucuronidase from tissue and assess its activity using p-Nitrophenyl-β-D-glucurinide as substrate
b) Determine protein concentration of tissue homogenate using the Bradford Dye-binding Protein Assay.