Semester Project

The Semester Project is designed to provide students with practice using the **scientific method.** As students continue toward their educational goals, more emphasis will be placed upon their ability to work independently and think critically. Successful completion of this project will require that students **plan ahead**, **perform research**, **carry out technical procedures, analyze their results**, **draw reasonable conclusions** and **write a scientific paper** suitable for publication. Projects are **not** to be simply library research projects, although some research will be required.

It is important that you reach a **reasonable balance** between this project's time demands and your other academic responsibilities. You may have to limit the scope of your proposed project in order to be successful within the time available to you between the beginning and end of the semester. Therefore it is required that you discuss this project with your instructor prior to beginning your activities. The instructor will assist you in determining the practicality of your proposal, suggesting reasonable alternatives, and providing necessary equipment and supplies as available within the department.

Some helpful hints for success:

- Work independently, or in groups of no more than 3 people.
- Begin and finish activities one step at a time.
- Don't be afraid to make mistakes! Recognize that you may have to repeat some steps.
- Start early – meet those deadlines!
- Record the results of your investigation ***in detail*** (temperature, time, media, etc.)
- Keep track of the status of your organisms – don't let them die!

Suggestions for project topics:

1. **Identification and characterization of microorganisms from the environment.** Organisms may be isolated from air, soil, water, food materials, fomites, plant or animal surfaces. **DO NOT COLLECT ORGANISMS FROM DISEASED INDIVIDUALS** (human or other animal). Bacteria vary considerably in their virulence, and we do not want to propagate highly virulent animal pathogens in this laboratory. **THANK YOU!**

2. **Enrichment for and isolation of specific bacteria.**
   Enrichments typically require the preparation of unique media and sometimes involve temperature and/or atmospheric modifications. Detailed information is available in the Bergey's Manual of Systematics of Archaea and Bacteria (BMSAB) and online.

3. **Investigation or experimentation involving microorganisms.**
   Unique investigations may be assigned/selected, exercises available in our laboratory manual may be modified, or students may use experiments adapted from other sources.

4. **Environmental Survey – Eukaryotic Microorganisms.**
   At least fifteen different types of eukaryotic microorganisms must be identified and **original** representations of these (illustrations, still photomicrographs or video images) must be recorded. **DO NOT PLAGIARIZE!** A written description of each organism type and the environmental **niche** it occupies must also be included.

Project outline and checklist:

_____ 1. Establish your group, choose your project and approve it with your instructor.

_____ 2. Sample collection, Hypothesis and Introduction.
- Obtain living organisms from some environment.
- Perform a literature search to generate a hypothesis (organism types expected). The written hypothesis, references used and notes taken will be worth points.
- Write the introduction for your project report (see #9 below) and have your instructor read/critique it.

_____ 3. Organism isolation
- Select one isolated colony per student from the organisms collected, and establish one or more pure cultures (you **must** have a pure culture to complete #5 below).
- You will be required to turn in a live culture at the end of the semester – DO NOT LET YOUR CULTURE DIE OR BECOME CONTAMINATED!
- Restreak your culture every 3 or 4 lab periods.

_____ 4. Begin morphological characterization of your culture (cell and colony).

_____ 5. Obtain chromosomal DNA from your isolate(s) and prepare it for DNA sequencing (A joint venture involving students and their instructor).
- You must have a fresh (less than one week old), pure culture to do this.
- Boil cells in Tris Buffer and if the culture is Gram-positive, beat with glass beads.
- Arrange for the completion of a PCR, gel electrophoresis and DNA purification.

_____ 6. Have DNA sequenced – Samples will be taken to the ^{UC}DNA Sequencing Facility in bulk lots, not one at a time, so DO NOT PROCRASTINATE!

_____ 7. Obtain a tentative identification for your culture through the NCBI BLAST.
- Look for entries including species names and accompanied by publications.
- Record the accession number(s) of entries with sequence data most similar to that obtained from your isolate(s).
- Access and read publications describing new species (sp. nov.) to determine the tests needed to characterize these organisms.

_____ 8. Conduct tests to confirm the identity of your culture.
- You will use the Bergey's Manual or scientific literature to determine the tests appropriate, and do only those tests. DO NOT WASTE MEDIA!
- Quick example: Your DNA sequence data indicates you have *E. coli*. You look up *E. coli* in Bergey's and find these are Gram-negative, rod-shaped bacteria that are lactose and indole positive but H_2S and acetoin negative. You then run an MR-VP, an SIM and a lactose carbohydrate deep to determine the characteristics of your isolate.

_____ 9. Write your report paper using the template provided by your instructor.
- Students that ask instructors to read/critique their rough drafts usually produce the best papers (and therefore earn the best grades).
- Avoid plagiarism!

_____ 10. Submit your paper electronically and turn in your living pure culture(s).

This project will be worth 70 points, so take it seriously and avoid "put off until later" tendencies. The best results will be obtained if you start your project **early**.

Semester Project Write-Up Guidelines

The write-up for the Semester Project must be completed in a scientific format. Scientific writing is not like literary writing. Correctly written scientific reports are succinct and to the point, without dialogue, symbolism, foreshadowing, simile, metaphore or mood. In a scientific report, it is more important that you follow the scientific method, rather than trying to earn a Pulitzer Prize. That is, the report must describe what you did, what you saw, and what you think those observations mean. For examples of completed student reports, see the "Sierra College Journal of Microbiology", located in the laboratory and available through the Biological Sciences website. For full credit, each write-up must have the following components.

Title
This must accurately and succinctly describe your project in a single sentence, e.g., "Isolation and Identification of Lysinibacillus boronitolerans from Automotive Cabin Air." or "A Comparison of the Effectiveness of Household Cleaners on Selected Gram-negative Bacteria."

Abstract
This is a brief description of the entire project, i.e., what you did, how it was accomplished, the results obtained, and your conclusions. Think of this as a 4 to 6 sentence synopsis of the entire paper. You must write this section **LAST**.

Introduction
This section introduces the project to your reader. Start by explaining the importance of your work (why you were conducting the investigation). Think about including potential impacts on human health, agriculture, or the ecosystem, and include some specific information. This section must also include your hypothesis (what you expect to find). For example, if you are isolating organisms from the environment, list several specific organism types you would expect to find and why they are significant.

Materials and Methods
This section describes what you did in enough detail for another Bio. Sci. 4 student to repeat your investigation. For procedures we have completed as a laboratory exercise, it is not necessary to include every step. Instead, refer to the laboratory syllabus, e.g., "Finally, a Gram stain was performed (Wilson, 2017)". This section must NOT include results; it is for procedures only. This is the easiest section of a scientific report to write, and most scientists write it **FIRST**. Hint – Be thorough here and use it as a guide as you complete your project.

Results
This section will contain your data (what you saw) and your results (what the data told you). Analysis of your results does NOT belong here. For example, it would be appropriate to describe carbohydrate deep data as "Following incubation, a sucrose deep inoculated with the organisms appeared yellow and lacked cracks." It would also be appropriate to describe the results as "acid positive and gas negative". It would NOT be appropriate for this section to say, "The culture can ferment sucrose forming acid, but cannot produce gas from fermentation." That kind of statement is an analysis of what your results meant and is reserved for the Discussion section. If you choose to include pictures, tables, graphs, etc. in this section, be sure to label them (Table 1, Figure 1, etc.) and refer to them in the text. For example: "The organisms were bacilli, i.e., display rod-shaped morphology (Fig. 1)."

If you are identifying a specific type of organism, be sure to include all relevant BLAST data (score, percent nucleotide similarity, ratio of nucleotides matched, accession number and taxonomic lineage of the authenticated organism type the query sequence matched most consistently with).

Discussion
The discussion section contains your interpretation of the results obtained, i.e., what your results meant. In this section you must convince your reader why you came to specific conclusions, e.g., that your organism type was indeed what you said it was (compare your findings to reference documents). If you performed an experiment; discuss briefly the implications of your findings (again – think human health, agriculture, the ecosystem, etc.). This is also the place to discuss any conflicting or confusing results.

Acknowledgements
The acknowledgement section is optional, but usually contains a brief sentence or two thanking individuals or organizations for funding or otherwise assisting with your project. Your instructor can provide specific information about grant funding.

Literature Cited
References used during the preparation of your report are to be **cited in the text** and listed at the end of the report, alphabetically by author. You must include **no less than three** "non-web-based" references. When citing references within the text, use the appropriate author's name and the year of publication, at the end of the sentence. Like this:

Clostridium botulinum produces a toxin that causes the illness known as botulism (Bauman, 2004).

In the Literature Cited section, arrange your sources in alphabetical order by first author's last name. Depending on the source, each reference will use one of the following four formats:

1. **Books** are referenced like this:

   Holt, J. G., N. R. Krieg, P. H. A. Sneath, J. T. Staley, and S. T. Williams. *Bergey's Manual of Determinative Bacteriology*, Ninth Edition. Lippincott Williams & Wilkins, 2000.

   (Author or authors. *Title*, Edition. Publisher, Year published.)

2. **Books that are collected works** (i.e., Bergey's Manual of Systematic Bacteriology) are referenced like this (the example used is for the genus *Leuconostoc*):

   Garvie, E. I., Genus *Leuconostoc* van Tieghem 1878, 198 emend mut. Char. Hucker and Pederson 1930, 66. *In* P.H.A. Sneath, N.S. Mair, M.E. Sharpe and J. G. Holt (Eds.), *Bergey's Manual of Systematic Bacteriology*, First Edition, vol. 2, pp. 1071-1075. Williams & Wilkins, 1986.

   That looks like a mess, but there is method in the madness! Let's look at the above reference in greater detail:

   Garvie, E.I., Genus *Leuconostoc* van Tieghem 1878, 198 emend mut. Char. Hucker and Pederson 1930, 66. *In* P.H.A. Sneath, N.S. Mair, M.E. Sharpe and J. G. Holt (Eds.), *Bergey's Manual of Systematic Bacteriology*, First Edition, vol. 2, pp. 1071-1075. Williams & Wilkins, 1986.

   The first section (highlighted upper portion) is the reference for the article (Author, Title.). Typically, you will find this information on the first page of the article describing the Genus or Family of the organism type you are investigating. In this case the author is E. I. Garvie, and the title is quite long: "Genus *Leuconostoc* van Tieghem 1878, 198 emend mut. Char. Hucker and Pederson 1930, 66."

After the article is cited, it is customary to type the word "In" in italics to indicate that this is an article in a collection. Then the book containing the collection is cited (highlighted lower portion).

Garvie, E. I., Genus *Leuconostoc* van Tieghem 1878, 198 emend mut. Char. Hucker and Pederson 1930, 66. *In* P.H.A. Sneath, N.S. Mair, M.E. Sharpe and J. G. Holt (Eds.), *Bergey's Manual of Systematic Bacteriology*, First Edition, vol. 2, pp. 1071-1075. Williams & Wilkins, 1986.

In (Editors, *Title*, Edition, Volume, Pages, Publisher, Year published.)

3. **Journal articles** must be referenced in the Journal of Bacteriology style. Like this:

Warren, S. M. and G. M. Young. 2005. An amino-terminal secretion signal is required for YplA export by the Ysa, Ysc, and flagellar type III secretion systems of *Yersinia enterocolitica* biovar 1B**.** J Bacteriol. **187**:6075-83.

(Author. Year. Title. Journal. **Volume**:pages.) This information is usually available and abbreviated for you at the top of each PubMed Abstract.

4. **Journal articles that are published online prior to print** (i.e., "ePub ahead of print"). These have not been assigned a volume yet, and do not have page numbers. These should be referenced like this:

Zhang, M. 2012. Translational control in *Plasmodium* and *Toxoplasma* parasites. Eukaryotic cell. Advance online publication.

(Author. Year. Title. Journal. Advance online publication.)

Fredrickson, B. L. (2000, March 7). Cultivating positive emotions to optimize health and well-being. *Prevention & Treatment, 3,* Article 0001a. Retrieved November 20, 2000, from http://journals.apa.org/prevention/volume3/pre0030001a.html

(Author. (Date published). Title, *Name of site*. Date retrieved, from URL)
Please do NOT just list the URL.

DO NOT REFERENCE PUBMED! It is a **database**, not a source. If you have a reference that you found using PubMed, it should be cited as a journal article (see #3 & #4 above).

DO NOT REFERENCE NCBI BLAST! NCBI is a database, not a source, and the BLAST is an algorithm used as a tool to access and compare data stored within NCBI and other databases throughout the world. When you have identified a species as being most similar to your isolate (based on nucleotide sequence), use the accession number to access author and submission information (article title, PubMed number if available, etc.). Proceed from there to a specific reference and cite it as a journal article (see #3 & #4 above).

Details, Details:

- The Gram stain is named after H.C. Gram, and must be capitalized.
- All Genus and Species names are written in *italics*!
- All sections must contain text. Tables and pictures are meant to enhance, not replace the text.
- The term "bacteria" is plural, i.e., indicating more than one, like apples, peaches, and Toyotas. Statements such as "this bacteria is a potential pathogen" or "a bacteria is a very small organism" are **INCORRECT**, and will be graded accordingly.

Semester Project Data Record

Project plan:
My independent project will involve _____
_____

All cultures collected in association with student projects are considered for inclusion in the Sierra College Culture Collection. Data relating to these organisms will be recorded and maintained as part of an ongoing research project. Please complete the data record indicated below for each pure culture isolated in association with your independent project, and deliver or email the information to your instructor.

Collection location:
City _____, State _____, environment sampled (air, water, soil, animal mouth, etc.) Please be specific. _____
_____

Cell morphology:
Please include cell size, shape, arrangement and Gram stain character (stain and KOH).
_____

Colony morphology: (Note – The culture must be pure.)
Please include colony form, margin, elevation, surface texture, optical character, pigmentation, size in mm and any unique character – be sure to include agar type used. _____
_____
_____

DNA processing:
Extraction of chromosomal DNA – Date completed _____
Method used (boil vs. boil & beat) _____
Polymerase Chain Reaction (PCR) amplification – Date completed _____
Gel electrophoresis of PCR product – Date completed _____

Electropherogram analysis and Genebank comparison with the NCBI, BLAST:
Date completed _____
Identity of species showing greatest similarity _____
Taxonomic lineage _____
_____
Query length _____ Gene bank length _____
% similarity _____ Number of bases matching pairwise _____
Score _____ Accession number _____

Unique features of this culture as indicated in reference materials (NCBI, Bergey's Manual):
_____
_____

Data and Results obtained from enzymatic testing as applicable:

Record type of metabolism and gas requirements as determined by completing the Oxidation/Fermentation test (Gram-negative organisms) or MR-VP tests (Gram-positive organisms). Note that for *Bacillus* species a numerical value is required for the methyl red test (use pH paper)

_____

MR (acid) _____ (numerical value obtained with pH paper may be required)
VP (acetoin formation) _____
Catalase (test with 3% hydrogen peroxide) _____
Oxidase – Cytochrome C (treated filter paper) _____
Citrate utilization (Simmon's citrate slant) _____
Urea hydrolysis (urea agar slant) _____
Esculin hydrolysis (Esculin slant) _____
Bile esculin hydrolysis (Bile esculin slant) _____
Starch hydrolysis (test with Grams iodine) _____
Gelatin hydrolysis (may require two or more weeks of incubation) _____
Indole formation (SIM) _____
Hydrogen sulfide formation (SIM or TSI) _____
Nitrate reduction (nitrate agar, reagents A & B, plus zinc) _____
Motility (SIM or wet mount) _____
Lysine decarboxylation (fermentative cultures only) _____
Coagulase test (*Staphylococcus* only) _____
CAMP test (if specified) _____
Aerobic acid formation on carbohydrate slants (specify each type used) _____
_____
_____
Acid and gas formation from fermentation in carbohydrate deeps (fermentative cultures only) specify each type of carbohydrate used _____
_____
Utilization of single carbon sources other than citrate (specify each type used). _____
_____

Describe growth on alternate media types used as indicated (record non-growth also).
Mannitol salt agar (MSA) _____
Eosin Methylene Blue agar (EMB) _____
Tergitol-7 agar (T-7) _____
MacConkey's agar (MAC) _____
Mueller Hinton agar (MHA) _____
Tryptic Soy agar (TSA) _____
Sheep Blood agar _____
Brain Heart agar (BHA) _____
Other enzymatic tests not listed above (specify type) _____
_____

Conclusion:
Was the data collected sufficient for making an accurate identification? _____
If not, what could be done to improve this project? _____
_____