The Wild Yeasts Biodiversity Project

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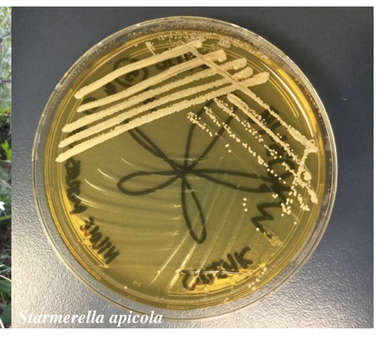
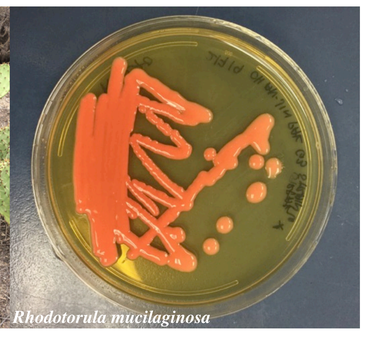
Marah macrocarpa (manroot/wild cucumber)


Starmerella apicola


Pseudognaphalium californicum (everlasting)


Meyerozyma guilliermondii


Opuntia sp. (prickly pear)


Rhodotorula mucilaginosa


Encelia californica (CA brittlebush)


Sporidiobolus salmonicolor

Abstract

We describe the Wild Yeasts Biodiversity Project, an inquiry-based module developed for a biology lab class at the University of California, San Diego. Over the course of this 10-week project, students work to isolate strains of wild yeast from the chaparral at a local nature reserve. On the initial collecting trip, students explore the habitat and choose their own samples, which increases the variety of samples and also students' ownership of their data. After several weeks of culturing and observing their strains, students extract genomic DNA and genetically barcode their strains to identify them. All yeast strains are preserved for future study, building a living archive of microbial biodiversity at the nature reserve that also serves to document changes over time in this ecologically sensitive habitat. The class pools their data and students work in pairs to look for patterns; students write and present a short proposal for further research to test the pattern they found. The protocols are derived from the Hittinger lab at the University of Wisconsin-Madison[1,2] but have been substantially expanded into a 50-page lab manual that guides students through collecting, culturing, phenotyping, isolating, freezing, DNA barcoding, and identifying their wild yeast strains.

Learning objectives

Laboratory skills

The module teaches an array of laboratory skills:

- Basic microbiology skills for liquid and agar plate culture including inoculating, plating lawns, streaking to isolation, etc.
- Aseptic technique.
- Pipetting with micropipettes and serological pipettes.
- Microbiology safety (because we culture unknown microorganisms, we use Biosafety Level 2 protocols and strongly emphasize lab safety).
- Ability to use standard lab equipment: centrifuges, vortexers, balances, filter-sterilization units, etc.
- Molecular biology skills: PCR, gel electrophoresis, genomic DNA preparation.
- Basic bioinformatics skills: analyzing and cleaning up trace files, searching GenBank and related databases, analyzing matches.

Former students have reported that these skills, including the BSL2 protocols, were useful in their subsequent positions in research labs.

Scientific practice

In addition, the module gives students the opportunity to practice authentic science:

- Collecting authentically novel data: The Scripps Coastal Reserve represents an important and threatened ecosystem (coastal sage scrub). Efforts have been underway to document its invertebrate residents but we are the first to build a comprehensive picture of its yeast biodiversity. We will add our results to the national database of wild yeasts maintained by the Hittinger Lab where it can be used by other researchers. In addition, we are building a collection of cryogenically preserved wild yeasts that serves as longitudinal data that we can use to test ecological and evolutionary questions.
- Generating hypotheses, designing experiments: Students comb through the extensive dataset that the class has generated over the quarter, looking for associations. They develop a hypothesis (e.g., bee-associated yeast species are most likely to be isolated from flowers) and design an experiment to test that hypothesis.
- Science communication: Students collaborate to produce written research proposals based on the associations they found in class data and present them orally.

Student investment and ownership

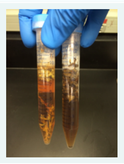
Each student chooses what they wish to sample at the Scripps Coastal Reserve natural area (with some safety-related exceptions) and many of them get inspired by this freedom, sampling bees or caterpillars as well as leaves and flowers. The students then work individually to culture and isolate strains from their sample. As the lab progresses, students become invested in their strains. There is genuine excitement and disappointment when the gels eventually reveal which strains can be sequenced (mostly excitement — the success rate for the genome extraction and PCR is high), and students are enthusiastic about finding out the species identifications and looking up what is known about the yeast's ecology. In addition, we have found a number of species that are not in GenBank and thus may represent novel species; SRS will collaborate with the Hittinger lab to try to characterize and describe these.

Timeline of the project

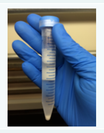
The Wild Yeast module is one of three projects that students complete in the 10-week course.

Week 1: Background and aseptic technique.
- Students learn the background of the project and how to collect samples aseptically.

Week 2: Field collecting.
- Each student takes 7 samples from the Reserve natural area: leaves, flowers, insects, etc.
- Students practice aseptic serological pipetting of media.



Week 3: Liquid media culture (round 1)
- Students make and filter-sterilize wild yeast liquid media.
- Students aliquot media, add antibiotics, and inoculate media with their samples.
- Cultures incubate in conditions that favor yeast and discourage molds and bacteria: limited oxygen (capped tubes, no agitation), 8% glucose, antibiotics, 30°C.



Week 4: Liquid media culture (round 2).
- After 5 days, students record observations about their cultures and check for signs of fermentation.
- Students passage cultures to new aliquots of wild yeast media.

Weeks 5-7: Agar plate culture.
- Students spread liquid cultures onto agar plates with rich yeast media (YPD).
- Students pick colonies and re-streak as needed until each yeast strain has been successfully isolated.
- Average success rate: 2-3 isolated yeast strains per student.

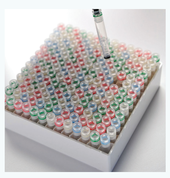
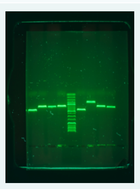


Photo: Fisher Scientific

Week 8: Extract genomic DNA; freeze strains for long-term storage.
- Students inoculate rich liquid yeast medium with each isolated strain and grow the cultures overnight.
- Students prepare a sample of each culture for cryogenic storage at -80°C, contributing to our living archive of microbial diversity from this natural area.
- Students extract genomic DNA from the cells and PCR the barcoding locus (intergenic transcribed spacer and 5.8S regions of a ribosomal gene).
- Students photograph stock plates and make detailed observations on colony morphology.



Week 9: Analyze PCR products, sequence barcoding loci, document strains.
- Students run PCR products on electrophoresis gel, choose the most promising ones, and prepare samples for sequencing.
- An outside company sequences the PCR products.
- Students collaborate to create a shared gallery of yeast colony morphology photographs that will be archived.
- Students record all their collecting, culturing, and colony morphology notes in a spreadsheet, using standardized language to make it easier for other students to interpret. All spreadsheets are pooled together into one shared class document.

Week 10: Analyze sequence data, research species, analyze pooled class data, write research proposal.
- Students clean up and analyze the sequence of their strains, then search databases to identify the species if possible.
- Students research ecology, known range, commercial uses, human health effects, etc. of their species.
- Students collaborate to search pooled spreadsheet of all class yeast data to find patterns and potential associations (e.g., particular yeast taxa associated with particular plant species or plant parts).
- Students write and present research proposals for follow-up experiments that would test the associations they found in the class data.

Interested in using this activity in your class?

I would be happy to share the materials for this activity with interested instructors. Please contact Sarah Stockwell at **sarahs@ucsd.edu.**

Works cited

1. Sylvester et al. Temperature and host preferences drive the diversification of *Saccharomyces* and other yeasts: a survey and the discovery of eight new yeast species. *FEMS Yeast Research* 2015; 15(3):fov002.
2. Hittinger lab. Official Wild YEAST Isolation Guide.

Acknowledgements

SRS would like to thank Chris Hittinger for advice in developing the module.
Photos of *Encelia californica, Rhus integrifolia,* and *Artemisia californica*: Heather Henter.


Astragalus trichopodus (Santa Barbara Milk Vetch)

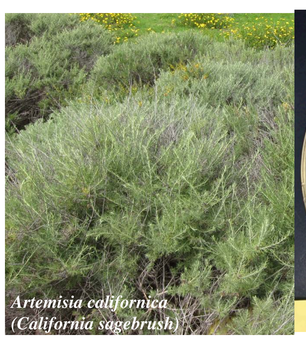

Aureobasidium melanogenum

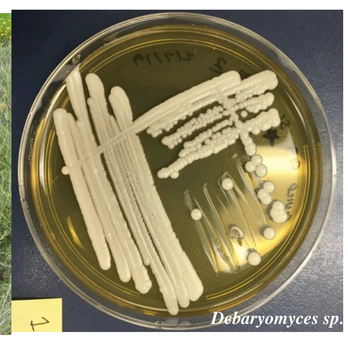

Rhus integrifolia (lemonadeberry)


Pseudosydowia eucalypti


Heteroteca grandiflora (telegraph weed)


Aureobasidium melanogenum


Artemisia californica (California sagebrush)


Debaryomyces sp.