Phaff Collection News

Fall 2014

phaff collection UCDAVIS

Inside:

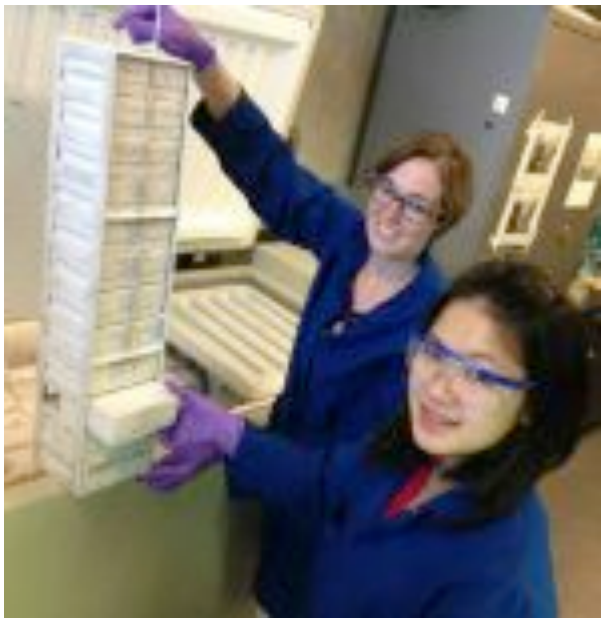
"Our heirloom yeasts are facilitating modern discoveries in ecology, biotechnology, agriculture and medicine."

-Kyria Boundy-Mills, PhD
Curator

Tapping Biodiversity for Discovery

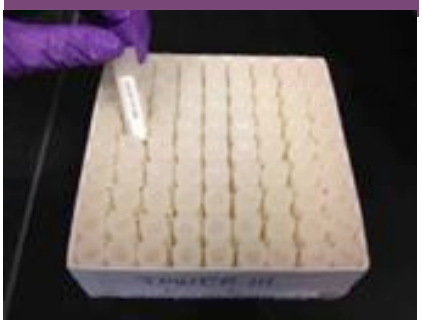
Long before genetic modification methods were developed in the laboratory, Nature honed methods for generating and optimizing genetic diversity. The Phaff collection has captured a large slice of known yeast species diversity. The tremendous diversity of species, and of strains within a species, allow comparative research approaches that are possible at very few institutions in the world. Phaff collection yeasts are used at UC Davis in research projects including those described in stories in this newsletter on ionic liquid, glycolipid secretion, and many other projects. 2014 was a great year for research productivity. Boundy-Mills was lead or co-author on eight publications (listed on Page 7) that referenced the Phaff collection and its yeasts.

NSF Funds Phaff Collection Overhaul



PHAFF COLLECTION STATS

- 7000 Strains
- 850 Species
- 80% of strains not available elsewhere

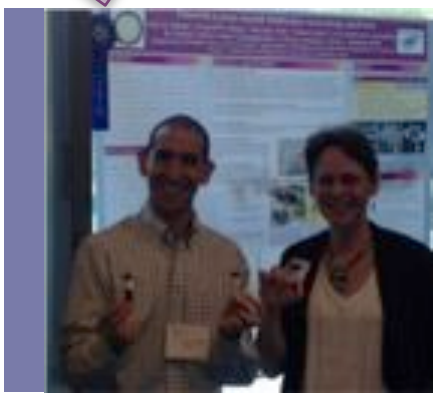


The last major upgrade of the Phaff collection was about 12 years ago, and included a full inventory, and transfer of stocks from antiquated agar slants to cryopreservation. The Phaff collection is long overdue for a major overhaul. This year, funding was obtained from NSF for major collection improvements. Between now and 2017:

- All yeasts will be revived to check their viability and purity.
- Over 50 years of unpublished data generated by Herman Phaff will be posted in an online database.
- Yeasts will be re-identified using modern, ribosomal DNA sequencing methods. sequences will be deposited in GenBank.
- A third copy of the collection is being archived at a remote facility in Colorado operated by USDA. For free!
- The collection will get a top-notch database and website, using the BioloMICS application developed by and for the CBS fungal collection in the Netherlands and used by several top yeast collections in the world.

There are many benefits of this project. Users of the collection will have access to greatly expanded information about the yeasts. Many undergraduate students are receiving training in basic microbiology and yeast identification. And, most importantly, the stocks will be stabilized for future discoveries.

Note the blue ribbon!

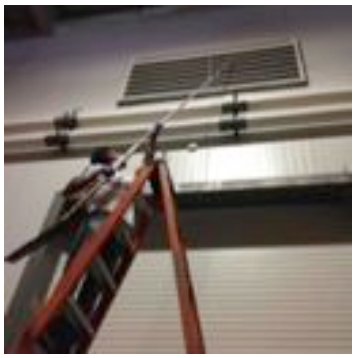


First Place Poster

In September 2014, the UC Davis Robert Mondavi Institute held the Third Annual Poster Competition for graduate students in the Viticulture & Enology and Food Science & Technology departments. Third year PhD student Luis Antonio (Tonio) Garay presented a poster on his thesis focus: "Towards a Yeast-Based Multivalue Technology Platform". His poster won first prize in the competition! His research, focusing on valorization of multiple co-products from Phaff collection yeasts, was very compellingly portrayed in his poster, and in his eloquent description of the project. The small vials of yeast culture, oil and protein that he displayed added a nice touch. The award consisted of a generous cash prize plus a plaque. Congratulations, Tonio!







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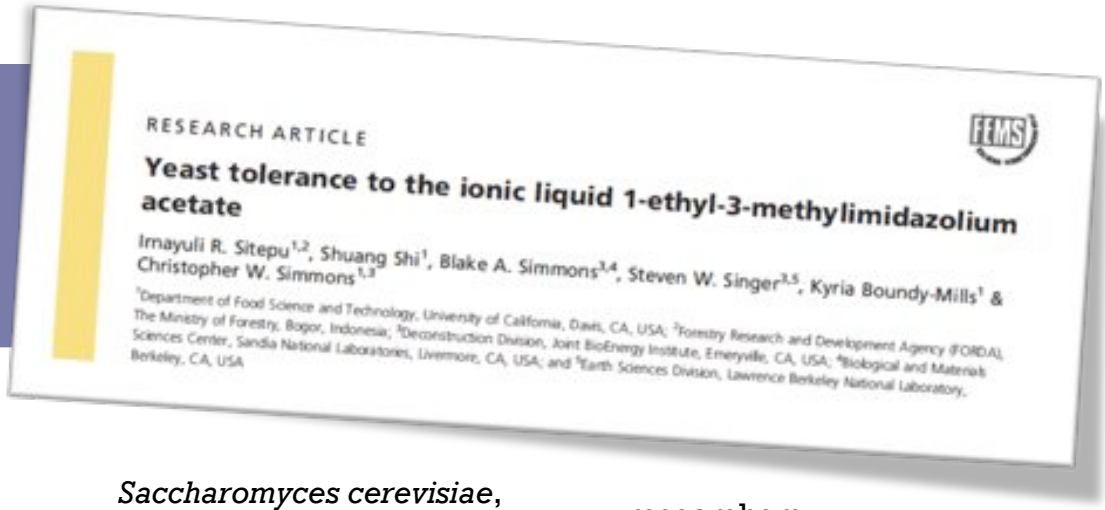
Microbiomes:

Bacteria and fungi in wineries and dairies

The Sloan Foundation's Microbiome of the Built Environment program is currently funding a collaboration led by David Mills, Viticulture and Enology, UC Davis. Aims of this project are to correlate building conditions (humidity, temperature, construction materials, etc) with bacterial and fungal microbiomes in three wineries, a fluid milk processing facility and a cheese facility. Roger Boulton (Viticulture & Enology) and Andre Knoesen (Computer and Electrical Engineering) are leading the development of environmental sensors.

Kyria Boundy-Mills is coordinating the microbiome sampling segment of the project. Students in the Boundy-Mills lab are collecting hundreds of samples from each facility over two years. DNA sequences will reveal what types of bacteria and fungi (including yeasts) are on the building surfaces, processing equipment, input materials, and products.

Discovery of Ionic Liquid-Tolerant Yeasts



RESEARCH ARTICLE

Yeast tolerance to the ionic liquid 1-ethyl-3-methylimidazolium acetate

Irnayuli R. Sitepu[1,2], Shuang Shi[1], Blake A. Simmons[3,4], Steven W. Singer[3,5], Kyria Boundy-Mills[1] & Christopher W. Simmons[1,3]

[1]Department of Food Science and Technology, University of California, Davis, CA, USA; [2]Forestry Research and Development Agency (FORDA), The Ministry of Forestry, Bogor, Indonesia; [3]Deconstruction Division, Joint BioEnergy Institute, Emeryville, CA, USA; [4]Biological and Materials Sciences Center, Sandia National Laboratories, Livermore, CA, USA; and [5]Earth Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA, USA

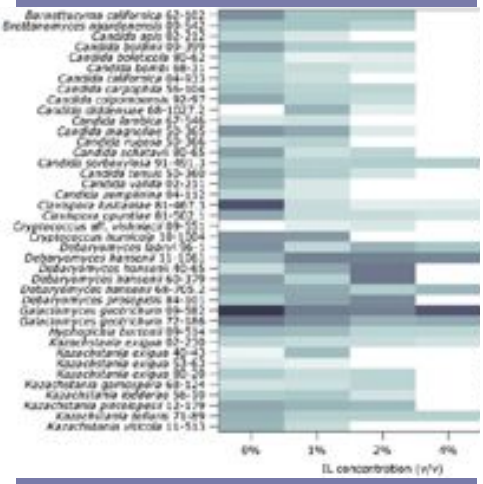
Ionic liquids (IL) are very strange materials: they are salts that are liquid at room temperature. They are very effective in deconstructing plant matter prior to conversion to biofuels by yeasts, and are being studied for production of cellulosic ethanol. Unfortunately, yeasts that are currently used to make ethanol are very, very sensitive to traces of IL that remain after washing the plant material.

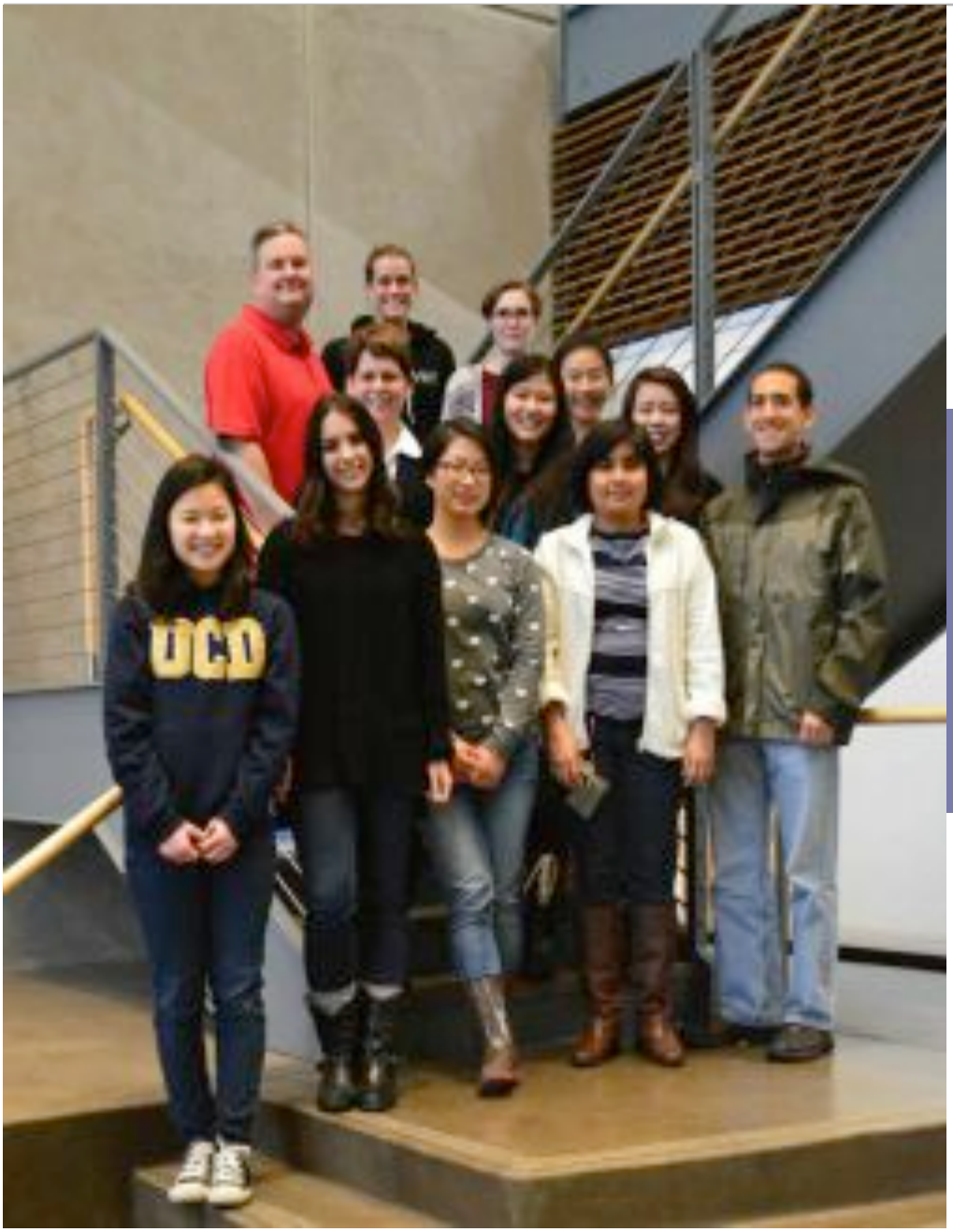
To get around this limitation, we wanted to see if we could find any natural *Saccharomyces cerevisiae* strains or other ethanol-producing yeasts that might be tolerant of IL. This would reduce both process costs and water consumption. In a project co-led by Chris Simmons (Food Science & Technology) and Kyria Boundy-Mills, with collaborators at the Joint BioEnergy Institute, we measured the IL tolerance of 180 yeasts from the Phaff collection. These included 30 independent, natural strains of *Saccharomyces cerevisiae*, other *Saccharomyces* species and relatives, and dozens of species in other taxonomic clades. We hoped to find one or two strains that could tolerate the 4% IL that remains in plant matter after washing off the IL.

Though most *S. cerevisiae* strains were unable to grow even in the lowest concentration of IL, we were pleased to discover 2 mildly IL-tolerant *S. cerevisiae* strains. But, we were even more delighted to discover dozens of other highly IL-tolerant species, some that could grow in 5% IL! It turns out that *S. cerevisiae* is a particularly IL-sensitive species. This work was recently published in FEMS Yeast Research. Because Chris Simmons is such an excellent wordsmith, this manuscript was accepted as submitted, without revisions, which is unprecedented in our experience.

This work was possible because Phaff and other University of California researchers isolated, characterized, and preserved thousands of yeasts from all over the world. Continued maintenance of the Phaff collection will allow these discoveries to continue.

+ Heat map of IL tolerance





Phaff Collection Personnel

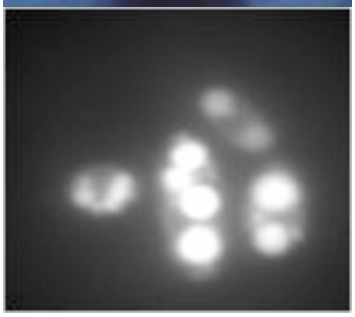
The Phaff Collection is maintained and used by University of California Davis personnel including faculty, staff and students. Many undergraduate students are fortunate to receive training in microbial ecology, fungal taxonomy and biotechnology.

Front row: Shanny Krisna (Food Science undergrad), Lauren Enriquez (Microbiology undergrad), Ting Lin (Food Science undergrad), Irnayuli Sitepu (postdoc), Tonio Garay (Food Science PhD student).

Second row: Kyria Boundy-Mills (Specialist), Christie Hartanto (Food Science undergrad), Florencia Chua (Food Science undergrad).

Back row: John Butler (Food Science undergrad), Jen Lincoln (Viticulture & Enology undergrad), Erin Cathcart (technician), Vania Rahardjo (Food Science undergrad)

Yeast oil: A new replacement for petroleum?



Over the past four years, we have been comparing the ability of yeasts in the Phaff collection to convert sugars to oil. Yeast oil is very similar in composition to the plant oils such as soy or sunflower oil that are used for cooking, and for biodiesel and other chemicals.

When we grow yeasts in the lab, most species will either ignore excess sugar, or convert it to waste products such as ethanol, or store it as carbohydrates. A few dozen species can convert sugar to oil, some accumulating over 60% oil by dry weight! To put that into perspective, soybeans contain about 18% oil. Yeasts grown in bioreactors grow much faster than plants, take up less land, can be grown on waste products, and are not subject to weather and climate conditions. If developed, this technology could result in a renewable, sustainable replacement for petroleum and for plant oils that are part of our food system.

In the last few years, the Phaff collection has played a big part in developing yeast oil technology.

- In the last three years, we have discovered 17 new high-oil species. (Spoiler alert: more will be published soon!)
- We have characterized the properties of dozens of high-oil yeasts, including their ability to use different carbon sources and tolerate inhibitors present in the plant materials they could be grown on.
- We are working on ways to extract the oil out of the yeast cells.

These discoveries have been possible at UC Davis because thousands of yeasts, representing hundreds of species, have been gathered and preserved at UC Davis.

How to Access the Phaff Collection

The Phaff Yeast Culture Collection at the University of California Davis is the fourth largest public collection of wild yeasts in the world. Yeasts are distributed worldwide to academic, government agency and industry researchers for use in a broad range of applications such as biofuels, validation of clinical diagnostic kits, discovery of new yeast species, hosts for heterologous protein expression, studies of stress tolerance, and much more.

You can access the online catalog and ordering system at phaffcollection.ucdavis.edu.

The Phaff Collection hosted the Fall 2014 meeting of the US Culture Collection Network.

USCCN Meeting: Boundy-Mills is on the steering committee of the US Culture Collection Network <http://usccn.org>, led by Kevin McCluskey (curator of the Fungal Genetic Stock Center, Kansas State University). This network, funded by NSF since 2012, has helped to promote interaction and synergy among US microbial culture collections. The fall 2014 meeting was held at UC Davis, and brought together curators of 13 biodiversity and genetic stock collections of algae, bacteria, fungi, and yeasts. In addition, a targeted group of users of microbial collections participated in the meeting, which focused on uses of microbial culture collections for genome sequencing projects. Discussions and visions for the future were summarized in a meeting report that will be submitted for publication soon. Contact curator Kyria Boundy-Mills if you would like to see the current draft of this report.

Boundy-Mills announced that **Phaff Collection yeasts are available for free** to researchers who would use them for genome sequencing as part of the Thousand Fungal Genomes Project, led by Joey Spatafora of Oregon State University.

Contact Information

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URL: http://phaffcollection.ucdavis.edu

2014 scientific publications involving the Phaff collection:

1. **Sitepu I, Shi S, Simmons BA, Singer S, Boundy-Mills K, Simmons C.** 2014. Yeast tolerance to the ionic liquid 1-ethyl-3-methylimidazolium acetate. FEMS Yeast Res **In Press**.
2. **McCluskey K, Bates S, Boundy-Mills K, Broggiato A, Cova A, Desmeth P, DebRoy C, Fravel D, Garrity G, del Mar Jiménez Gasco M, Joseph L, Lindner D, Lomas M, Morton J, Nobles D, Turner J, Ward T, Wertz J, Wiest A, Geiser D.** 2014. Meeting Report: 2nd Workshop of the United States Culture Collection Network. May 19-21, 2014, State College, PA, USA. Standards in Genomic Sciences **In press**.
3. **McCluskey K, Wiest A, Boundy-Mills K.** 2014. Chapter 4. Genome Data Drives Change at Culture Collections, p. 81-96, Fungal Genomics. Springer Berlin Heidelberg.
4. **Sitepu I, Garay L, Sestric R, Levin D, Block DE, German J, Boundy-Mills K.** 2014. Oleaginous yeasts for biodiesel: Current and future trends in biology and production. Journal of Biotechnology Advances **32:**1336-1360.
5. **Sitepu I, Selby T, Zhu S, Lin T, Boundy-Mills K.** 2014. Carbon source utilization and inhibitor tolerance of 45 oleaginous yeast species. Journal of Industrial Microbiology and Biotechnology. **41:**1061-1070.
6. **Sitepu I, Jin M, Fernandez J, Sousa L, Balan V, Boundy-Mills K.** 2014. Identification of oleaginous yeast strains able to accumulate high intracellular lipids when cultivated in alkaline pretreated corn stover. Applied microbiology and biotechnology **98:**7645-7657.
7. **Garay L, Boundy-Mills K, German J.** 2014. Accumulation of high value lipids in single cell microorganisms: A mechanistic approach and future perspectives. Journal of Agricultural and Food Chemistry **62:**2709-2727.
8. **Boundy-Mills KL.** 2014. Methods for the Isolation and Investigation of the Diversity of Cold-Adapted Yeasts and Their Ex Situ Preservation in Worldwide Collections, p. 23-45, Cold-adapted Yeasts. Springer Berlin Heidelberg.

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Giving to the Phaff Collection

Researchers in a variety of areas continue to make important discoveries using the Phaff Collection. You can donate to help ensure the continued maintenance of this resource. Make checks out the "UC Regents", include "Phaff Collection" on the memo line, and mail to: Kyria Boundy-Mills, Food Science and Technology, UC Davis, One Shields Ave, Davis CA 95616. Contact the curator for more information.