



# 2017 ASBC Annual Meeting

## Atlas of Yeast Diversity, The Quest for Hidden Yeast

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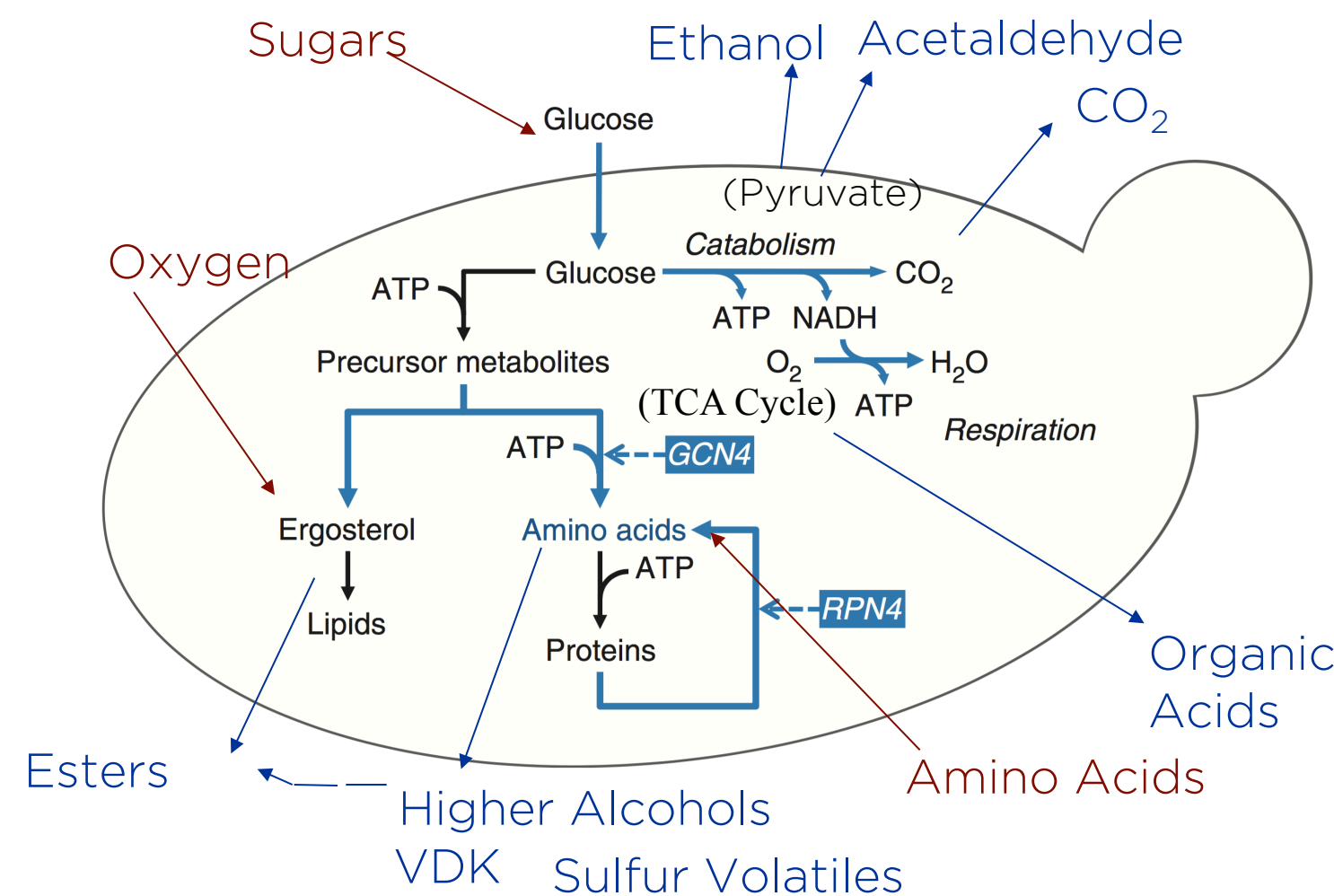
2017 ASBC Annual Meeting

June 4-7, 2017

Sanibel Harbour Marriott  
Fort Myers, FL, U.S.A.

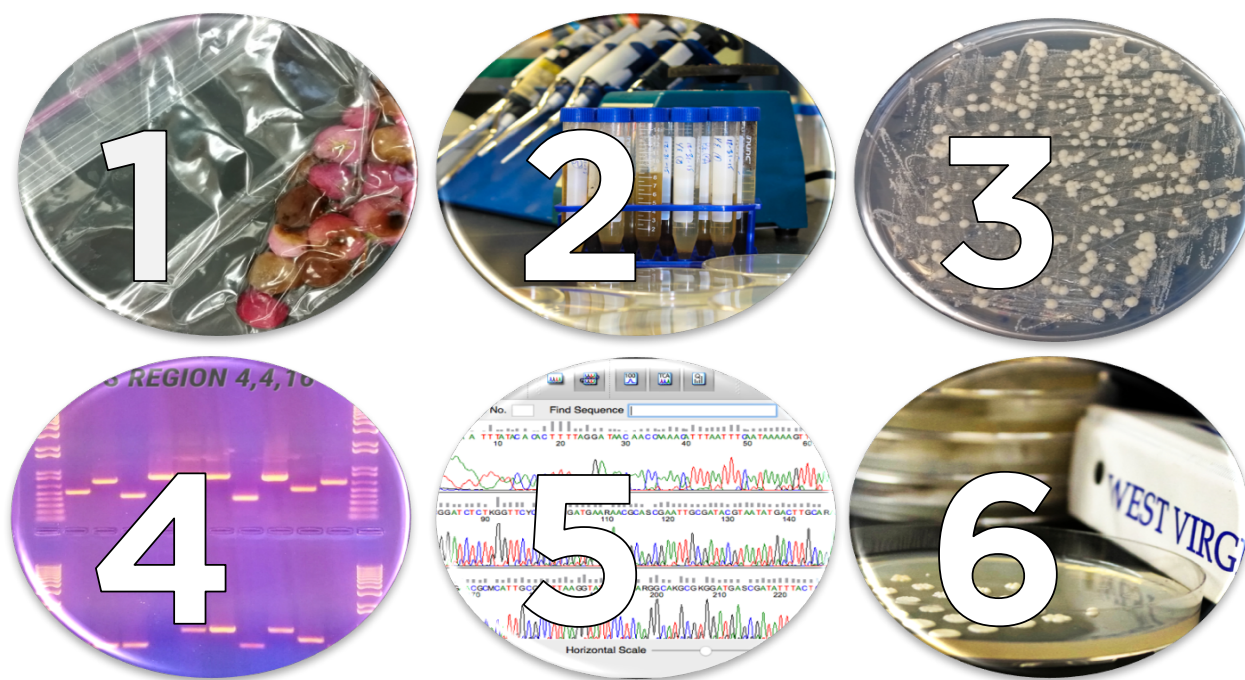
### YEAST IN NATURE, BREWERIES, & LABORATORYS

- Yeast primarily metabolize sugars found on oak tree extrudes, fruits, wasp stomachs, and throughout soil.
- Domestication of *Saccharomyces cerevisiae* by brewers and bakers predates the discovery microbes.
- Genomic & phenotypic assays of modern *S. cerevisiae* strains separate into 5 distinct sub-lineages apart from wild strains (fig.5).
- Industrial fermentation pressures select for strain with pleasing flavor profiles, tolerance to stress, and sugar fermentation capabilities.
- Human domestication has aided in unusual degradation of yeast natural survival ability by hindering their sexual cycle and genomic stability in hybrids in several cases.
- Unique genomic signatures can be detected from strains used in fermentations including the wine circle cluster, copy number variations in the *MAL* (maltose) gene, *RTM* (resistance toxic molasses), nonsense mutations in *PAD1* & *FDC1* (associated with production of phenolic 4-vinyl guaiacol (4VG)) among others.
- Wine Circle Cluster obtained from wild yeasts presumably in spontaneous fermentations including *Zygosaccharomyces bailii*, *torulaspora microellipsoides*, and an unknown species.



**Figure 1.** Yeast metabolize sugars imported from their environment to be catabolized into the molecules they need to live. Modified from (Canelas, 2010).

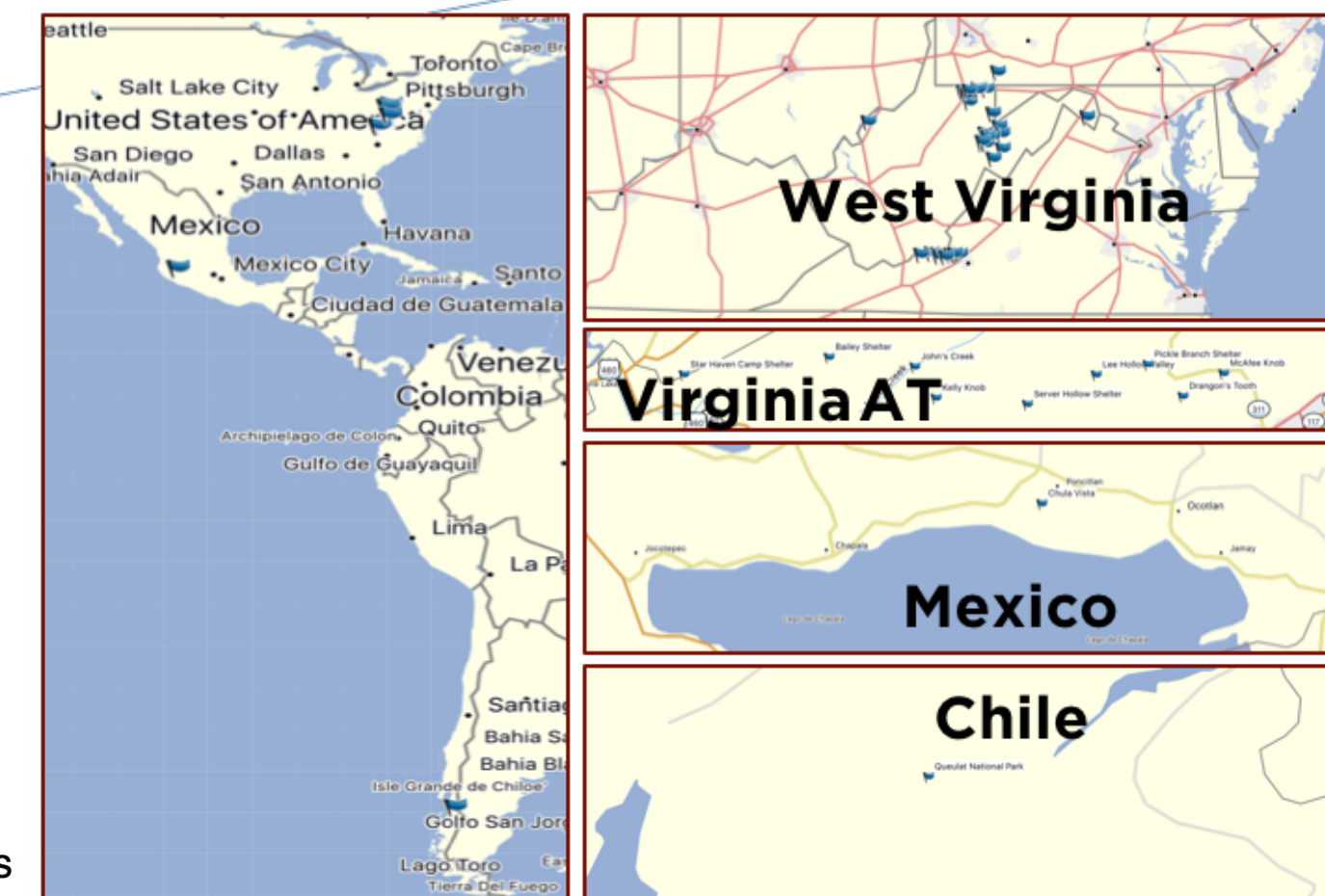
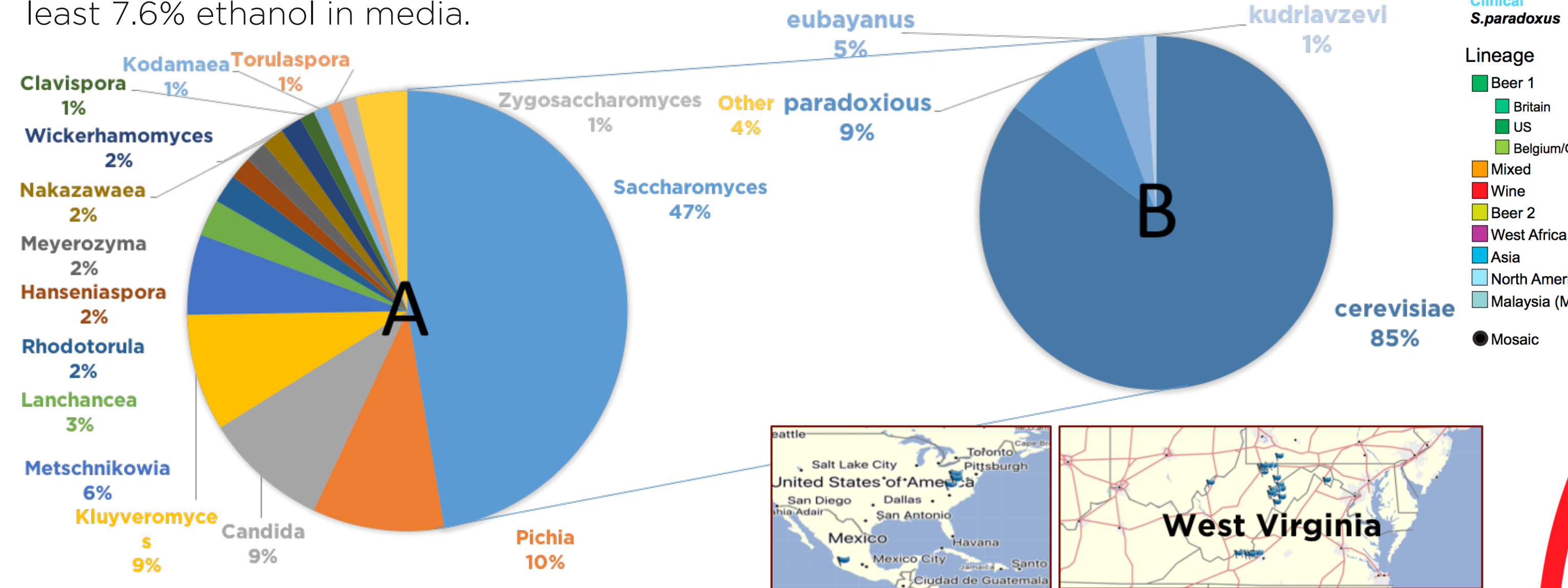
### HOW WE ISOLATE, ID, AND SEQUENCE YEAST



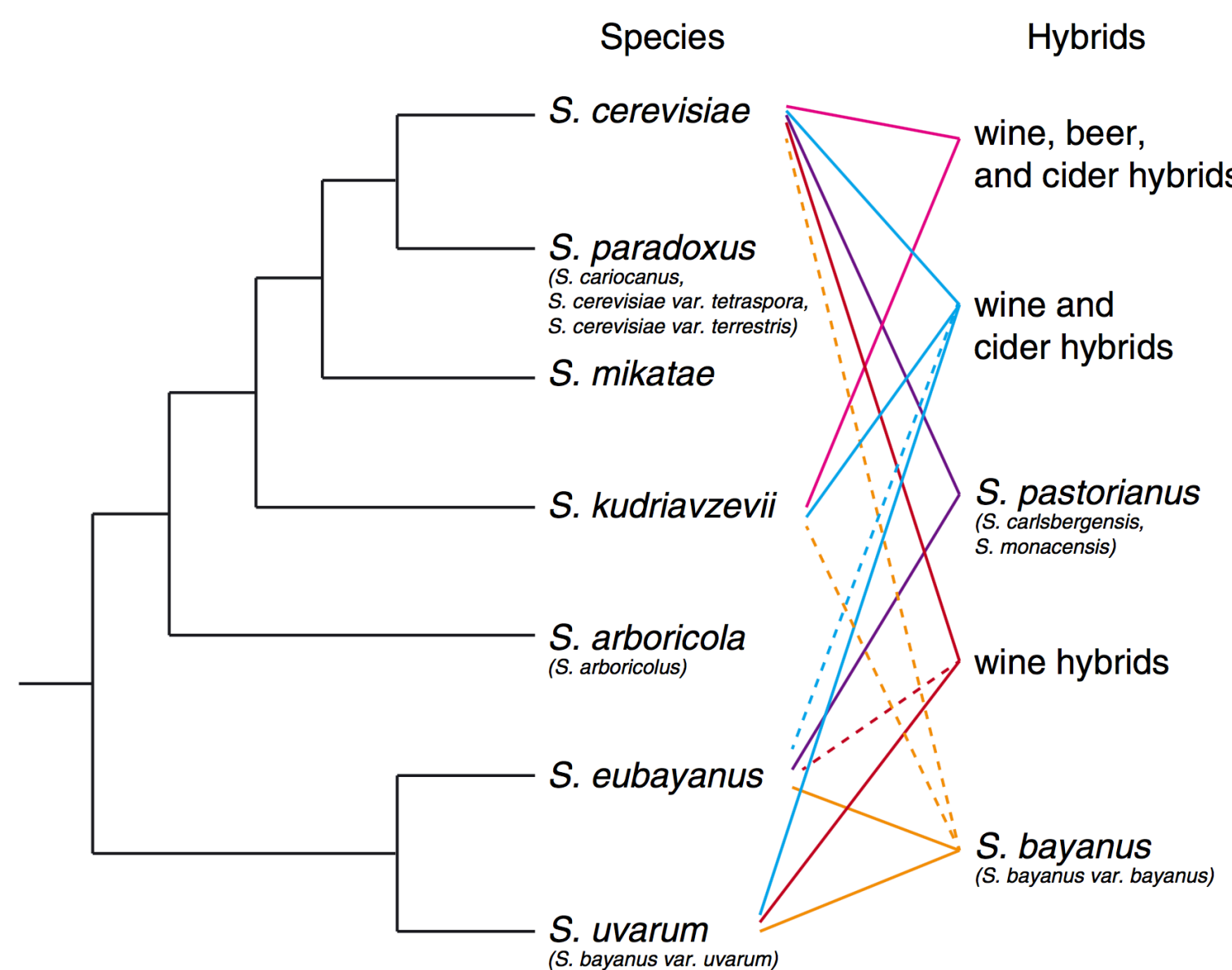
1. Collection of environmental samples from various sources and locations such as plants, animals, and soils in nature.
2. Extraction of wild yeast from the samples with modified culture media.
3. Selection of pure colonies is performed based on colony morphology, incubation temperature, and simple aroma sensory profiling.
4. Polymerase Chain Reaction (PCR) of genomic deoxyribonucleic acid (DNA) with primers for the ribosomal DNA (rDNA) regions of internal transcribed spacer (ITS) and D1/D2 ribosomal large-subunit. Gel electrophoresis separates the ITS or D1/D2 amplicon by size in an agarose gel, the bands are excised, and purified.
5. Sanger sequencing is performed on the amplicons and checked against the NCBI RefSeq database through BLAST nucleotide.
6. Downstream applications include trial fermentations, genetic engineering, hybridization, and challenging media.

### WHAT WE HAVE FOUND SO FAR AND WHERE

On going research has compiled 186 non domesticated yeast strains from 373 Appalachian samples, 9 Mexican samples, and 6 Chilean samples totaling 388 samples. Our attempts at isolating wild yeast shows a success rate of 50% and 23% of any attempt yielded a *Saccharomyces* member. All strain represented here were capable of utilizing maltose, dextrose, and sucrose as the only carbon source and tolerating at least 7.6% ethanol in media.



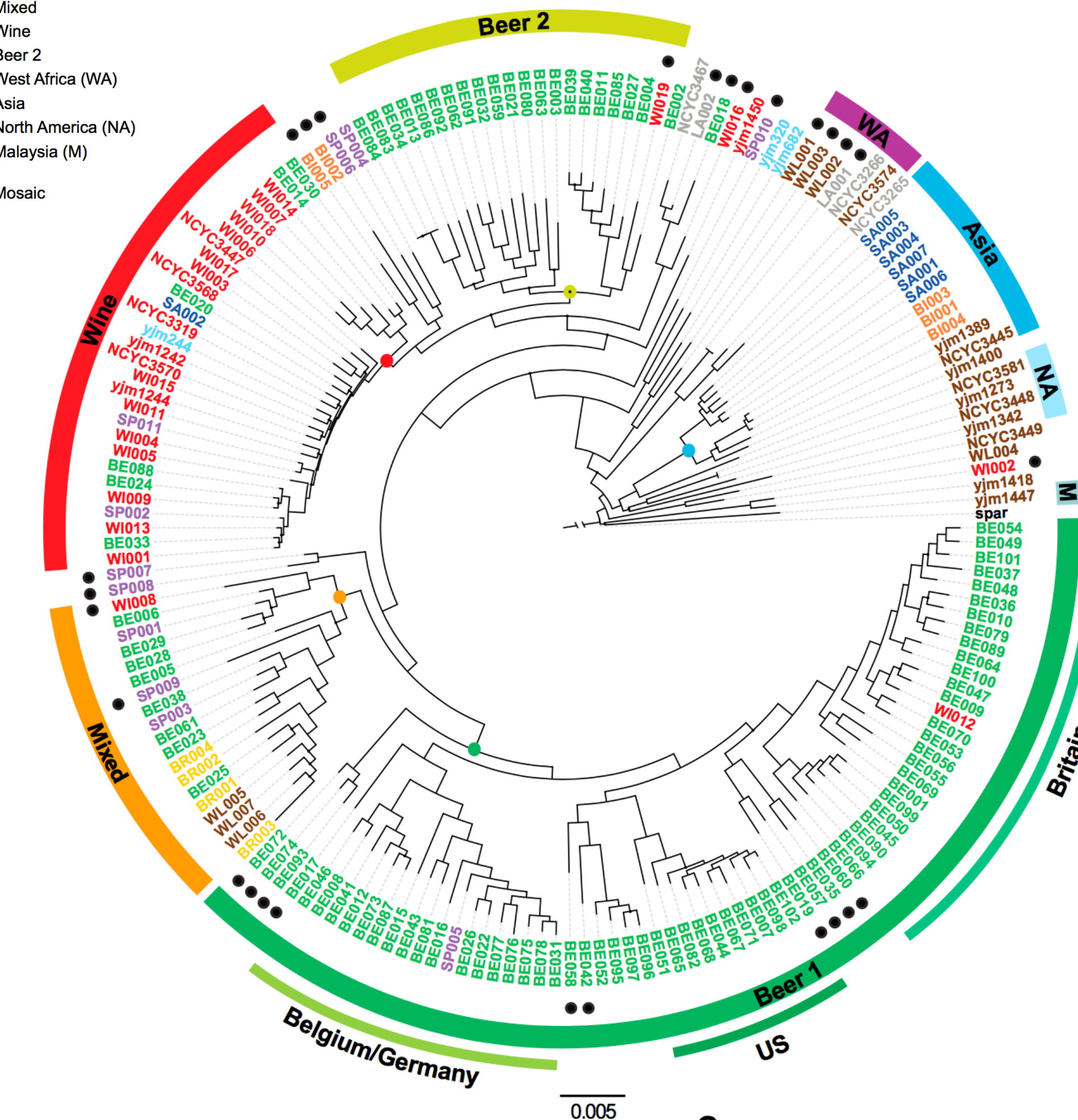
**Figure 3.** World map showing sampling locations in the United States, Mexico, and Chile.



Special thanks to MB, Barney, and Dr. Liti.

### SO WHAT ARE WE GOING TO DO WITH ALL THIS?

Wild strains and industrial strains of *S. cerevisiae* will be hybridized with the newly discovered *Saccharomyces arboricola* species during the summer of 2017 at Saga University in Saga City, Japan under my sensei, Dr. Hiroshi Kitagaki with funding through NSF and JSPS. Fermentations will be characterized with gas chromatography-mass spectrometry (GS-MS).



**Figure 5.** Maximum likelihood phylogenetic tree comparing modern yeast strains who have document sequences from studies Liti, *et al* 2009, Strobe *et al* 2015, and Gallone *et al* 2016. *S. paradoxus* serves as the outgroup (Gallone, 2016).