Population structure, admixture, and migration of *Saccharomyces eubayanus* and their lager-brewing alloploid hybrids

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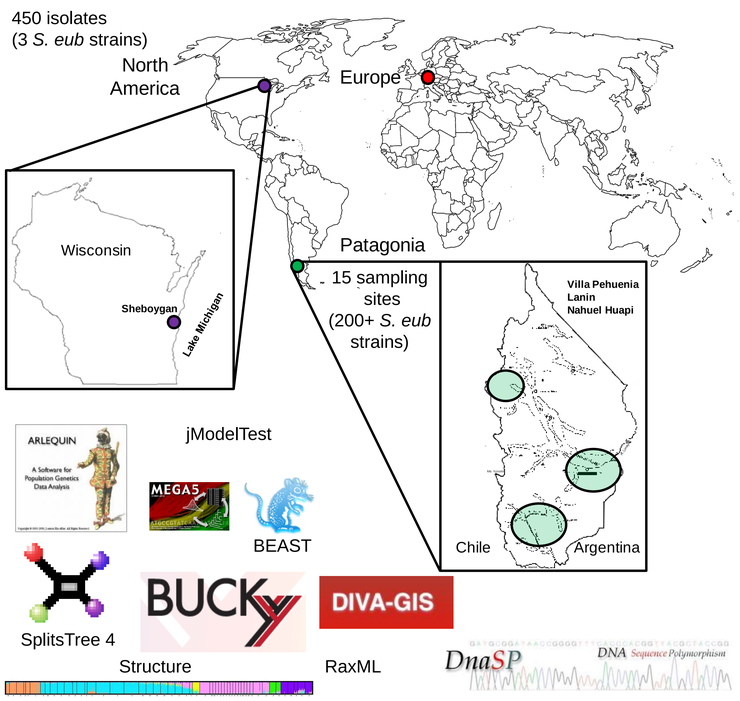
ABSTRACT

Lager beer is brewed with *Saccharomyces pastorianus*, an alloploid hybrid of *S. cerevisiae* and a species we recently discovered in Patagonia, Argentina, called *S. eubayanus*. However, little is known about the geographic distribution and diversity of *S. eubayanus*. The aim of this study was to isolate *S. eubayanus* strains from South and North America and to determine their population structure using a multilocus phylogenetic approach. To infer their evolutionary relationships with wild *S. eubayanus*, we compared representative strains from the Frohberg and Saaz groups of lager-brewing alloploid hybrids, as well as "*S. bayanus*" triple-hybrid brewing contaminants. Our findings showed a high efficiency of isolation of *S. eubayanus* in Nothofagus trees from Patagonia and, for the first time, a rare isolation from Wisconsin, USA. Multilocus phylogenetic analyses indicate high genetic diversity among *S. eubayanus* from Patagonia and support two differentiated populations, the "Patagonia" and "Patagonia-Lager" populations. The Patagonia-Lager population is closely related to lager-brewing alloploid hybrids and to the *S. bayanus* triple hybrids, suggesting that European brewing strains and contaminants are derived from crosses involving this population or a close subpopulation. Finally, the genetic characteristics of the new strains found in North America are consistent with the admixture of Northern and Patagonia-Lager populations and the recent expansion to North America by an unknown vector. In conclusion, the range of *S. eubayanus* is more widespread than expected, but the non-Patagonian strains have low genetic diversity and appear to have originated by the admixture or hybridization of migrant strains.

INTRODUCTION

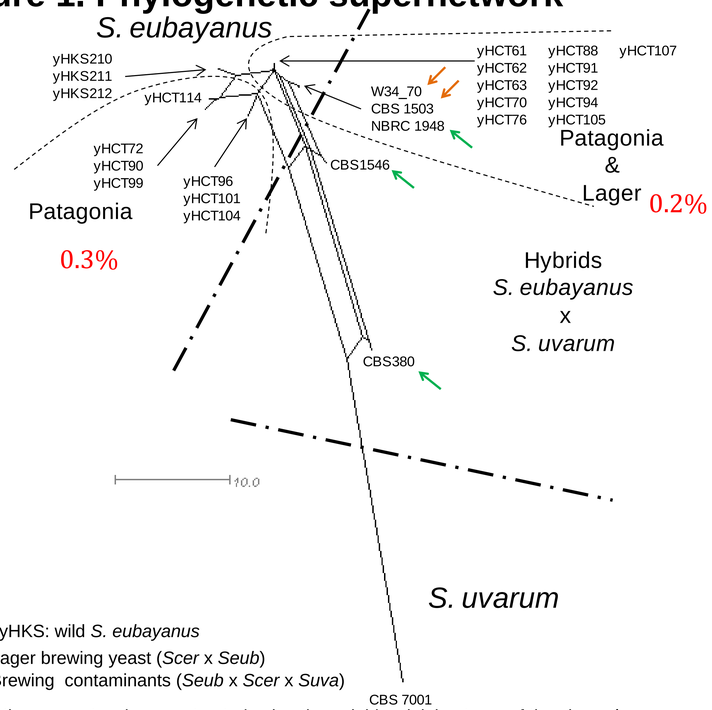
The *Saccharomyces* genus includes seven species. Hybrids have been described in biotechnological environments. The most studied hybrid is the lager brewing *S. pastorianus*[1] (*S. cerevisiae* x *S. eubayanus*). The application of new methodologies for yeast isolation has allowed the identification of the one unknown wild genetic stock of *S. pastorianus,* the new species *S. eubayanus*[2]. However, little is known about the diversity of this species, its distribution, or how it contributed to the evolution of lager brewing yeast. As a result of the Wild YEAST (Yeast Exploration and Analysis Science Team) Program[3] and the application of many phylogenetic methods, we were able to explore the diversity of *S. eubayanus* strains and their phylogenetic relationship with their hybrids.

MATERIAL & METHODS



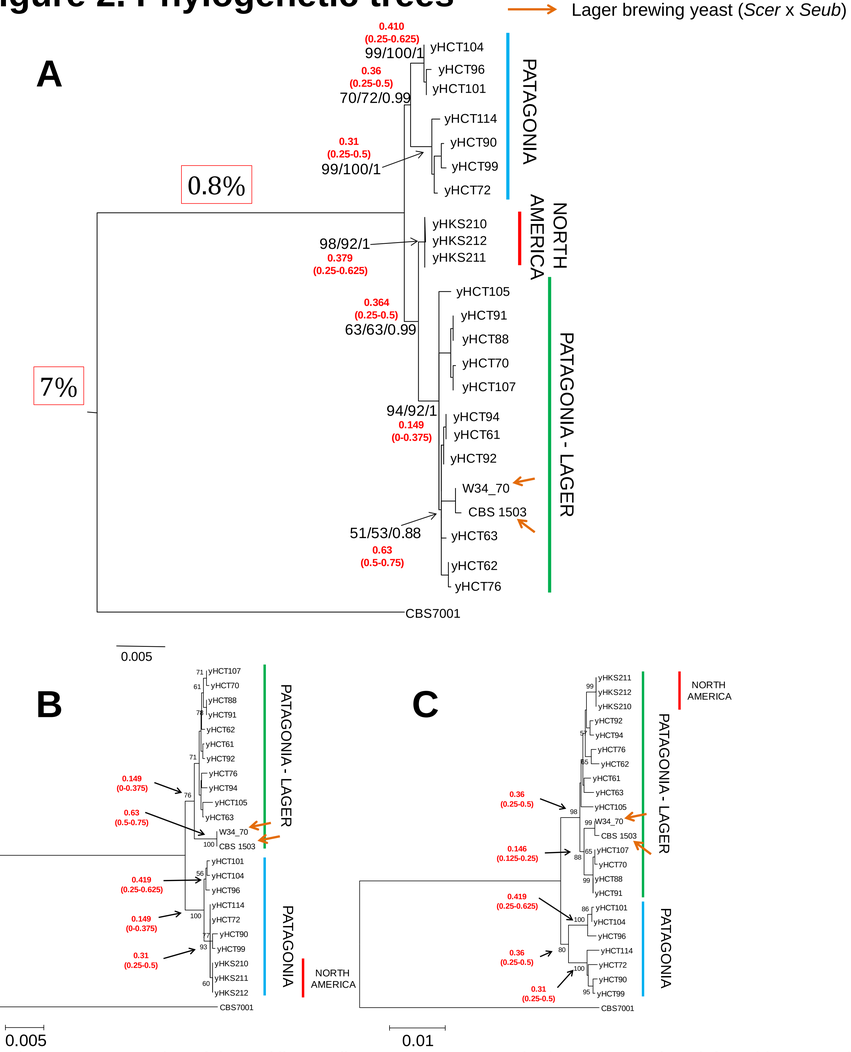
RESULTS

Figure 1. Phylogenetic supernetwork



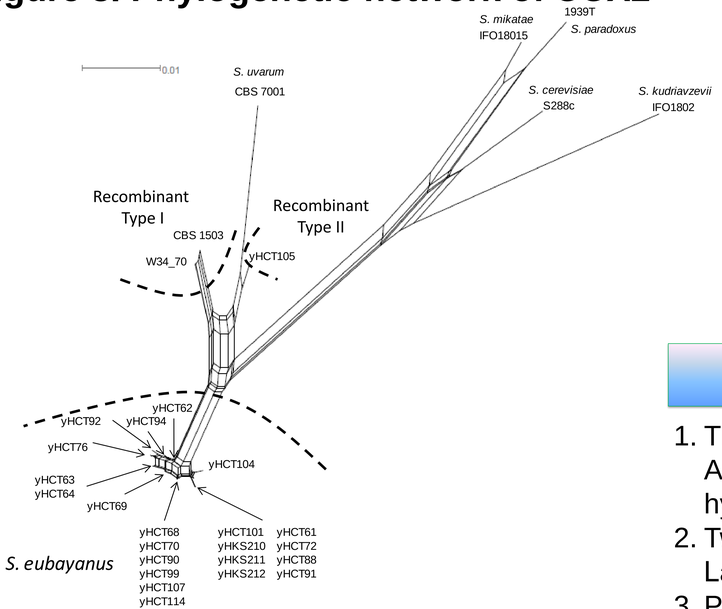
Phylogenetic supernetwork reconstructed using the neighbor-joining trees of the six nuclear genes (*FSY1*, *FUN14*, *GDH1*, *HIS3*, *MET2*, *RIP1*) by the Z-closure method implemented in SplitsTree 4. Incongruent topologies are displayed adding new edges to the network. Filter was set up to two to represent the splits found in at least two phylogenetic trees. Scale bar represents the edge's weights inferred using the tree size weighted means options, a measure similar to those from branches in a phylogenetic tree. STRUCTURE analysis supported two cluster/population (Patagonia and Patagonia-Lager (data not shown). Nucleotide diversity of each population is displayed in red color.

Figure 2. Phylogenetic trees



A) Phylogenetic tree reconstruction of the multilocus alignment sequences. The best tree topology inferred in RaxML is shown. Branch support is represented by bootstrap values performed in RaxML and MEGA after 1000 pseudo-replicates and posterior probability obtained in Beast: Bs RaxML/Bs MEGA/Pp Beast. Outgroup CBS7001 correspond to a *S. uvarum* strain. B) NJ tree reconstructed using the concatenation of nuclear genes (*FSY1, FUN14, RIP1, URA3*) that support the clustering of North American strains with Patagonia strains. C) NJ tree using the concatenation of nuclear genes (*DCR1, GDH1, HIS3,* MET2) that support the clustering of North American strains with the Patagonia-Lager group. In red color is represented the average and, in parenthesis, the standard deviation of the concordance factors (a measure of proportion of trees that support a specific branch) obtained by BUCKy software. Red squares represent nucleotide divergence between *S. uvarum* and *S. eubayanus,* and Patagonia and Patagonia-Lager. Scale is given in nucleotide substitutions per site.

Figure 3. Phylogenetic network of *COX2*



Phylogenetic Neighbor-Net network reconstructed from partial mitochondrial *COX2* gene sequences, which contain a recombination hotspot[4]. Species specific cluster is displayed using *COX2* gene sequences from type strains.

CONCLUSIONS

1. The genetic diversity of *S. eubayanus* strains are higher in Patagonia, Argentina than among European brewing isolates, all of which are interspecies hybrids.
2. Two populations exist in sympatry South America, "Patagonia" and "Patagonia-Lager".
3. Phylogenetic incongruence could be due to admixture and hybridization.
4. North American strains appear to be closely related and originated by admixture between Patagonia and Patagonia-Lager strains.
5. *COX2* could indicate ancestral hybridization between *S. eubayanus* and *S. uvarum*.

LITERATURE

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