

Genomic adaptations of dominant sugarcane fermenting yeast strains

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Sugarcane is by far one of the largest current sources of biofuels. More than half of the world's bioethanol production relies on the efficient fermentation by the yeast *Saccharomyces cerevisiae* of sucrose-rich broths such as sugarcane juice and molasses. These raw materials are also used for the production of baker's yeast, and for production of several distilled alcoholic beverages, including the Brazilian *cachaça*. Studies of the microbiological dynamics of the industrial fermentors revealed a very rapid succession of yeast strains, and consequently the original "starter" yeast (usually commercial baker's yeast strains) was completely replaced by other strains in a matter of weeks. Nevertheless, a few highly productive yeast strains then tended to dominate the fermentor during the entire production season, allowing efficient and stable fermentations. Despite their economic importance as highly efficient ethanol producers, almost nothing is known about the genes, traits and/or physiological adaptations that allow these selected yeast strains to produce large amounts of ethanol while apparently thriving in the competitive and stressful fermentor environment. Using microarray-based comparative genome hybridization (aCGH), we have determined gene copy number variations (CNVs) common to 12 industrially important fuel ethanol and *cachaça S. cerevisiae* strains responsible for the production of billions of gallons of ethanol per year from sugarcane. Most CNVs were observed at telomeric or subtelomeric chromosomal regions, and generally correspond to genes that are depleted in copy number relative to the laboratory strain S288C. Our results also show that these sugarcane-fermenting yeast strains have significant amplifications of the telomeric *SNO* and *SNZ* genes, which are involved in the biosynthesis of vitamins B6 (pyridoxine) and B1 (thiamin), or have amplifications in genes (*THI71*, *THI72*) involved in the uptake of the pyrimidine (pyridoxine) moiety of thiamin. These genetic changes have likely been adaptive and selected for in the industrial environment, and may be required for the efficient utilization of biomass-derived sugars from other renewable feedstocks. Finally, despite the relatively large number of amplified genetic elements found exclusively in the sugarcane yeasts, unexpectedly very few of these strains showed amplification of the *SUC2* gene, which encodes the extracellular invertase that is responsible for sucrose hydrolysis and fermentation. Widespread presence of *SUC* genes at multiple telomeric positions has been shown to be a common feature of both baker's and distillers' yeast strains, and is postulated to be an adaptation to sucrose-rich broths. Thus, despite sucrose being the predominant sugar found in sugarcane juice and molasses, our results indicate that invertase activity probably does not limit sucrose fermentation during industrial ethanol production by the Brazilian sugarcane yeast strains.