ANALYSIS OF PHENOTYPIC CONSEQUENCES OF LOSS-OF-HETEROZIGOSITY DURING BIOETHANOL FERMENTATION.

Rodrigues A.1; Duarte F. M.1; Netto O. V. C.1,2; Zeidler A. F. B.2; Argueso J. L.2; Pereira G. A. G.1

1 – Universidade Estadual de Campinas, Brazil; 2 - Colorado State University, USA

Karyotype instability has been frequently observed among industrial yeast strains, including PE-2, a strain widely adopted in sugarcane bioethanol distilleries. We have analyzed the karyotypes of yeast clones isolated from a 60-day industrial fermentation started from a single cell of strain JAY270, a purified isolate of the commercial PE-2 stock. Eleven of these isolates, displaying at least one chromosomal size polymorphism relative to JAY270, were analyzed and the data indicated that the predominant mechanism for karyotypic variation was allelic mitotic homologous recombination leading to loss-of-heterozigosity (LOH). We then evaluated the eleven LOH strains for fitness during continuous sugarcane fermentations in direct competition against their fully heterozygous JAY270 parent. For this purpose, we first marked the JAY270 parent strain and the LOH derivatives with different drug resistance, therefore allowing the monitoring of the relative LOH to parent cell ratio in the population during co-culture. Fermentation experiments were carried out with these marked strains inoculated in 18% sugarcane syrup for 30 consecutive cycles. The starting inoculum was prepared by mixing the same percentage of pure JAY270 and each of the eleven derivatives (1:1 starting ratios). At the end of each fermentative cycle (~24 hours), a small sample of the cultures was transferred to fresh media to start new cycle. Every five cycles, the samples were plated on YEPD containing the specific antibiotics and the LOH:parent ratio was determined. These assays showed that ten of the isolates did not have a change in fitness, while one of them (FDY6), showed a marked advantage in competition. We are currently using the whole genome sequence data to create a genome-wide map of LOH in JAY270 and to identify the specific genomic regions associated with the fitness gain in FDY6.

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