Identification of genetic variations in engineered yeast for xylose consumption and acetic acid resistance applied to second generation ethanol production

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The second-generation ethanol is a new and promising technology that can dramatically reduce the costs and increase the production. But, while the first-generation is based on fermentable sugar (glucose, fructose and sucrose) from sugarcane using industrial yeast, the second is based on hydrolyzed biomass consisting of the residual non-food crops such as leaves, stems, grass, etc. The biomass deconstruction process generates glucose, non-fermentable sugars (mainly xylose) and inhibitors of yeast growth (acetic acid, furfural and HMF). In order to increase the yield and productivity of yeast in the second generating process, it is necessary to identify industrial robust yeast for growth inhibitors, perform genetic modifications to allow the xylose consumption by insertion of endogenous xylose pathway genes and make use of evolutionary engineering approach to improve some characteristics by several rounds of cell growth and recycling on selective growth media. In this study, genetically modified industrial yeast for xylose-consuption was submitted for three rounds of evolutionary engineering using xylose as carbon source and adding acetic acid in the last round. For each round, two evolved strains were isolated and inoculated in the next one. In a total of seven strains (including parental) were submitted for genome sequencing and bioinformatics analysis to identify all mutations in the evolved strains in comparison with parental genome. For that was necessary to develop a set of bioinformatics analysis: (1) parental genome was assembled and submitted for gene prediction and annotation; (2) sequenced reads from evolved strains were aligned into parental genome allowing mismatches; (3) copy number variation (CNV) analysis using aligned reads and Poisson distribution (cn.MOPS); (4) SNP/Indel calling using the combination of GATK and Freebayes; (5) SNP/Indel annotation using Variant Effect Predictor (VEP). The CNV analysis revealed an increase of xylose pathway genes over evolutionary timeline which can explain partially the xylose consumption profile over three evolution rounds. Moreover, the mutation analysis identified several non-synonymous SNPs distributed over the rounds contributing to a better understanding of metabolic bottleneck of xylose consumption and acetic acid tolerance in industrial yeast.