Development of Industrial Yeast for Second Generation Bioethanol Production

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The cost of lignocellulose-based bioethanol needs to be reduced, in order to commercialize this clean and sustainable fuel substitute for fossil fuels. A microorganism that can completely and efficiently convert all the sugars in lignocellulose into ethanol is one of the prerequisites of a cost effective production process. In addition, the microorganisms should also have a high tolerance towards the inhibitory compounds present in the lignocellulosic hydrolysate, which are formed during the pretreatment of lignocellulose. Baker’s yeast, Saccharomyces cerevisiae, is generally regarded as a robust microorganism and can efficiently ferment glucose. But it lacks the ability to ferment xylose which comprises 20-35% of lignocellulose. Naturally xylose-fermenting yeast such as Pichia stipitis is much more sensitive to inhibitors than S. cerevisiae and it requires accurately controlled microaerophilic conditions during the xylose fermentation, rendering the process technically difficult and expensive. In this study, a novel xylose fermenting yeast Spathaspora passalidarum displayed fast cell growth and efficient xylose fermentation under anaerobic conditions. In contrast, P. stipitis was almost unable to utilize xylose under the same conditions. It is further demonstrated that S. passalidarum converts xylose by means of NADH-preferred xylose reductase (XR) and NAD+ dependent xylitol dehydrogenase (XDH). Thus, the capacity of S. passalidarum to utilize xylose under anaerobic conditions is possibly due to a balance between supply and demand of cofactor through this XR-XDH pathway. Only one other XR with NADH preference has been reported so far. Unfortunately, S. passalidarum also has a low tolerance towards inhibitors generated during pretreatment, which prevents immediate use of this yeast in industrial application. S. passalidarum is able to convert the inhibitor furfural to furfuryl alcohol in a synthetic medium when the addition of furfural is low. The enzymes involved in furfural and 5-hydroxymethylfurfural (HMF) reductions by this yeast have both cofactor preferences for NADH. Due to the low inhibitor tolerance, the growth of S. passalidarum was completely inhibited in the liquid fraction of pretreated corn stover and wheat straw. The inhibitor tolerance of S. passalidarum was improved by the method of genome shuffling including UV mutagenesis and protoplast fusion. The protoplast of a UV-induced furfural-resistant mutant of S. passalidarum (S. passalidarum M7) was fused with the protoplast of a robust yeast S. cerevisiae ATCC 96581. The finally selected hybrid strain (FS22) has desired phenotypes derived from both parents, namely the ability to ferment xylose from S. passalidarum and an increased tolerance to inhibitors from S. cerevisiae ATCC 96581. Phenotypic and molecular analysis indicated that S. passalidarum M7 was the dominant parental contributor to the hybrid. Rearrangement of DNA segments from the other parental strain S. cerevisiae ATCC 96581 possibly occurred in FS22. The inhibitor tolerance of the robust yeast S. cerevisiae ATCC 96581 was further improved by sequentially adapting this strain into media with increasing amounts of the liquid fraction of pretreated corn stover (CSLQ). The adapted strain completely fermented glucose in 100% CSLQ and the ethanol yield was 0.48 g/g glucose, while the parental strain was unable to ferment under this condition. Co-fermentation of this adapted strain with the selected protoplast fused hybrids (FS2 or FS22) in the pretreated wheat straw hydrolysate improved the final ethanol yield by 11% and 26%, respectively, due to partial conversion of xylose in the hydrolysate by the xylose fermenting hybrids. Co-fermentation with one robust C6 fermenting yeast for detoxification and one C5 fermenting yeast for converting xylose into ethanol could be a viable strategy for lignocellulosic bioethanol production.