

Currently almost all the fuel ethanol produced in the United States is generated from processes using corn as the feedstock. However, in order to increase production other viable feedstocks must be developed. Efficient ethanol production from soft wood biomass at titers suitable for an industrial process is challenging for a variety of reasons. One challenge is the production of inhibitory chemicals from the biomass as it is pretreated prior to enzymatic digestion. These chemicals are then present in the fermentation media where they inhibit the activity of the fermenting organism. To overcome this we have developed a *Saccharomyces cerevisiae* yeast that is capable of producing ethanol from greater than 17.5% dry weight per volume of pretreated loblolly pine at ethanol yields over 90% of the theoretical maximum. This strain, AJP50, was shown to have higher inhibitor tolerance than the original parent strain, XR122N. This may explain in part the more efficient ethanol production observed with AJP50 at high concentrations of pine wood. A culturing method was developed that allowed for the acquisition of isolated colonies from AJP50. These isolates were then studied to assess the stability of the AJP50 phenotype when cells were cultured in rich liquid media. Different isolates had different phenotypes after culturing, which suggests that AJP50 is not a single strain of uniform genotype, but rather that it is a genetically diverse population of cells. Certain isolates retained the ability to ferment 17.5% dry weight of pine after culturing on rich media, while others did not. Transcriptomic sequences from isolate GHP4 which retained the high solids fermentation ability were compared to sequences from isolate GHP1 which could no longer ferment high pine solids after growth in rich media to determine the gene expression differences that may be important in high pine solids fermentations. Many expression differences were found. Some of these genes had been previously described as important for inhibitor tolerance or efficient biomass fermentation; while other observed gene expression differences have not been previously described as having a role in improved performance in these environments.