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## ABSTRACT

Ethanol can be used as a complete fuel or as an octane enhancer, and has the advantages of being renewable and environmentally friendly. Ethanol produced by a fermentation process, generally referred to as bioethanol, is considered to be a partial solution to the worldwide energy crisis. Traditionally, industrial bioethanol fermentation involves two major steps: starch hydrolysis and fermentation. Since the key microorganism, *Saccharomyces cerevisiae*, lacks amylolytic activity and is unable to directly utilize starch for proliferation and fermentation, it requires large amounts of energy and starch-hydrolyzing enzymes to hydrolyze raw starch into fermentable sugars.

It has been suggested that genetically-engineered yeast which express amylolytic enzymes could potentially perform simultaneous starch hydrolysis and fermentation. This improvement could greatly reduce the operational and energy costs in current bioethanol plants and make bioethanol production more economical. In this study, a novel yeast strain was genetically engineered in such a way that barley α-amylase was constitutively expressed and either secreted or anchored on the cell surface. This particular α-amylase was selected based on its superior kinetic properties and its pH optimum that make it compatible with the pH of yeast culture medium during fermentation.

Both secreting and anchored strains showed the ability to hydrolyze soluble starch under batch fermentation conditions. In all of the batch fermentations performed, the secreting strain showed 1.8-2.7 times higher starch hydrolysis rates than the anchored strain. The expression of barley α-amylase was improved by 2.9-6.3 times by spiking the medium with 5 g $L^{-1}$ glucose. With the addition of 80 U $L^{-1}$ exogenous glucoamylase, ethanol yields up to 92% of the theoretical maximum could be generated by the secreting strain. By integrating the gene cassette into the ribosomal DNA locus of the yeast genome, 100% mitotic stability of the integrated gene was achieved under non-selected conditions. With the addition of 5 U $L^{-1}$ glucoamylase, the integrated strain that secreted barley α-amylase was able to completely hydrolyze 20 g $L^{-1}$ raw wheat starch over a 120 h period and produce 1.4 g $L^{-1}$ ethanol. The findings indicate that secreting enzyme systems are more efficacious than anchored systems, and that integration of the gene cassette into multiple copy loci needs to be strongly considered when designing amylolytic yeast strains.

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To my mother and father in China
To my supervisors Dr. William Roesler and Dr. Gordon Hill

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| Generally Regarded As Safe | GRAS |
| Glyceraldehyde 3-Phosphate Dehydrogenase | GAPDH |
| Glycosylphosphatidylinositol | GPI |
| Luria-Bertani | LB |
| Luria-Bertani Ampicillin | LBA |
| Multiple Cloning Site | MCS |
| Phosphoglycerate Kinase 1 | PGK1 |
| Ribosomal DNA | rDNA |
| *Saccharomyces cerevisiae* | *S. cerevisiae* |
| Sodium Dodecyl Sulfate | SDS |
| Starch Binding Domain | SBD |

| | |
|---|---|
| Terrific Broth | TB |
| Yeast extract, peptone and dextrose | YPD |