# N-propyl Alcohol Synthesis through Saccharomyces Cerevisiae

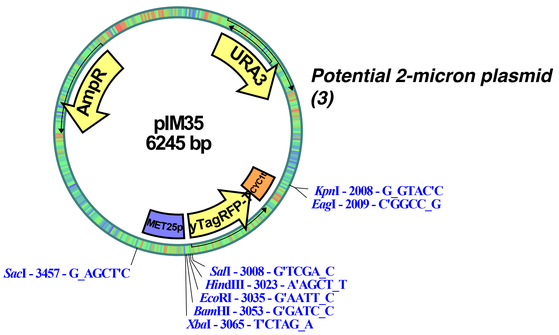
## Abstract

The need for replacement of modern combustion fuel sources are increasing annually. To efficiently replace hydrocarbon fuel systems, a product will need to be biologically produced in a sustained environment. Saccharomyces Cerevisiae (brewers yeast) can hold the key to a sustainable biofuel production cycle. N-propyl alcohol otherwise known as propanol is an alcohol molecule that has been labeled as a biofuel for the future. Yet there remains no efficient production methods of n-propyl alcohol. S. Cerevisiae can produce minute concentrations of propanol under specific conditions in a chemical solution known as “Fusel Alcohols” within the Ehrlich pathway. Through the addition of a 2-micron plasmid containing the genes associated with propanol production S. Cerevisiae can be modified to biologically synthesis increased propanol concentrations. The specific genetic sequences are ARO10 and SFA. This theoretical experiment follows the potential of inserting the genes that correlate to n-propyl alcohol synthesis into a S. Cerevisiae genome. The acceptance of these genes will induce a synthetic duplication of the gene ratio and thereby increase the concentration of propanol that can be created.

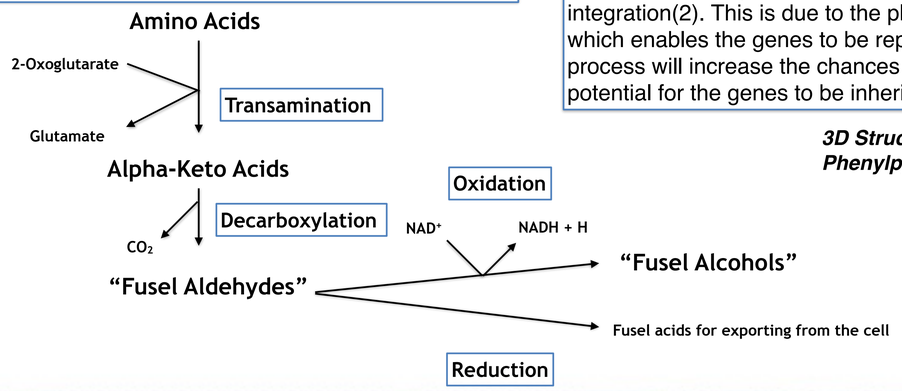
## Methods and Results

### Utilizing the Ehrlich Pathway

The biochemical process that Saccharomyces Cerevisiae utilizes to produce n-propyl alcohol is through the Ehrlich pathway. The Ehrlich pathway is able to render by-products of fermentation of cyclic sugar compounds. These by products are often referred to as “fusel alcohols”. Traditionally these compounds are considered impurities and create distinct flavors or aromas in alcoholic beverages. One of the molecules that comprise the fusel alcohol solution is propanol, which varies roughly from 1 to 10%. To produce fusel alcohols the Ehrlich pathway is activated in S. Cerevisiae only under several specific environmental conditions. These factors are: low pH, a limited nitrogen source, a limited glucose supply and at higher temperatures (1). Amino acids with low nitrogen concentrations are metabolized through the Ehrlich pathway. These amino acids are leucine, phenylalanine and methionine. The amino acids will under go transamination (1). Once converted the newly generated alpha-keto acids will be treated to decarboxylation.Decarboxylation will remove the corresponding carboxylic acid group from the compound and produce a varying array of aldehyde molecules that can be synthesized into fusel alcohols. The fusel alcohols will contain a certain percentage of propanol which could under further treatment be removed from the S. Cerevisiae organism.




*Potential 2-micron plasmid (3)*




### Influencing translation

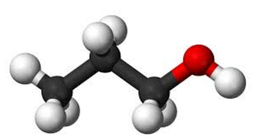
In order to orchestrate the adequate production of propanol from a S. Cerevisiae specimen, the specific genes related to transamination of the Ehrlich pathway need to be amplified. Through duplication the genes can be synthetically increased, which will create a biochemical cascade. Specifically the genes that will be duplicated will contain sequences that have been correlated with the decarboxylation and oxidation steps of the Ehrlich pathway. In previous experiments and analysis, S. Cerevisiae cells with only access to low nitrogen amino acids increased translation of BAT1, BAT2, ARO8 and ARO9 genes(1). These sequences are directly related to biosynthesis of transaminases. With an increase rate of translation through modification of the cellular environment gene amplification is not needed. The genetic sequences of interest have been previously linked to the Ehrlich pathway(2). These genes include the ARO10, SFA1. Each sequence will need to be spliced into the 2-micron plasmid to induce integration into the S. Cerevisiae genome. The ARO10 gene resides within chromosome IV and is 1908 base pairs in length. This gene encodes a phenylpyruvate decarboxylase enzyme that contains the unique ability to potentially bind to a variety of alpha-keto acids(4). With ample quantities of this particular enzyme within the cell there lies an increased chance of metabolizing the idle propanol precursor. The SFA1 sequence is 1161 base pairs in length and is also located within chromosome IV(5). SFA1 sequences synthesize an alcohol dehydrogenase enzyme that contains dual functionality. This particular enzyme is able degrade aldehyde and formaldehyde molecules(5). This alcohol dehydrogenase is also key to the production of higher chained alcohols which include n-propyl alcohol.

To properly incorporate these two genes into the genome of Saccharomyces Cerevisiae a 2-micron plasmid will be utilized. This plasmid sometimes referred to as 6.3 kd Yeast Episomal Plasmid or YEp would be the ideal candidate for integration(2). This is due to the plasmids ability to contain the ARS sequence which enables the genes to be replicated autonomously(2). This replication process will increase the chances of the target sequences insertion and the potential for the genes to be inherited in the F1 generation.

*3D Structure of Phenylpytuvate Decarboxylase*

## Discussion

The manipulation of the environment of Saccharomyces Cerevisiae and addition the SFA1 and ARO10 gene duplications could establish the increased translation of these enzymes. However each gene will need to added with two separate plasmids. The enzymatic activities from these genes are a necessity to develop a proper n-propyl alcohol yield. Through the proper utilization of the Ehrlich pathway through amino acid catabolism of a biological source of sufficient n-propyl alcohol can be achieved. This can only be done through artificial duplication of the correct gene sequences to enhance synthesis. With adequate research and design the SFA1 and ARO10 genes can be amplified to determine the exact potential S. Cerevisiae may contain within it’s genome to generate a future biofuel source.



*3D representation of n-propyl alcohol*

## References

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