**Interspecific Hybridization of Lager Yeast Strains**

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**The *Saccharomyces pastorianus* hybrid is one of the most used yeast in brewing. In this article hybrids made from breeding two strands, *Saccharomyces cerevisiae* and *Saccharomyces eubayanus*, were characterized against their parents in wort fermentation. The hybrids were shown to inherit beneficial properties from their parents; producing a beer with a higher alcohol content as well as fermenting faster. Results conclude that production of yeast hybrids are beneficial for brewing practices.**

**The process of making beer is a task that has been around for centuries, utilizing many different ingredients and processes. Finding faster ways to produce and maximize results is always a good thing. This article by Krogerus et al. talks about a hybrid interspecific yeast, Saccharomyces pastorianus; which is the offspring of cross breeding two other strands of Saccharomyces. This hybrid was tested for many different properties and compared to its parental strains to see whether it is beneficial to use. Saccharomyces pastorianus hybrid inherited many properties from both parents that increased its ability to thrive. When tested against the parents, Saccharomyces pastorianus showed to ferment faster and produced a beer with alcohol content higher than that of its parental strands. These discoveries can lead to improving brewing practices.**

**In brewing there are many different types of yeast used depending on the type of beer wanting to be produced. For example, pale lager, which is the most popular style in the world, uses the hybrid Saccharomyces pastorianus yeast in its fermentation process. The two parental strands Saccharomyces cerevisiae and Saccharomyces eubayanus are used to create this hybrid based on favorable properties they possess. The resulting hybrids were fermented into beer and were tested for flocculation and sugar content.**

**The first step was to create the hybrid from two parental strains. One parent, S. cerevisiae, was obtained from an ale type beer in the United Kingdom while the other, S. eubayanus, was sourced from a culture collection in Finland. The Parental strands were first tested to see if they were satisfactory and then processed and allowed time to sporulate. After spores of the parents were ready it was then time to produce the interspecific hybrids. Spores of both parents were put together into tubes and processed to the desired product. The product obtained from mixing the two parents was the hybrid. After measuring cell concentration, the product was spread onto plates to be incubated until colonies formed. The colonies were then purified by replating.**

**The hybrids needed to be confirmed and was done so using PCR and PFGE (pulsed-field gel electrophoresis). The parents, S. eubayanus and S. cerevisiae, showed a 3 and 4-band pattern respectively. To identify hybrids, restriction fragments from enzyme digestion were looked at. Hybrids that were successful showed a combination of the 3 and 4 band parents, resulting in a 5 band pattern. Of the hybrids S. pastorianus produced, 4 were chosen and named H1-H4 for further characterization.**

**Further characterization was done through small scale fermentation with wort produced from the VTT Pilot Brewery. Hybrids H1-H4, and the parental strains were put through duplicate fermentation with 1.5 L of wort medium in a 2-L stainless steel fermenting unit. Per every liter of wort, 4 g fresh yeast was used. The fermentation process went for 11 days, unless there was no change in samples extracted for 24 hours. Samples were taken throughout the process from each of the fermentation units and placed on ice to stop all processes and then the yeast was separated from the wort through centrifugation.**

**Once the yeast was separated and ready to be characterized, the first step was to test for flocculation through the process of an assay. Flocculation was measured by an absorbance reading at 600nm and a percentage was determined by difference of absorbance between the hybrids being tested and controls. The previously mentioned centrifuged samples were also used for specific gravity, pH and alcohol level using a density meter with alcolyzer beer and pH modules. Dry mass of the yeast was determined after a washing, centrifuging and then being dried overnight and allowed to cool in a pre-weighed crucible and then being measured for change of mass.**

**More characterization was performed by measuring the concentrations of fermentable sugars using a waters separation module and waters system interphase liquid chromatograph with a differential refractometer. Analysis of flavour compounds were measured through gas chromatography with flame ionization. The compounds were determined by comparing them with standards and evaluated using a standard curve.**

**Through the process of determining the hybrids by PCR and amplifying the DNA, 5 bands were noted from all the isolated hybrids being tested, confirming that the parents were represented. The status of the hybrids was fully noted with the PFGE process. PFGE showed that the hybrids had received a full set of chromosomes from both parents. Hybridization of this type has a low frequency and it is not fully understood the portions of the parents that the hybrids received. The data collected is not sufficient to determine how much DNA was received from each parent and there is possibility that that the hybrids obtained more DNA from one parent than another. Not knowing the real proportions of DNA inheritance could be related in the properties of the hybrids.**

**Hybrid strains were fermented completely and showed higher rates of fermentation than the parents. As explained by Krogerus et al., fermentation improvement could be from meiotic segregation of spores or from differing inheritance of mitochondrial DNA, which is only inherited from one parent. Higher alcohol percentage was also seen from the hybrid strains (5.6%) as compared to the parents (4.5%). With the success of fermentation and growth performed at 12 C, normally done at 20 C, Krogerus et al. estimated that the hybrids obtained a higher cold tolerance from S. eubayanus parent strand. Flocculation testing showed strong results for all the hybrids (H1-H4) and the parent S. cerevisiae, whom is most likely where the property came from.**

**Concluding the research, the mating of the strains S. cerevisiae and S. eubanyanus, to create interspecific yeast hybrids was a success. The hybrids produced inherited many beneficial properties from the parents; sugar utilization, heat and cold tolerance, flocculation and more. Inheriting such properties increased the practicality of producing such hybrids, having a higher alcohol content and fermenting faster are both qualities admired. The main purpose of this research was to “suggest that interspecific hybridization is suitable for production of novel non-GM lager yeast strains with unique properties and will help in elucidating the evolutionary history of industrial lager yeast” (Krogerus et al., 2015).**

**Of all the tests performed, the hybrids results were compared to the original strains of the parents. Krogerus et al. notes that research on properties of DNA proportions and inheritance, mitochondrial DNA, and production of aromas is incomplete. More studies on these topics is necessary to clarify the origins of industrial lager yeast strains.**

**References**

Krogerus, Kristoffer, et al. “New lager yeast strains generated by interspecific hybridization.” *Journal of Industrial Microbiology & Biotechnology*, vol. 42, no. 5, 2015, pp. 769–778., doi:10.1007/s10295-015-1597-6.