

New Yeasts, New Beers: Non-GMO Technologies for New Beer Flavours (Part 3)

DESIGNING YEAST | In this concluding section of our three-part series (part 1 and 2 see BRAUWELT International No. 5, 2018, pp. 354-356 and No. 6, 2018, pp. 430-432) we outline the various non-GMO methods by which new brewer's yeast are being created to drive beer flavour and aroma innovations. By applying the classical technique of selective breeding – used for millennia in the domestication of species – it becomes possible to re-imagine brewer's yeast, thereby enhancing and expanding yeast's natural ability to define beer styles and flavours.

ANTON CHEKHOV famously wrote, "Knowledge is of no value unless you put it into practice." In the last two articles in this series, the importance of yeast to the final sensory profile of beer was highlighted, an examination was made of how yeast produce these compounds, and an overview was provided of how brewers can modulate environmental, chemical and biological factors to produce the beers they desire. Sometimes however all the knowledge in the world is useless unless it is applied correctly. When it comes to beer that means starting with the right *Saccharomyces* yeast. Sometimes Mother Nature is kind and the right choice is already out there. If not, however, *Saccharomyces* yeast strains can

be built from the ground up using classical, non-GMO yeast development techniques.

■ Building Better Yeasts

Today, it is possible to quickly and accurately determine the presence and concentration of sensory compounds on large permutation sets of *Saccharomyces* yeast strains and wort/growing conditions. High-throughput technologies such as ultra-performance liquid chromatography (UPLC), gas chromatography–mass spectrometry (GC–MS) and spectrophotometry can be used to analyze the organoleptic profile of any *Saccharomyces* yeast strain growing in any given condition. While not as chemically precise, sensory evaluation by trained professionals can, as discussed, achieve similar results through the use of a yeast-derived chemical compound database and flavour wheel.

One potentially untapped source of novel sensory profiles is in wild *Saccharomyces* yeast, as it likely features more biodiversity than hops, barley or even industrial *Saccharomyces* yeast strains. This is evidenced by the diverse and unique beers produced through wild/spontaneous fermentations. On the other hand, there are significant

downsides to wild fermentation, including inconsistent fermentation kinetics, production of off-flavours and, most significantly, a lack of consistency in the production processes employed by modern brewers. That being said, once a suitable wild *Saccharomyces* yeast strain is characterized as possessing a favourable sensory profile by the aforementioned technologies or sensory panelling, it is possible to use classical breeding, adaptive evolution and rare phenotype selection to produce more economically viable versions of these strains.

These three tools – classical breeding, adaptive evolution and rare phenotype selection – are cornerstone techniques used by humans since before the dawn of agriculture, and have been applied to the do-



Lead Development Scientist Jessica Swanson measures the specific gravity of beer using a hydrometer

Authors: Jason Hung, Associate Scientific Research Writer, Matthew Dahabieh, Ph.D., Chief Science Officer, Renaissance BioScience Corp., Vancouver, Canada

mestication of plants, animals and microbes. Furthermore, since these techniques obviously far pre-date the introduction of GMO technologies (by thousands of years, in fact), they are therefore considered non-GMO techniques.

Dog Breeding Through the Millennia

As an interesting parallel, these three techniques were used in the domestication of dogs from wolves. Today, according to the World Canine Organization, there are 340 distinct dog breeds, ranging all the way from Chihuahuas to Great Danes and everything in between; this vast diversity is believed to have originated from the domestication of a single gray wolf some 20 000-40 000 years ago. Classical (also called selective) breeding of dogs, whereby breeders select for specific traits by choosing parents who display those characteristics, is highly common. The Welsh Corgi, for instance, is believed to have been developed as a herding dog with short legs (making them less vulnerable to being kicked by livestock) by breeding Welsh herding dogs, a breed known for their herding ability, with the Swedish Vallhund, a breed that likely carries specific genetic mutations resulting in short-leggedness.

Classical breeding in *Saccharomyces* is fundamentally the same. Two parental *Saccharomyces* yeast strains with desirable traits can be bred to produce progeny that feature the characteristics of both. For example, a wild *Saccharomyces* strain might produce high levels of favourable esters but also high levels of methyl-mercaptan (cabbage, garlic, pungent, sulfur). An industrial *Saccharomyces* yeast strain that produces no or low levels of methyl-mercaptan could be used as the other parent in order to produce hybrids

with high ester formation but low methyl-mercaptan production. Given the semi-random nature of selective breeding (the reason that non-identical siblings are genetically different), the most promising hybrid strains can then be refined through the process of backcrossing, whereby hybrids are bred back to one of the parents (to create new, similar hybrids) but with the addition of the selected trait. In such a case, the final hybrid strain would display all of the positive characteristics of the wild *Saccharomyces* yeast strain in terms of ester production, but would “act” like an industrial strain.

Adaptive Evolution on the Tibetan Plateau

Adaptive evolution is another technique that allows for growth in a specific environment to aid in the development of novel phenotypic and genotypic changes. Going back to the example of dogs, it was recently discovered that the Tibetan Mastiff has 12 gene mutations including the gene EPAS1 that allow it to survive better in high-altitude, hypoxic conditions such as those on the Tibetan Plateau. It is believed that these gene mutations originally happened by chance but was subsequently selected for by the environment, allowing Mastiffs to survive and thrive in these conditions. Intriguingly, Tibetans themselves also appear to have a very rare version of the EPAS1 gene that allow them to better survive on the Tibetan Plateau as well in a likely case of convergent evolution.

In *Saccharomyces* yeast, this technique relies on knowing what environmental conditions are necessary to produce a certain phenotype. Luckily, with recent advancements in genetic and bioinformatic technologies, it is now easier than ever to dis-

Glossary

Selective breeding – Targeted crossing between choice parent individuals to generate hybrid progeny with traits of both parents.

Backcrossing – Repeated selective breeding in which choice hybrid progeny are isolated and crossed to one parent.

Adaptive evolution – Iterative adaptation of an organism to selective growth conditions or environments over many generations because of accumulated mutations that increase fitness.

Direct mating – Targeted crossing of two specific, mating-type compatible, haploid yeast parents (cells and/or spores).

Rare mating – Untargeted crossing of two specific, mating-type incompatible yeast parents (haploid or diploid) enabled by random, low-frequency mating-type switching events.

cover which genes are responsible for a specific phenotype, as well as which metabolic pathways and intermediate compounds can be used to modulate the growing environment in order to produce advantageous phenotypes. Using the example from our previous “Better Yeast, Better Beer” article in BRAUWELT International 2018, No. 2 in which *Saccharomyces* yeast converted ferulic acid into less toxic guaiacol, it would be possible to use adaptive evolution to develop a “clove-bomb” *Saccharomyces* yeast strain that produces high levels of guaiacol.

Unfortunately, since ferulic acid is toxic to *Saccharomyces* yeast, simply growing yeast in high concentrations of ferulic acid is unlikely to produce positive results. However, the metabolic pathway linking ferulic acid and guaiacol is well studied in *Saccharomyces*, and it is known that the less-toxic chemical p-coumaric acid uses the same metabolic pathway as ferulic acid. Therefore, one could culture a *Saccharomyces* yeast strain with ever-increasing levels of the less-toxic p-coumaric acid in order to overexpress the genes necessary to convert ferulic acid into guaiacol, thereby producing beers with high levels of clove aroma.

Rare Phenotype Selection of Shar Peis and Lagers

Finally, rare phenotype selection techniques exist in cases where an uncommon, usually spontaneous, genetic mutation that produces beneficial or novel characteristics is maintained or further exaggerated by human intervention. This is somewhat similar to adaptive evolution, except that humans must take the initiative to notice/maintain the novel characteristic, instead of relying on the environment to do so. In dog breeding, it’s likely that one dog was born with a mutation in the HAS3 gene that caused its

skin to be wrinkly. This trait doesn’t provide dogs with any particular advantage, so left alone it is unlikely nature would have propagated the mutation. Instead, the phenotype was probably noticed by early Chinese dog breeders who selected for and exaggerated this trait over multiple generations out of simple curiosity, ultimately producing the modern Shar Pei that is well known for its wrinkles.

In *Saccharomyces* yeast development, one specific method of rare phenotype selection is called rare mating, an atypical event by which some *Saccharomyces* yeast cells spontaneously switch mating type (equivalent to gender in humans), with the result of enhancing fertility. Rare mating is particularly useful in the development of *Saccharomyces pastorianus* hybrids or other *Saccharomyces* yeast strains that have difficulty breeding. Lager yeast in particular is a hybrid of the standard brewer’s yeast *Saccharomyces cerevisiae* and *Saccharomyces eubayanus*, a non-canonical brewing yeast strain believed to have originated in Patagonia. This hybridization makes *Saccharomyces pastorianus* basically sterile, resulting in all *Saccharomyces pastorianus* strains being genetically similar: this is one of the main reasons all lager yeast tends to make the same type of beer (specifically, the clean and neutral profile standard to lagers). Rare mating has been shown to successfully overcome low fertility and therefore it is possible to use rare mating with classical breeding in order to produce novel hybrids. Take the standard lager strain W-34/70, known to have low fertility and is known to produce phenolic off-flavours (POF). POF is a trait common to all *S. eubayanus* strains, and the likely source of POF in *S. pastorianus*. However, it has been recently reported that *S. eubayanus* strains have been developed through non-GMO methods

that do not produce POF and these strains can be used to recapitulate *S. pastorianus* through rare mating with *S. cerevisiae*.

Conclusion

In conclusion, *Saccharomyces* yeast is not the enigma brewers often assume it to be. In reality, *Saccharomyces cerevisiae* is a foundational model organism, and in fact possibly the most academically well-studied one on the planet. Indeed, the amount of publicly available genetic, phenotypic, metabolomic and proteomic information on *Saccharomyces* yeast is staggering, and brewers need only exploit this wealth of knowledge.

Simply put, there is no hyperbole in saying that today’s yeast scientists now have the power to develop *Saccharomyces* yeast strains that feature any unique combination of desired traits. All that’s needed in the mix is the creativity, ingenuity and vision of brewers to see what’s possible using non-GMO *Saccharomyces* yeast development techniques such as selective breeding, phenotype selection and rare mating, among others. Add to this a desire to produce better-quality beer and broaden the choices for consumers by inventing entirely new beer styles, and the possibilities for beer lovers become limited only by the breadth of brewers’ collective imagination. ■

Sources

- Melina, R.: “The Incredible Explosion of Dog Breeds”, Live Science, August 5, 2010, URL: <https://www.livescience.com/8420-incredible-explosion-dog-breeds.html>.
- Botigue, L.; Song, S.; Scheu, A.; Gopalan, S.; Pendleton, A.; Oetjens, M.; Taravella, A.; Seregely, T.; Zeeb-Lanz, A.; Arbogast, R.R.; Bobo, D.; Daly, D.; Unterlander, M.; Burger, J.; Kidd, J.; Veeramah, K.: “Ancient European dog genomes reveal continuity since the Early Neolithic”, Nature Communications, URL: <https://www.nature.com/articles/ncomms16082>, DOI: 10.1038/ncomms16082.
- Hill’s Pet Nutrition, URL: <https://www.hillspet.ca/en-ca/dog-care/dog-breeds/welsh-corgi-pembroke>.
- United Kennel Club, URL: <https://www.ukcdogs.com/pembroke-welsh-corgi>.
- Parker, H.; VonHoldt, B.; Quignon, P.; Marguiles, E.; Shao, S.; Mosher, D.; Spady, T.; Elkhahloun, A.; Cargill, M.; Jones, P.; Maslen, C.; Acland, G.; Sut-

- ter, N.; Kuroki, K.; Bustamante, C.; Wayne, R.; Ostrander, E.: "An expressed *fgf4* retrogene is associated with breed-defining chondrodysplasia in domestic dogs.", *Science*, URL: <https://www.ncbi.nlm.nih.gov/pubmed/19608863>, DOI: 10.1126/science.1173275.
18. Li, Y.; Wu, D.; Boyko, A.; Wang, G.; Wu, S.; Irwin, D.; Zhang, Y.: "Population Variation Revealed High-Altitude Adaptation of Tibetan Mastiffs", *Molecular Biology and Evolution*, URL: <https://academic.oup.com/mbe/article/31/5/1200/997994>, DOI: 10.1093/molbev/msu070.
19. Huerta-Sanchez, E.; Jin, X.; Bianba, Z.; Peter, B.; Vinckenbosch, N.; Liang, Y.; Yi, X.; He, M.; Somel, M.; Ni, P.; Wang, B.; Ou, X.; Huasang; Luosang, J.; Cuo, Z.; Li, K.; Gao, G.; Yin, Y.; Wang, W.; Zhang, X.; Xu, X.; Yang, H.; Li, Y.; Wang, J.; Wang, J.; Nielsen, R.: "Altitude adaptation in Tibetans caused by introgression of Denisovan-like DNA", *Nature*, URL: <https://www.nature.com/articles/nature13408>, DOI: 10.1038/nature13408.
20. Adeboye, P.; Bettiga, M.; Aldaeus, E.; Larsson, P.; Olsson, L.: "Catabolism of coniferyl aldehyde, ferulic acid and p-coumaric acid by *Saccharomyces cerevisiae* yields less toxic products", *Microbial Cell Factories*, URL: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4578848/>, DOI: 10.1186/s12934-015-0338-x.
21. Olsson, M.; Meadows, J.; Truve, K.; Pielberg, G.; Puppo, E.; Mauceli, E.; Quilez, J.; Tonomura, N.; Zanna, G.; Docampo, M.; Bassols, A.; Avery, A.; Karlsson, E.; Thomas, A.; Kastner, D.; Bongcam-Rudloff, E.; Webster, M.; Sanchez, A.; Hedhammar, A.; Remmers, E.; Andersson, L.; Ferrer, L.; Tintle, L.; Kindblad-Toh, K.: "A Novel Unstable Duplication Upstream of *HAS2* Predisposes to a Breed-Defining Skin Phenotype and a Periodic Fever Syndrome in Chinese Shar-Pei Dogs", *PLOS Genetics*, URL: <http://journals.plos.org/plosgenetics/article?id=10.1371/journal.pgen.1001332>, DOI: 10.1371/journal.pgen.1001332.
22. Libkind, D.; Hittinger, C.; Valerio, E.; Goncalves, C.; Dover, J.; Johnston, M.; Goncalves, P.; Sampaio, J.: "Microbe domestication and the identification of the wild genetic stock of lager-brewing yeast", *PNAS*, URL: <http://www.pnas.org/content/108/35/14539>, DOI: 10.1073/pnas.1105430108.
23. Krogerus, K.; Magalhaes, E.; Vidgren, V.; Gibson, B.: "Novel brewing yeast hybrids: creation and application", *Applied Microbiology and Biotechnology*, URL: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5203825/>, DOI: 10.1007/s00253-016-8007-5.
24. Didreich, J.; Weening, S.; van den Broek, M.; Pronk, J.; Daran, J.: "Selection of Pof- *Saccharomyces eubayanus* Variants for the Construction of *S. cerevisiae* × *S. eubayanus* Hybrids With Reduced 4-Vinyl Guaiacol Formation", *Frontiers in Microbiology*, URL: <https://www.frontiersin.org/articles/10.3389/fmicb.2018.01640/full>, DOI: 10.3389/fmicb.2018.01640.