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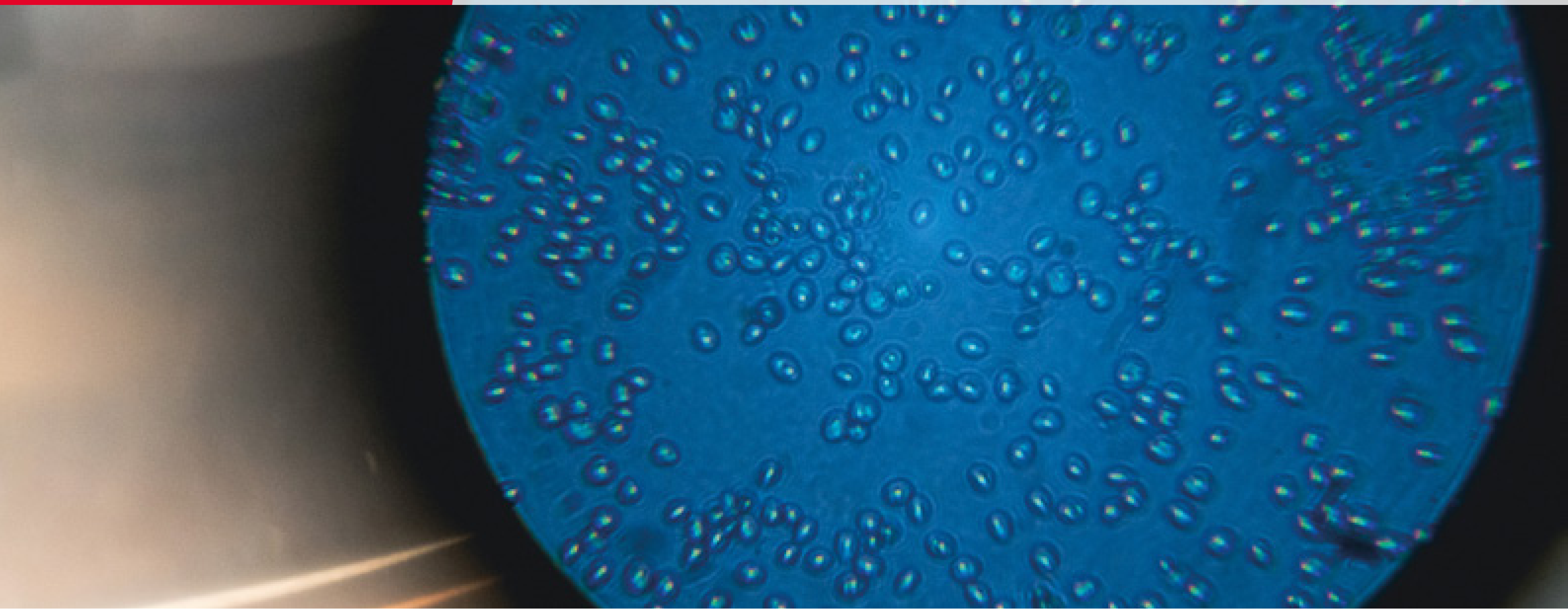
THE OFFICIAL NEWSLETTER
OF ALL THINGS LALLEMAND BREWING

FROM PITCH TO RE-PITCH

Maximizing Yeast Efficiency

EDITION #16



BE PASSIONATE
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YEARS OF EXPERTISE

We recently conducted a survey among the Lallemand Brewing Team members to learn about their individual experiences in the brewing industry. We asked them to share how long they have been working in the industry and sum up their answers: it's been 500 years! This shows the level of expertise and passion the team possesses in supporting brewers.

#bepassionatebelallemand

EDITORIAL

Repitching brewing yeast has long been a subject of nuanced dialogue within the brewing community, and a topic that resonates deeply with our team at Lallemand Brewing. The practice of reutilizing brewing yeast is well established in certain regions, while emerging as a novel pursuit in others, paralleling the dissemination of advanced educational resources. When executed carefully, the act of repitching yeast emerges as an economic benefit for brewers, saving the brewer money and keeping brew schedules on track.

An incontrovertible truth regarding repitching brewing yeast is that it requires effort from both the yeast producer and the brewer alike. In essence, for a yeast strain to merit the "repitchable" designation, the yeast producer must ensure genetic stability across successive generations, coupled with adherence to the most stringent quality and microbiological standards. It's important to remember that not all yeast performs the same; some exhibit slower growth rates, while others are higher flocculators, for example. Once a high-quality stable yeast is in the hands of the brewer, more work needs to be done to ensure consistent beverage with repitched yeast.

In this newsletter, we will look at what it takes for a yeast manufacturer to produce a "repitchable yeast" as well as what the brewer must do to ensure consistent beer with repitched yeast. Here at Lallemand, we have made significant improvements in our production processes to ensure the genetic stability and high quality of our dry brewing yeast products. This progress is supported by continuous research in the areas of yeast propagation and drying technology. We will take a look at the latest data from a repitching research project in conjunction with Nottingham University as well as hear from industry experts about repitching approaches, best practices and some tips and tricks for repitching both at home and commercially.

Brent Jordan
President & General Manager
of Lallemand Brewing

YEAST GENETIC STABILITY

By Avi Shayevitz,
R&D Research Scientist

The rise of domesticated brewing yeast

The modern brewing yeast is the result of millennia of selective breeding. The slow domestication process from spontaneous grain fermentations in neolithic pottery to modern stainless steel, in sterile mega-breweries has resulted in a species of yeast that is very different from its wild ancestors. The result of this domestication is that *Saccharomyces cerevisiae* has become well adapted to niche environments created by humans that do not exist in nature. Unlike wild yeast, the genome of *S. cerevisiae* is often quite 'messy'. It's the result of artificial selection and environmental pressures in human-made environments such as breweries. A common example of these adaptations is loss of functional PAD1 and FDC1 genes, which, in their functional form, are responsible for producing phenolic "POF" flavors in beer. Additionally, domesticated *Saccharomyces* species have developed unique mechanisms to rapidly metabolize complex carbohydrates such as maltotriose, a valuable energy source found in brewing wort that is not typically metabolized by wild yeasts. Other unique characteristics, such as an abnormal number of chromosomes, pervasive genetic homozygosity (genes are the same on each chromosome), and an inability to successfully mate and undergo meiotic rearrangement through sporulation, contribute to making domesticated *S. cerevisiae* so useful to us.

When billions of cells are dividing, living, metabolizing, mutations are not merely a possibility, but an inevitability

Consistent beer through yeast genetic stability

The utility of yeast domestication to modern brewers cannot be understated. These adaptations come at enormous biological costs, often making it very difficult for domesticated brewing yeast to sexually reproduce, further diminishing the capacity to adapt and evolve to different environmental conditions. This makes domesticated yeast less competitive in the wild, but extremely useful for a brewer as this genetic stability leads to consistent fermentation performance.

In practice, "genetic stability" is the ability to minimize observable changes in fermentation performance and flavor during normal industrial use, despite slowly collecting mutations over long term use. This is a highly desirable trait of brewing yeasts and key to their ability to produce consistent and reliable results from batch to batch.

Genetic drift is inevitable

While brewing yeasts are resistant to change, it is also important to understand that genetic mutations are inevitable. Every time a cell divides, there is a chance that an error occurs while replicating the DNA – resulting in some altered behavior of the new cell. When a yeast is propagated for use in brewing, the population increases by more than six orders of magnitude from just a few hundred cells per milliliter of wort to one billion cells per milliliter (or more!). When billions of cells are dividing, living, metabolizing, etc., mutations are not merely a possibility, but an inevitability. Fortunately, most mutations will not alter the desired behavior of the yeast. Occasionally, a mutation will occur in an important metabolic pathway that impacts the whole brewing process in unforeseen ways.

Genetic stability during yeast production

As genetic drift is inevitable, what can we do to best ensure consistent yeast performance? Thankfully, a lot! As yeast producers, we carefully manage our culture collection to ensure the long term stability of our yeast strains through rigorous testing and documentation.

STRAIN CHARACTERIZATION

When a new strain is collected, or developed, it is subjected to a mating test to determine its ability to sporulate and mate. Documentation of mating capacity can provide valuable insight into the chances of rare mating events or spontaneous chromosomal rearrangements in the brewery. This also gives us an opportunity to explore novel strain development through hybridization.

Although genetic changes are inevitable in any biological system, we mitigate that risk through rigorous quality assurance.

At present, there are two primary methods through which entirely novel strains may be developed: hybridization and bioengineering.[1] Hybridization is a complex process which, if done incorrectly, may cause whole genome dysfunction – resulting in a genetically unstable organism that is completely unfit for brewing or other industrial applications. The same concepts apply to bioengineered yeast since genetic modifications may result in reduced fitness and introduce selective pressure against this modification. Careful selection and screening of offspring are required to ensure that the resulting organism can fully integrate any changes made to its genome, and successfully pass these traits onto subsequent generations without any loss of function. In our labs, we rigorously test novel traits in bioengineered yeast often exceeding 100 microbial generations – approximately equivalent to 25 repitches in a brewery.

YEAST BANKING AND STORAGE

Banked yeast strains are stored long term in a dormant state at cryogenic temperatures of ≤ -150°C, which ensures that random mutations are kept to a minimum. These cryogenic yeast banks serve as a "doomsday" backup so that strains are never lost in case of a disaster. All cultures for commercial production are derived from our culture bank and managed carefully to minimize the number of generations from the original banked culture and guarantee manufacturing consistency. Under these conditions, it is expected that major genomic changes will not occur. Nevertheless, our culture collection team performs routine genetic sequencing of our culture strains to monitor for rare genetic changes during storage. This can be achieved by initial whole genome sequencing when the culture is first submitted, and then routine sub-genome sequencing of important metabolic pathways.

YEAST PRODUCTION QUALITY CONTROL

During yeast production, when cell division is high, our production facilities keep an eye on key regions of the yeasts' genome through genetic sequencing. By minimizing selective pressures during the propagation process, we ensure that Lallemand Brewing Premium dry yeast is genetically consistent from one production to the next, ready for use and subsequent re-pitching.

Although genetic changes are inevitable in any biological system, we mitigate that risk through rigorous quality assurance. Through careful management of our yeast culture collection, we ensure that when a culture begins to drift in undesirable ways, we are able to provide a fresh culture with the desired, original genetic profile.

Monitoring genetic drift in the brewery

Despite the inherent stability of any yeast strain that we bring to market, genetic change is inevitable. The stresses of a normal beer fermentation will result in a very low level of genetic drift in the yeast culture. Most breweries are not equipped to sequence DNA or properly manage a yeast culture bank. So what can you do as a brewer to manage the normal risk of genetic drift in yeast?

To ensure consistent high-quality beer, monitor the yeast for any changes in performance over time. Changes in fermentation kinetics, lag-phase, and attenuation may indicate that the yeast has a reduced ability to import and metabolize certain sugars. Small colonies on agar plates may indicate "petite mutations", which results from mutations to the mitochondrial DNA. Changes in sensory profile could indicate mutations to one of the many biochemical pathways involved in production or metabolism of esters, phenolics, fusel alcohols, sulfur compounds and diacetyl. It is important to monitor flocculation closely since the repeated harvesting of yeast from the cone or surface of the beer can change flocculation behavior. Harvesting from the cone will select for fast-settling cells, whereas top-cropping will select for cells that prefer to stay at the top of the tank. As always, test for wild yeast or bacteria contamination and check the yeast under the microscope to assess cell viability and morphology. Minimize your risk by limiting the number of re-pitch generations (5-8 max) and refresh your culture with a first-generation pitch if you have any doubt about yeast performance. By following yeast handling and re-pitching best practices[2], you will achieve consistently high-quality beer.

[1]: https://www.lallemandbrewing.com/en/united-states/resources/whats-new/innovative-methods-for-selecting-novel-yeast-strains-for-brewing-unique-beers

[2]: https://admin.lallemandbrewing.com/wp-content/uploads/2023/07/LAL-bestpractices-Repitching_Eng_A4.pdf

A QUEST FOR DATA: YEAST STRAIN REPITCHING

By Molly Browning,
Technical Support Manager

Our R&D team enjoys quests – one recently completed expedition involved answering the question that has surrounded our dry yeast range for years: repitchability of the LalBrew® range.

To start, we conducted a survey of the previous studies done on dry yeast repitching. In 2010, our Senior R&D manager, Tobias Fischborn, conducted a study with industry colleague Chris Powell to investigate repitching LalBrew Diamond™, a German lager strain. Published in the *Journal of American Society of Brewing Chemists,* they found consistent fermentation performance with no genetic variance or mutation across these repitches.

Beer styles and brewing strains have significantly advanced in the past 13 years, including in our own portfolio. Also, this previous study only examined a lager yeast. Would ale strains perform differently?

To answer this question, we continued our expedition in partnership with the University of Nottingham, with the goal of examining different ale yeast strains through multiple repitches.

As we wanted to capture a variety of beer styles, five yeast strains were selected for this study: LalBrew Verdant IPA™, LalBrew New England™, LalBrew Wit™, LalBrew Belle Saison™, and LalBrew Nottingham™.

What did we discover? One, that all strains were healthy with no significant difference in fermentation character across multiple generations. Specifically, live cell concentrations increased throughout the generations for all strains, apart from LalBrew Nottingham™ where live cell concentrations remained the same from generation 1 and 2.

This study highlights the high quality of LalBrew strains and gives confidence to brewers that these strains maintain high viability when repitched. But our R&D explorations do not end here. For example, this year we are excited to collaborate with White Labs on various research projects that offer additional technical insights into the recently introduced dried strains in their range.

Furthermore, we continue to partner with other research institutions in our journey to explore the depths of fermentation. Our research encompasses areas such as: nutrition and fermentation performance, yeast strain influence on thiols and effective biotransformation, as well as the development of new and exciting yeast strains to cater to diverse brewer requirements. Onward.

5 TIPS FOR REPITCHING YEAST AT HOME

By Eric Abbott,
Technical Support Manager

While pitching a fresh sachet of dry yeast is the easiest way to achieve consistent fermentation performance, a homebrewer can maximize their investment by harvesting and repitching yeast. Here are some tips for achieving the best results when repitching at home.

- **Use sanitized equipment.** Ensure that the harvesting spoon and the receiving container have been thoroughly cleaned and sanitized (by heat, if possible).
- **Avoid harvesting trub and hop particles.** This can be achieved by top-cropping during high krausen or bottom cropping after transferring to a secondary fermenter. Avoid harvesting yeast after dry-hopping.
- **Pitch only fresh, highly viable yeast.** Count viable yeast cells if you have a microscope. Otherwise, keep your yeast happy by storing it in the fridge for up to a week. Avoid harvesting from stressful fermentations like high-gravity or sours.
- **Don't overpitch.** You can have too much of a good thing. Pitching too much yeast can result in off-flavors. Use about 150-300ml of yeast slurry per 19L (5 gallons) carboy, depending on the thickness and viability of the slurry. Save some of the yeast in the fridge, you can always add more if the fermentation is slow to start.
- **Limit the number of repitches to less than 5.** With each generation, the cost savings are less and the risk of contamination or changes to the yeast flavor profile becomes greater. After five repitches (maximum), start again with a fresh pitch to ensure your beer is always consistent and high quality. The cost of a new sachet is less than the cost of materials and your time for a brew that has to be dumped.

SITTING DOWN WITH WHITE LABS

At Lallemand Brewing, we're thrilled to share some exciting news from our valued partner, White Labs, a global leader in liquid brewing yeast. They've just released two of their beloved liquid yeast strains in a dry form, produced by Lallemand, making them available worldwide. Our collaboration with White Labs has sparked enriching discussions on the science of repitching, comparing experiences with both liquid and dry yeast. In this article, Eric Abbott, Technical Support Manager, Lallemand Brewing, sits down with Troels Prahl, Director of Innovation of White Labs Global, Managing Director of White Labs Copenhagen, to dive into the fascinating topic of repitching brewing yeast.

EA: Repitching yeast is an important tool for brewers to reduce costs, which is more and more important these days with the rising cost of, well, pretty much everything. But many brewers are intimidated by the process. What would you say to a brewer who wants to start repitching yeast in their brewery?

TP: I want to say that some brewers tend to be overly cautious when it comes to repitching. You don't need to have a lab or be a trained microbiologist. A yeast slurry is much less susceptible to contamination than a freshly aerated wort at the start of fermentation because there is some alcohol present, no fermentable sugars, and there are billions of yeast cells to compete with any contamination. If a brewer is comfortable with cleaning and sanitizing a fermenter, then they already know enough about brewery sanitation to repitch yeast.

Would you say that all brewing yeast is repitchable?

In principle, all brewing yeast is repitchable. As long as the fermentation is healthy and the yeast is viable, the yeast can be reused again and again. Good yeast handling practices are important though, and there are some cases where repitching is not recommended. For example, some beer styles such as high ABV, dry hopped IPAs, or sour beers make it difficult to harvest a good amount of highly viable yeast. There are also challenges related to carryover of color or flavor with the repitched yeast, for example yeast from a very dark or very bitter beer repitched into a lighter flavored beer. And fermentation performance and sensory may change over time due to genetic drift. But the repitchability does not depend on the strain itself.

Is there a limit to how many generations the yeast can be repitched?

Of course, this may vary on a case-by-case basis. I know some breweries that have repitched their culture for decades and hundreds of batches without issues. This is rare and specific to their yeast handling practices and brewing process. As a rule of thumb, a commercial brewer should be able to get to five generations, or up to 10 generations, with good yeast handling and favorable conditions. Beyond this, we see a significant drift in the culture that is independent of yeast handling practices, and additional cost savings are minimal.

That's in-line with our own recommendations as well. What should a brewery look for in terms of yeast performance when determining whether to repitch, or to start again with fresh yeast?

The best strategy is to look at the fermentation data from the previous batch. There is so much good information in the kinetics of the fermentation graph. A longer lag phase, slower fermentation, or incomplete attenuation are all signs that you should pitch fresh yeast. Consistent fermentation graphs over consecutive batches are usually associated with consistent flavor characteristics of the final beer.

What causes a yeast culture to slow down after multiple generations?

For each yeast pitch, you need to make sure that majority of yeast cells are young, fresh daughter cells from the previous fermentation. One of the most common issues with repitching is overpitching, leading to less cell growth and therefore an older and less optimal population to carry on. This will cause issues in the second or third or fourth generation of repitching because there are older soldiers in the mix. The result is a slower fermentation or incomplete attenuation.

What are some things a brewer can do to improve their repitching practices?

It is important to pitch an adequate amount of viable yeast, so use a microscope to count cells and measure viability. If you don't have a lab, you can get a crude measurement of yeast quality by measuring the total amount of biomass present at the end of fermentation. More biomass suggests more cell division, more young and fresh daughter cells, and higher viability. After collecting yeast for repitching, use a flow meter, or simply measure the number of buckets of slurry dumped from the fermenter. The total biomass is usually consistent, so any changes will give you information about optimizing aeration rates, pitching rates, or nutrition.

White Labs now has dry versions of some strains available. While liquid yeast is pitched by cell count, dry yeast is generally pitched by weight. Has this led to any confusion with customers?

It's quite important to remember that harvested yeast slurry has undergone an alcoholic fermentation, so the physiological condition of a culture after a beer fermentation is quite different from the culture coming out of a lab. Since the yeast physiology is different, they will perform differently, and this is true for both liquid and dry yeast. The brewer can compensate by customizing the pitch rate and fermentation conditions for the first-generation pitch to achieve more consistent fermentation performance and flavor. For the most consistent results, follow the manufacturers' recommendation about pitching rates for each strain.

Yeast should be used ASAP and stored for no longer than 2 weeks.

Do you have any tips for achieving greater flavor consistency from one batch to the next?

Experienced brewers know that first generation yeast, from any source, performs slightly different compared to subsequent generations. For both liquid and dry yeast, there is a signature flavor in the first generation, and the second and third generations are generally producing better beer. Flavor differences can be minimized by blending the first generation with other batches from repitched yeast. If you can't blend the first generation beer out, optimize your pitch rate and fermentation conditions to achieve consistent fermentation kinetics and biomass yield to minimize some of those flavor differences.

Do you have any nutrient recommendations with regards to repitching?

Repitching success is very much related to the cell growth, which depends on the nutritional state of the wort and the nutritional conditions of the media that the yeast was propagated in. It depends a lot on the malt quality and the malt supplier, and whether you are using adjuncts. All-malt wort has almost everything that a yeast cell needs to be happy. Zinc is the most important element that can be deficient in wort to varying degrees depending on the harvest year and growing conditions. Zinc enriched inactivated yeast such as Servomyces™ provides great benefit for propagating and repitching yeast. I recommend doing comparative fermentations with and without nutrients and then looking at the fermentation profile. If there's a difference, then it's probably a sign that you need to add that nutrient, and zinc would be the first nutrient type I would recommend.

How long can harvested yeast be stored before repitching, and under what conditions?

It's a very good question, and it's very strain dependent. It's important that the brewer gets to know their yeast strain well since some are very robust and some are more sensitive and need to be used more quickly. Yeast should be used ASAP and stored for no longer than two weeks. At the end of fermentation, the yeast builds up glycogen levels inside the cell, which acts as a carbohydrate source to keep the yeast alive over time. Glycogen is very quickly depleted in the presence of oxygen, so it is important to harvest yeast as oxygen-free as possible. Keep the yeast as cold as possible (2-4°C) to slow the yeast metabolism. Yeast is a very good insulator which can allow for hot spots to develop due to metabolic activity in the yeast slurry. The slurry can be mixed to ensure the temperature is homogenous. The storage vessel should be vented to allow CO_2 to escape and avoid pressurizing the culture, which would cause yeast stress. Don't store yeast in a normal brewery keg, it must be modified to allow CO_2 to be released and prevent dangerous levels of pressure to build up. At White Labs, we developed the FlexBrink®, which is essentially a heavy duty, sterile plastic bag that allows you to harvest yeast without having any ingress of oxygen, store easily, and pitch in line via peristaltic pump.

Do you have any advice for how to harvest yeast from a NEIPA?

NEIPA is often dry-hopped during active fermentation, so generally it is best to avoid harvesting yeast from these beers to avoid carryover of hop material. However, we have seen many breweries that are able to successfully repitch for many generations from hazy beers by finding ways to crop the yeast before they dry hop, even if that means harvesting before the fermentation is complete. This goes against the normal best practices of yeast harvesting, but with some experience you can bend the rules with these types of beer with good results.

Can you speak a bit about your ongoing R&D programs related to repitching?

We have 28 years of knowledge about how to best reuse the liquid cultures in our collection. We are working now to build up some of that same data for our dry yeast strains to understand how they repitch over time. We should be ready to share these results early 2024. We're extremely excited about this new dry format for the strains that we've known for so many years.

Troels Prahl holds a degree in biotechnology (University of Copenhagen), specializing in fermentation science. Prahl has since the early 2000's dedicated his working life to brewing and fermentation science, and its application in Europe, UK and USA. Prahl has worked closely with fermentation leader White Labs on yeast research and development project management since 2007. Prahl has presented at major technical conferences and taught technical classes with renowned brewing science schools, such as Siebel Institute, and the Scandinavian School of Brewing in Copenhagen. Prahl was also recognized as an award-winning head brewer during his time at London's Camden Town Brewery. In 2014 Prahl relocated from California, back to Denmark where he, apart from his role in the mother corporation as Director of Innovation, is the managing director of White Labs Copenhagen. White Labs Copenhagen is a satellite production and lab facility servicing the European market's yeast and fermentation needs.

BUSTING MYTHS ON DRY YEAST

By Keith Lemcke,
Marketing Director, Siebel Institute of Technology

Even just 25 years ago, commercial brewers were reluctant to consider the use of dry brewing yeast. If you were in conversation with a brewer at a technical conference and the topic turned to using dry yeast, many brewers would share their concerns that dry yeast had issues related to performance and purity. In today's brewing market, brewers now embrace the advantages of dry yeast in all their products. So, what changed? While dry yeast quality and variety has improved over the years, the greatest change has involved the dispelling of myths surrounding these important fermentation products.

The homebrewing myth hangover

Homebrewers have used dry yeast ever since it was first introduced. Before brewing-specific strains were created, it was common to use baking yeast for creating homemade beer, wine, cider, grog, hooch, and more! People fermented with whatever dry yeast was available at their local supermarket. Eventually, homebrew kits came onto the market with many including a sachet of unbranded "mystery yeast" that lacked any details on the contents, especially something as important as an expiration date.

By the time homebrewing took hold in the USA in the late 70s and early 80s, followed by the rise of commercial craft brewing, books on the craft of small brewing were setting the tone for how beer was made. Sometimes the information provided in the books lacked a real scientific foundation, yet some of the information they introduced lives in brewing lore to this day. A good example is what was said about dry yeast in the 1995 book "Dave Miller's Homebrewing Guide", one of the biggest-selling homebrew books of its time. After a short, somewhat antiquated, overview of how dry yeast used to be made, Miller offered this observation:

"The viability of cells and the degree of contamination vary widely. Even among the better brands, there are great differences from one lot to the next."

Not all dry yeasts are equal

While the first homebrew beer kits contained "mystery yeast" of questionable quality, commercial brewers (and some savvy homebrewers) were using high-quality dry brewing yeast to brew high-quality beers. Yet statements like these in popular homebrewing books have led to a persistent mythology that dry yeast is still somehow inferior to its liquid counterparts. One such myth is that dry yeast lacked the ability to be repitched and reused for subsequent batches of beer. In fact, dry yeast doesn't differ from yeast that originated as a liquid culture. When repitching a slurry that originates from a dry culture, the same handling techniques and precautions should be used as if repitching from a culture of liquid origins.

Over the three decades since Mr. Miller's book was introduced, dry yeast has established itself as a trusted tool for breweries large and small, with performance that meets or exceeds that of its liquid counterparts. Throughout the manufacturing process, every key factor of purity and performance undergoes testing to assure the finished products offer the highest level of consistency in brewing application. Combine this with the remarkable shelf-stability, and ease of measurement and use that only active dry yeast can provide, dry yeast has advantages that make it the obvious choice of today's brewer. With some of the world's best beers being produced with dry yeast, it's time to consider the early homebrew myths…busted.

DID YOU KNOW...

...yeast was being repitched by brewers centuries before we even knew what yeast was.

In medieval Germany, the 'hefner' was a person responsible for harvesting yeast from one batch to transfer to the next fermentation. Yeast was not identified as a single-celled organism responsible for fermentation until 1866 by Louis Pasteur, and the German Reinheitsgebot was not revised to include yeast as an ingredient until 1906.