

MICROBIAL CONTAMINATION AND IMPLICATIONS FOR DISTILLED SPIRITS PRODUCERS

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The basic ingredients used to make different distilled spirits are well known. Rum is distilled from fermented cane sugar. Bourbon whiskey is made with a combination of grains including corn, wheat or rye, and malted barley (among others). Vodka can be distilled from a wider range of fermented starting material including potatoes, various grains or sugar. Another ingredient of fermentation is the yeast, responsible for taking up the sugar and producing ethanol and other flavor components. However, there is another subset of “ingredients” present in almost every fermentation, but unlike those described above, these “other ingredients” were not put there intentionally. These “other ingredients” are what can be collectively referred to as microbial contamination. These are microorganisms (most often bacteria and/or yeast) that gain access to the fermentation via the grains, water, fermentation tanks, piping, backset, dust, and other sources. These contaminating microbes are important for several reasons. One of the most

significant consequences of microbial contamination is that it can severely affect distillery yields and production by competing with the yeast for sugars and nutrients. In addition, contaminating

bacteria and yeast produce various organic acids and other metabolic byproducts that can build to toxic levels, further impacting fermentation. Apart from potentially devastating yield and production issues, contaminating microbes can also influence flavor of the finished spirit. Here we discuss, in detail, microbial contamination with a focus on factors important to distillers and how this might contribute (positively or negatively) to the flavor profile of the finished spirit. We will also discuss methods for prevention and control and different ways to diagnose microbial contamination issues.

“HOUSE BUGS”

Often when distillers talk about microbial contamination it is done in very general terms. “House bugs” is one of the common phrases to describe the contaminating microbes that inhabit a distillery. Since there are literally thousands of different potential microbes and microbe combinations in any given contamination scenario, most of them involving wild yeast and/or bacteria, reference to these as “house bugs” hardly seems adequate considering the wide range of potential effects on fermentation and production. In addition, “house bugs” are often touted by distilleries as being crucial to the flavor profile of the finished spirit. Thus, a deeper look into what specific microbes make up those “house bugs” is appropriate and should be done at every distillery.

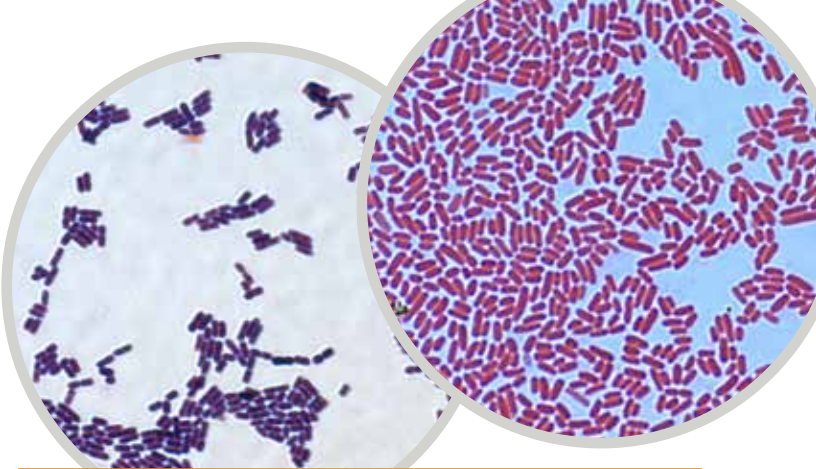


(FIGURE 1) Ferm Solutions maintains a repository of over 10,000 contaminating bacteria and several thousand wild yeast. Bacteria and wild yeast are isolated and stored cryogenically for further research and development.

MICROBES OF INTEREST

Bacteria and wild yeast that contaminate fermentation are typically those that are found naturally in grains and water. In our collection of over 10,000 bacteria from over 200 different distilleries (Figure 1), the vast majority were identified as “lactic acid bacteria” or LAB. These include bacteria in the genera *Lactobacillus*, *Lactococcus*, *Weissella* and *Pediococcus*, among others, which are Gram positive (result of a staining procedure that differentiates bacteria into two main categories based on cell wall characteristics). Within the genus *Lactobacillus* are many different species that can contaminate fermentation. Examples include *Lactobacillus fermentum*, *Lactobacillus delbrueckii*, and *Lactobacillus brevis*. Other Gram positive bacteria found in fermentation include *Enterococcus* and *Bacillus* species. *Bacillus* species are unique because these are one of the few bacteria known to produce endospores, survival structures that allow them to survive adverse conditions like desiccation and elevated temperatures. For this reason, *Bacillus* species are often cultured from samples collected from high heat areas such as during the cook process or from high temperature heat exchangers. Gram negative bacteria found in fermentation include *Acetobacter* and *Gluconobacter* species, but we also see on occasion members of the Family *Enterobacteriaceae* (*Escherichia coli*, *Salmonella*, *Klebsiella*, *Enterobacter*, etc). Various *Pseudomonas* species are also found, but like other Gram negatives are less common than their Gram positive counterparts. Figure 2 shows examples of gram positive and gram negative bacteria commonly isolated from fermentation at different distilleries.

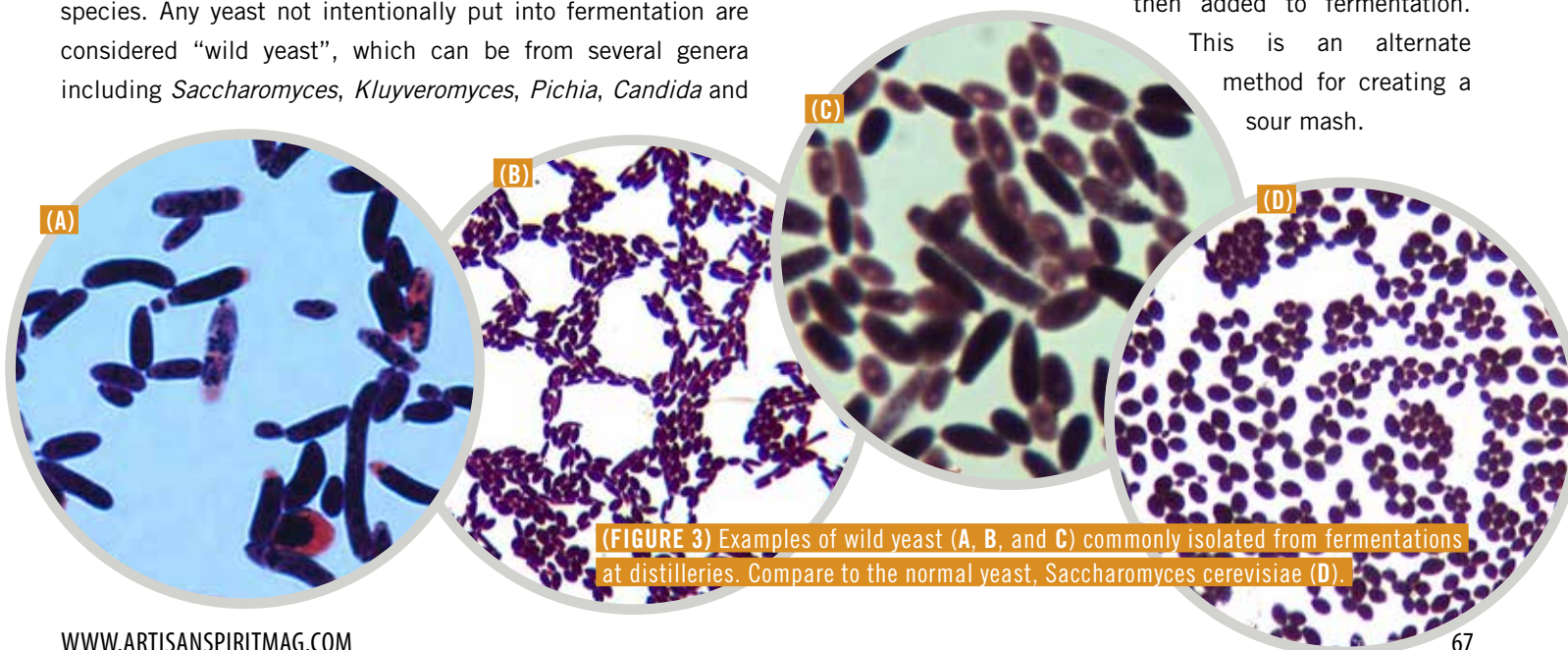
Apart from contaminating bacteria there are several yeast species. Any yeast not intentionally put into fermentation are considered “wild yeast”, which can be from several genera including *Saccharomyces*, *Kluyveromyces*, *Pichia*, *Candida* and



(FIGURE 2) Examples of Gram positive (purple) and Gram negative (pink) bacteria commonly isolated from fermentation at distilleries.

Brettanomyces, to name just a few. Wild yeast, like bacteria, can produce toxic organic acids or other metabolic byproducts that can affect fermentation. Examples of some of the different wild yeasts are shown in Figure 3 and are compared to the “normal” yeast *Saccharomyces cerevisiae*. Interestingly, some of the wild yeast that we find in fermentation that cause issues are useful in other applications. For example, *Kluyveromyces* species can be used to ferment lactose to make ethanol. Certain *Pichia* species are used to make ethanol from C5 sugars like xylose, which is a component of hemicellulose, another complex plant material found in fermentation. A *Brettanomyces* species that is causing major issues at a distillery might be used to make a sour beer at a brewery. These are good examples of how one distillery’s worst enemy can be another distillery or brewery’s greatest asset. The same is true for many of the bacterial contaminants, as LAB are well known and useful as probiotics. Some distilleries, rather than making a sour mash by recycling backset, will propagate a specific bacterium (*Lactobacillus brevis*, for example) in a separate vessel and once a pre-determined pH or level of lactic acid is reached, the culture is heat treated to kill the bacteria and then added to fermentation.

This is an alternate method for creating a sour mash.



(FIGURE 3) Examples of wild yeast (A, B, and C) commonly isolated from fermentations at distilleries. Compare to the normal yeast, *Saccharomyces cerevisiae* (D).

PROLIFERATION OF MICROBIAL CONTAMINATION DURING FERMENTATION AND IMPLICATIONS

Contaminating microbes get into fermentation through several routes described above. Our research has shown that in most cases involving reasonable cleaning and sanitation, populations of contaminating microbes are low in the beginning (less than 100 viable cells per ml of mash). In the case of grain-based fermentations, this is largely due to the high heat used in the cook process that initiates the breakdown of starch into fermentable sugars. The high heat and residence time of cook significantly reduces the total viable population of contaminating microbes present on the starting materials. Fermentation of molasses or cane sugar where heat is not required might start with higher background contamination, which is a major consideration when fermenting those feedstocks. Once these contaminating microbes make it into fermentation, low starting populations of bacteria and wild yeast can grow and multiply to higher populations. 1×10^5 viable cells/ml and greater are what we consider to be significant levels of microbial contamination. Any residues left behind in the fermentation vessels, heat exchangers, or associated piping, can serve as a source of inoculum for the next batch. The higher the beginning populations, the greater the potential for microbes to reach problematic levels during fermentation. For this reason, the primary method for controlling microbial contamination involves intensive cleaning and sanitation. This means scrubbing the tanks with soap and water after each batch or using hot caustic (sodium hydroxide). Another reason to better understand which bacteria make up your “house bugs” is that some are more likely to produce biofilms (*Pediococcus* spp., for example). Presence of these more prolific biofilm producers may warrant additional, more intensive cleaning requirements. Some of these bacteria can be pathogenic to humans and animals, which is another reason for knowing what

kinds of contaminating microbes inhabit your distillery. On one hand, having a homofermentative *Lactobacillus* species that makes lactic acid (organic acids condense with alcohols to make esters) may be a positive flavor contributor (and the foundation of the sour mash process). On the other hand, if your distillery is highly contaminated with the same bacterium that causes diarrhea or pneumonia, that may lead to a different, maybe not so favorable, flavor profile (not to mention it is just gross). Apart from flavor contributions, high levels of microbial contamination can cause severe production issues and yield losses because these contaminating microbes compete with the yeast for carbohydrates and nutrients. Lactic acid bacteria are so-called because they ferment sugars like glucose and turn it into organic acids like lactic and acetic acid. These organic acids reduce the pH of the fermentation and can become toxic when they reach certain levels. This issue is compounded in sour mash recipes if the backset/stillage being recycled already contains elevated levels of these or other organic acids. Once contaminating microbes reach a certain level, one of the possible outcomes is a stuck fermentation. In this scenario the fermentation stops prematurely from overgrowth of contaminants, resulting in leftover sugar and lower ethanol levels. Table 1 shows what a normal fermentation looks like compared to one with significant bacterial contamination.

DIAGNOSING MICROBIAL CONTAMINATION ISSUES

Before a distiller can diagnose a production issue, it is necessary to know what a normal fermentation looks like so that any deviations can be compared to a “normal” scenario. There are several pieces of information collected throughout fermentation that are helpful, the first being pH. Since bacteria and wild yeast can produce organic acids as byproducts, when contamination becomes significant this results in a lower pH. Thus, if your

TABLE 1 High Performance Liquid Chromatography (HPLC) analysis from a successful fermentation (A) compared to one with significant bacterial contamination (B). High levels of organic acids (lower pH), incomplete sugar utilization and lower ethanol are consequences of significant microbial contamination.

LINE		DP4+	DP3	MALTOSE	GLUCOSE	LACTIC ACID	GLYCEROL	ACETIC ACID	ETHANOL
A	Normal	0.464	0.111	0.245	0.012	0.110	0.699	0.013	8.598
B	Bacteria	0.564	0.113	1.230	0.926	0.876	0.678	0.120	6.234

DP4+= glucose polymers 4 glucoses or larger. DP3= maltotriose (polymer with 3 glucose subunits).



(FIGURE 4) High populations of bacteria growing from mash plated on agar-based growth media.


average terminal pH is 4.2 and then it drops to 3.6 in another batch, this may be a sign of microbial contamination. Sugar utilization is another helpful determinant, which is measured by Brix, Balling, gravity, etc. If your normal terminal Brix is 6.5 and now you are seeing a terminal Brix of 8.7 (indicating leftover sugar), this is a good indicator of an issue. Since there are other issues that can lead to higher residual sugars (spoiled yeast, temperature issues, etc.), coupling a high sugar reading with lower than average pH might together help with the diagnosis (if the issue is temperature related, you would likely not have the lower pH, for example). The aroma of fermentation may also be an indication that there are issues with microbial contamination issues as bacteria and wild yeast secrete organic acids and other metabolic byproducts into the mash, some of which have sour or otherwise foul odors. Other byproducts of microbial contamination can be favorable, as discussed above.

Other more sophisticated analyses may be employed for diagnosing and detecting microbial contamination including analytical and microbiological methods. Plating the mash onto semi-solid agar media to visualize bacteria and wild yeast populations is one option (Figure 4). If yeast counts are performed using a microscope, bacteria can sometimes be seen along with the yeast, which is another thing to look at when gauging the severity of a contamination event. HPLC (High Performance Liquid Chromatography; see Table 1) is another technique that measures sugars, ethanol and organic acids from a single sample. Most larger distilleries have these capabilities, but smaller distilleries may have to rely on outside labs for these kinds of analyses. Once microbial contamination is confirmed, the first order of business is to seek out the deficiencies in cleaning, implement solutions and regain control.

One of the problems with culture-based diagnosis is that it takes 24-48 hours to perform. If you are sending the sample to another lab for testing, sample transit time is added to the delay. By this time the batch in question may have already been processed. However, our experience has been that once a distillery becomes highly contaminated, it is likely to continue that way until strategies have been implemented to eradicate the causative organisms (again, this is normally some improvement

to cleaning). So, even though the results may not be available in time to save the batch that was tested, the information can be used to implement strategies to correct the issue for benefit of future batches. Another issue with culture-based approaches is that different microbes often require different conditions to grow. For example, some bacteria (certain *Lactobacillus* species) found in distilleries require anaerobic conditions to grow whereas others like *Acetobacter* require aerobic conditions. Other factors like pH, temperature and nutritional makeup of the growth medium can also vary for one microbe to the next. For this reason, we are looking at molecular techniques to help us detail the distillery microbiome (what bacteria and yeast are present and their populations relative to one another). This involves extracting and/or amplifying DNA and in some cases messenger RNA directly from the mash. This genetic material is then sequenced and bio-informatics are employed to identify and quantify any bacteria or yeast, even if they are present in low populations or are non-viable. While this testing is thorough and can be faster than traditional culture-based methods, it requires expensive equipment and highly trained technical staff to run it. As these methods are refined, they will gain utility in the distilled spirits industry.

CLOSING REMARKS

It is clear from the information above that microbial contamination plays a significant role in overall distillery production and quality of the finished spirit. We encourage distillers to take a deeper look into their “house bugs” to see if microbial contamination is significant enough for yield loss or if there are any microbes of concern. While many distilleries, such as those representing well-known brands, make high quality distillate, there may be a way to improve production and yields. In cases where significant microbial contamination has been confirmed, fixing the problem can sometimes result in a 10-25% yield improvement. That can translate to a lot of extra bottles and is a great way to increase production when compared to the expense of a distillery expansion. Although we have only scraped the surface of the complexities of how microbial contamination can affect distilleries, hopefully the above information will be useful for evaluating contamination at your distillery. 

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