Isolating WILD Yeast Strains

By Mike Lentz
“Wild” and spontaneously fermented beers are growing in popularity among homebrewers. Most of these beers are fermented by either pitching pure cultures of commercially available unconventional yeast and bacteria, or spontaneous fermentation using “captured” mixtures of local microbes. Spontaneous fermentation is unpredictable and can vary geographically as well as seasonally within a local environment. A small but growing number of homebrewers are experimenting with this method, with a mix of reported successes and failures.

Most homebrewers desiring to experiment with the wild side of brewing usually choose to ferment with one of the commercially available strains of Brettanomyces yeast (Brett), either as the sole fermenter or as part of a secondary fermentation. Compared to standard ale and lager yeast, limited strains are available for wild ales. Most of these strains can be traced back to one of the traditional sour ale breweries of Brussels or Flanders, or strains isolated from British stock ale, with a few newer isolates cropping up from other breweries. All of these available isolates have likely adapted to life in a brewery environment and have evolved there over decades or longer. One may wonder if they are still truly “wild.” It could be argued that they are no more wild than traditional ale and lager yeast, but rather tamed or domesticated versions of a different animal.

Comparing commercial Brett to commercial ale or lager yeast is more like comparing a house cat to a pet dog, rather than comparing the dog to a wolf, or the cat to a lion. The observation that spontaneous fermentations can make good beer suggests that some truly “wild” yeast have positive fermentation attributes. Can pure strains of new yeast isolates make good beer?
Isolation and Identification of Strains

Working in a university biology department allowed me to put this question to the test. This AHA REF project aimed to determine the potential of pure strains of newly isolated wild yeast to successfully ferment new and unique beers. Yeasts with fermentation potential are known to inhabit the surface of a wide variety of fruits and grains. Samples of yeast were collected by swabbing fruit and inoculating a simple sterile growth medium, or by dropping berries or grain kernels directly into small liquid cultures. Not surprisingly, growth was evident in one to two days in most cases.

In the lab, these mixed cultures were plated onto solid agar culture plates to try to isolate individual strains. In some cases, samples yielded a single variety of microbe, but from most sources a variety of yeast and bacterial colonies were evident. Although each strain consistently produced the same type of colony, the colony appearance varied widely from strain to strain. Cells from single colonies were observed by microscopy to make tentative identification as yeast. Similar variation was observed in the cells themselves, and there was considerable variation for cell size and shape even within a particular strain. In all, 13 yeast strains were selected for further analysis, collected from pindo palm fruit, hackberry, blackberry, loquat fruit, and pale malted...
harley. In Table 1, the strains are named for the original source genus and species names, followed by an isolate number.

We wanted to know what yeast we were working with. A standardized method has been developed to identify many yeast isolates down to the species level using molecular genetic techniques. We amplified a region of each strain’s DNA using the Polymerase Chain Reaction (PCR). The amplified DNA can then be analyzed and compared to published yeast databases. With this method, we were able to identify all of our isolates to the species level. Interestingly, nearly half are members of the *Brettanomyces* genus, with three isolates each of *B. bruxellensis* and *B. anomalus*. Four isolates are members of the *Candida* genus, two are *Pichia kudriavzevii*, and one was identified as *Issatchenkia terricola*. All of the identified species are common in the environment, and all are frequently isolated from alcoholic fermentations, especially wine.

**Analysis of Brewing Potential**

In order to succeed as a brewing strain, yeast must exhibit some degree of alcohol tolerance. This property was analyzed by growing yeast in liquid microculture in the presence of increasing concentrations of ethanol. Alcohol tolerance was determined by observing growth in the cultures, which turned cloudy over two days as yeast cells accumulated. Considerable
variation occurred among the different isolates for growth in ethanol. All of the *Brettanomyces* strains grew in 12-percent ethanol, the highest level tested. Other isolates varied in tolerance from poor growth above 4 percent ethanol, to efficient growth at 10 to 12 percent.

Yeast that naturally reside on fruit have ready access to simple sugars as a food source. Beer wort is a diverse mixture of simple sugars, maltose, other complex sugars, and dextrins. Efficient attenuation of beer wort will be required if new strains are to function in a brewery environment. Yeast isolates were used to ferment three different worts of original gravity (OG) 1.040, 1.048, and 1.056. The first two worts were prepared from Briess light dry extract and were unhopped, while the 1.056 wort was from an all-grain light amber mash hopped at 60 minutes to around 30 IBU. For the first two worts, fermentation took place in 50-milliliter culture tubes, while the third wort was fermented in 500-milliliter bottles (see next section).

Fermentation was allowed to continue for four weeks, after which apparent attenuation was determined using a refractometer. Considerable variation was found in fermentation potential among the different strains. Surprisingly, there was even variation within a single isolate for attenuation of the three different worts. These variations did not correlate with the OG, source of wort (all-grain or extract), or use of hops. Some strains may have particular preferences for oxygen content, which could not reliably be kept consistent in this experiment. One interesting observation is that the most efficient fermenter, Bc01 (*C. incommunis*) is also the least tolerant of growth in ethanol. Since our test worts would yield an alcohol concentration just within the tolerance range for this strain, higher gravity worts would likely result in poorer attenuation. Several isolates consistently fermented to apparent attenuation of at least 70 percent, indicating potential as a brewing strain.

**Brewing Wild Yeast Beer**

Any given yeast could be both alcohol tolerant and fermentation efficient, but would not be of use to a brewer if the 

Eric Luman (Green Room Brewing head brewer) adding fresh-squeezed citrus juice and honey at knockout.

Transfer of 30 gallons of cooled wort from the main fermenter to the pilot wild yeast brew barge.

Pitching wild yeast starter.

Finished 5 gallon "starter" beer with wild yeast. (right)
resulting beer smells or tastes bad! Wild yeasts are well-known for producing many metabolic by-products, in particular phenolic compounds that may have undesirable aroma and flavor properties. To assess potential for beer brewing, strains were used to ferment 400-milliliter test batches of the OG 1.056 wort described in the previous section. Fermentation was allowed to proceed for four weeks, after which the beer was bottled, carbonated, and chilled.

Beer characteristics were assessed by a panel of BJCP judges. Not surprisingly, many strains exhibited strong phenolic (plastic, medicinal, and burnt/smoky) characters. The strains that were shown to be poor fermenters exhibited the expected sweet, underattenuated worty character, in some cases with underlying fruity notes. But several of the strains produced beer with the complex, spicy, fruity, or “funky” character desired in wild ales. Due to the subjective nature of this analysis, the wild beers were categorized as having low, medium, or high potential as a brewing strain. The “low performing” group was dominated by undesirable characters in wild ales. Due to the subjective nature of this analysis, the wild beers were categorized as having low, medium, or high potential as a brewing strain. The “low performing” group was dominated by undesirable characters in wild ales.

### Table 1: Sources of yeast isolates, their identification based on rDNA RFLP analysis, and brewing potential. Yeast strains are identified by the name of the source plant.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Source</th>
<th>Identification</th>
<th>Beer Potential</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bc01</td>
<td>Pindo palm</td>
<td>Candida incommunisa</td>
<td>Low</td>
</tr>
<tr>
<td>Bc02</td>
<td>Pindo palm</td>
<td>Brettanomyces bruxellensi</td>
<td>Med</td>
</tr>
<tr>
<td>Bc04</td>
<td>Pindo palm</td>
<td>Issatchenka terricola</td>
<td>Low</td>
</tr>
<tr>
<td>Bc07</td>
<td>Pindo palm</td>
<td>B. bruxellensi</td>
<td>Low</td>
</tr>
<tr>
<td>Bc08</td>
<td>Pindo palm</td>
<td>C. diversa</td>
<td>Low</td>
</tr>
<tr>
<td>Bc10</td>
<td>Pindo palm</td>
<td>C. diversa</td>
<td>Low</td>
</tr>
<tr>
<td>Bc11</td>
<td>Pindo palm</td>
<td>B. bruxellensi</td>
<td>Med</td>
</tr>
<tr>
<td>Rs01</td>
<td>Blackberry</td>
<td>B. anomalusb</td>
<td>High</td>
</tr>
<tr>
<td>Cs01</td>
<td>Hackberry</td>
<td>B. anomalus</td>
<td>High</td>
</tr>
<tr>
<td>Hv01</td>
<td>Barley malt</td>
<td>Pichia kudriavezevi</td>
<td>Low</td>
</tr>
<tr>
<td>Hv01</td>
<td>Barley malt</td>
<td>P. kudriavezevi</td>
<td>Low</td>
</tr>
<tr>
<td>Ej01</td>
<td>Loquat</td>
<td>Candida sp. (tentative)</td>
<td>Med</td>
</tr>
<tr>
<td>Ej02</td>
<td>Loquat</td>
<td>B. anomalusb</td>
<td>High</td>
</tr>
</tbody>
</table>

| c This study, by sequence analysis. |

Anomalus strains. The “beer potential” category is included in Table 1.

### Scaling Up

With proper resources, it’s easy to isolate new strains of yeast from the environment. Based on the small sample size represented here, a small but significant portion of isolates are likely to be useful and interesting to the commercial or home brewer. It was somewhat surprising how frequently Brettanomyces strains were isolated from diverse environments. It is also of interest that all of the Brett isolates, none of the B. bruxellensis isolates produced “drinkable” beer, while all of the B. anomalus isolates could be used for brewing. A larger sample size may yield different results. Strains may also perform differently as secondary fermenters, or under different conditions of temperature or oxygen content. Further experiments will be needed to assess these different conditions.

Half-gallon batches of beer were brewed with the three B. anomalus isolates, which also served as starter cultures for five-gallon brews. The half-gallon brews were bottled and carbonated, then evaluated by the owners/brewers of Green Room Brewing in Jacksonville Beach, Fla. Based on this analysis, we brewed a “Florida saison” with orange blossom honey, Florida oranges, tangerines, and tangelos, and Florida-grown lemongrass. A 30-gallon portion of this brew was fermented with the B. anomalus yeast isolated from Florida loquat fruit (strain Ej02).

Florida Brett Saison was a popular beer in the taproom. In a final “step-up,” the yeast from this batch was used to ferment a full seven-barrel pale ale at Green Room Brewing. Eric Luman, Green Room’s head brewer, plans to keep the Florida Brett strain in regular rotation at the brewery.

Mike Lentz is a member and past president of the Cowford Ale Sharing Klub (CASK) of Jacksonville, Fla. He is also a BJCP Exam Director and Grand Master I beer judge. He lives in Jacksonville, Fla.