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Capturing Terroir: Isolation and Characterization of Local Wild Yeast

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ABSTRACT

Spontaneous fermentation is dependent upon the yeasts available in the local environment. In fact for generations, brewers, bakers and vintners relied on the ferments produced by microbes present on the grains/fruits, in the facilities and floating in the air. These wild yeasts can produce desirable ferments but often travel in the company of spoilage bacteria and molds. Due to the variability in using wild yeast, most production breweries and distilleries use commercially available yeast during fermentation to ensure production quality and consistency - however, we know that nuance in bouquet and flavor often come from terroir and the unexpected in fermentation. As the second-largest apple-producing state in the U.S., New York has no shortage of family orchards, some of which have turned to expanding their markets through the production of apple-based alcohols. We teamed up with orchard distilleries to collect wild yeast from the fruits during apple harvest season. Initial research isolated five potentially viable strains. The wild yeast strains were identified and then characterized for their glucose tolerance and consumption, ethanol tolerance and production, propagation potential and viability. These strains were then tested further in small-batch, beer-brewing trials.

INTRODUCTION

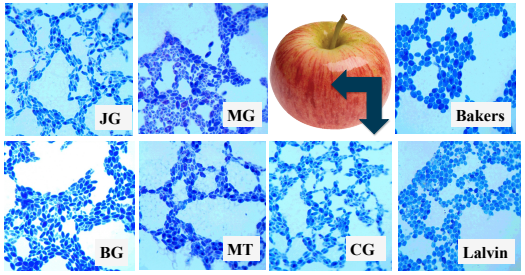
Today, many consumers are participating in the eco-conscious, farm-to-table movement by purchasing foods grown and produced within a limited radius of where they live. The goal being to preserve the environment and support the local economy by limiting foods mass produced through the global food system. Driven by these principles, many local businesses are aiming to sell products to consumers that are strictly sourced/produced locally. A specific example of this can be seen with the 2007 New York State Farm Distillery Law which authorized the manufacturing of liquor from farm and food products produced on site. The NYS ABC Law 61 has not only strengthened bonds between NYS farmers and distilleries, but has also led to direct economic growth. Indeed, of the 82 operating distilleries in NYS, 69 have opened in the last five years based on the issued date of the original distillery license[1]. One such business, Harvest Spirits Farm Distillery in Valatie, NY, creates unique liquors from their apple and fruit harvest. The distillery currently uses a commercially available yeast strain, Lalvin *Saccharomyces cerevisiae* K1-V1116, but desired to also create a distilled spirit using yeast obtained directly from their apple orchard ("wild yeast").

Ripe apples have been shown to have less than 500 yeast-like organisms per gram of sound fruit. The main organisms that can be found are: *Aureobasidium pullulans*, *Rhodotorula* spp., *Torulopsis*, *Candida*, *Metschnikowia*, and *Kloeckera apiculata*; *Saccharomyces* species and other sporulating yeasts are rarely found[2,3]. In addition to yeast, acid-tolerant bacteria are usually present, but lactic-acid bacteria are relatively rare. Not surprisingly, the quantity of microorganisms increases if the fruit is allowed to fall naturally or suffers damage to the skin. Interestingly, processing also increases yeast counts due to the indigenous flora of the factory and methods employed (*e.g.* a traditional rack and cloth apple press have been found to be a major source of microbial contamination)[2]. This study focuses on the characterization of five wild yeast strains, isolated from the orchard described above, and compares them to two commercially-available yeasts.

ISOLATION and IDENTIFICATION

Apple samples were collected from the Golden Harvest Orchard in Valatie, New York (Fall harvest). Five different types of apples were collected: Braeburn(B), Fuji(F), Macoun(M), Cortland(C), and Jona Gold(J). Two apples of each type were collected, one from the ground(G) and the other from the apple tree(T). Once collected, the apples were brought back to the laboratory for processing using a previously described protocol[4]. Briefly, apples were diced up and placed in Yeast Peptone Dextrose (YPD) broth containing 10 mg/ml of lysozyme solution (to selectively remove bacteria). After ten minutes, the apple/YPD/lysozyme mixture was centrifuged and the supernatant was plated on Yeast Extract Glucose Chloramphenicol Agar (YGC) before examination of colony morphology. After processing, 5 experimental yeast strains were selected for further analysis. Colony and cellular morphology of each revealed "yeast-like" samples[5]. Pure cultures from each of the individual colonies (subcultured on YGC agar plates) were run through the VITEK® automated system using the YST ID Card for identification based on genus and species (46 different biochemical tests)[6]. VITEK® results determined multiple wild-yeast samples.

Figure 1: Cellular morphology. *Cell morphology of all seven yeast strains (5 wild yeasts and 2 controls) stained with Methylene Blue and observed at 1000x magnification.*



Apple Collection Designation	Yeast Identification
Braeburn -ground (BG)	*Candida glabrata, Candida lipolytica*
Macoun -ground (MG)	*Candida lipolytica*
Macoun -tree (MT)	*Candida colliculosa, Saccharomyces cerevisiae*
Cortland –ground (CG)	*Candida lipolytica, Protheca wickerhamii*
Jona Gold –ground (JG)	*Candida sp. (krusei, lambica and inconspicua)*

Table 1: Yeast Identification with VITEK YST ID. Initial identification of each of the isolated wild yeast strains isolated from different apples, found in different locations.

CHARACTERIZATION

Figure 2: Yeast Fermentation Comparison Over 5 Days. *The average rate of three sensors for (A) Ethanol production, (B) CO_2 production and (C) Oxygen consumption.*

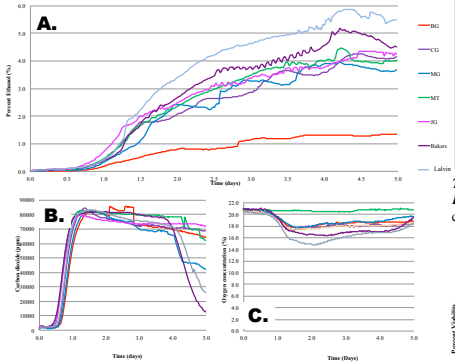
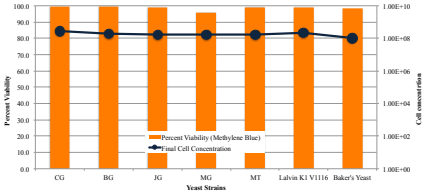


Table 2: Glucose Consumption and Ethanol Production after 5 day Fermentation Trials. Both the wild yeast strains and commercial strains consumed glucose and produced ethanol at similar levels between the start (day 0) and end (day 5) of fermentation trials.

	MT	JG	MG	CG	BG	Lalvin	Bakers
Average glucose mg/dL (% change over initial glucose reading of 3200 mg/dL)	234 (-93%)	126 (-96%)	108 (-97%)	128 (-96%)	110 (-97%)	124 (-96%)	51 (-98%)
Average EtOH by GC (top: using 4.5% standard) (bottom: using 6.0% standard)	4.3 (4.5)	4.5 (4.7)	4.2 (4.4)	4.0 (4.3)	4.4 (4.7)	5.2 (5.5)	5.7 (6.0)

Fermentation Studies: To examine the fermentation ability, the PASCO ME-6667 EcoChamber system was used. Fermentation potential of each yeast was done multiple times (n=6) and in varying medias (0.5M sucrose solution, YPD broth and Mott's original 100% apple juice). To do this, overnight cultures were made and cell counts performed by hemocytometer to ensure the total yeast cell concentration in each chamber would be the same (1.4 x10^8 yeast/chamber). EcoChambers were stirred continuously and monitored every 30 minutes using PASCO sensors and software. The rate of ethanol and CO_2 production, as well as the rate of O_2 consumption, was measured for a total of 5 days (120 hours). Before each run, each of the sensors was calibrated using standard PASCO protocols. Following the 5 day fermentation period, samples were taken from each trial for further analysis for total cell number, viability, final glucose concentration and ethanol concentration by gas chromatography.

Table 3: Total Cell Concentration and Viability After 5 day Fermentation. Percent viability ranged between 95.9-99.6 (bars) and cell count (circles) ranged between 1.06-2.94x10^8 by day 5.



CONCLUSIONS

The wild yeast strains isolated from the orchard showed similar cell morphology, EtOH and CO_2 production, as well as O_2 and glucose consumption as compared to the two commercial control strains over a 5-day fermentation. Additionally viability and total cell counts on all yeast were similar. Based on these results, we began brewing studies with the isolated yeast (SEE POSTER 188).

REFERENCES

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