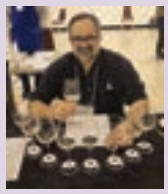
TRIALS & TROUBLESHOOTS

Terroir and Transparency Through Autochthonous Yeasts

Trinchero Family Estates and Bravium Play with Indigenous Yeasts as Inoculum

Bryan Avila



Meet the Author: This forum discusses how growers, vintners and experts use science and a systemic approach to innovate and overcome challenges in grape growing, winemaking and reducing their environmental impact through applied research. Bryan Avila is an experienced winemaker, educator, industry consultant and workforce advocate. Contact **Bryan Avila** at *bavila@santarosa.edu*.

PROGRAM LEAD: Derek Rohlffs, Bravium founder and winegrower, Trinchero Family Estates

Winegrower Derek Rohlffs discovered his calling after a life-changing tasting with Napa legend Bob Travers where he grasped the deep connection between land and wine. Of Cherokee descent, Derek has always felt drawn to nature, earning a degree in environmental studies from UC Santa Barbara before joining the wine industry. Rohlffs founded Bravium in 2007, crafting Pinot Noir and Chardonnay that express pure terroir with minimal intervention. His critically-acclaimed, neo-classical wines are a staple in many of the best restaurants in the U.S. and reflect a reverence for nature and a traditional approach rooted in his heritage and shaped by a belief that each bottle should tell a story of time and place.

BACKGROUND:

Dr. David Mills, a microbiology professor at UC Davis' Viticulture & Enology (UCD V&E) program, first reported the impact of soil microbes, which formally connected the effects of indigenous microflora on terroir[1]. This milestone was important because it provided concrete evidence that soil microbes also contribute to a wine's sense of place—more so than just grape variety, heat summation or weather events.

Traditional winegrowers understand that native yeasts can play a significant role in building complexity in fine wines; however, even winemakers who've mastered the art of native fermentation will admit that not inoculating with a commercial yeast strain comes with some risk. Indigenous microbial populations naturally fluctuate with changes in weather, soil, moisture levels and biodiversity from vintage to vintage. While it's not a problem for nature, it can present major challenges in wine quality and operational efficiencies, which can lead to heavy financial losses and insomnia for winemakers.

Unfounded or not, the use of commercial wine yeast can sometimes elicit a bad rap, which has inspired a flurry of movements from "clean" wine to natural wines, further baffling consumers and begging the question, "What's really in my wine?"

The Davis-based consulting company, Ferminkasi, is helping winegrowers capture an enhanced sense of place by enabling them to create their own inoculums from native yeasts, thereby eliminating the "commercial" association. Co-founded by Lucy Joseph and Vidhya Ramakrishnan—the microbiologists that curated the UC Davis V&E Department's Culture Collection—Ferminkasi is now assisting a handful of these producers.

Napa Valley winery Opus One was one of the early wineries to experiment with this technology, and it was described in a *WineBusiness Monthly* article published Oct. 2024. At the time, it seemed like a pie-in-the-sky idea that only a well-funded winery could pursue.

Independently of what was happening at Opus One, Derek Rohlffs had already begun exploring the microflora of his own vineyard. Fully understanding the risks of scaling uninoculated fermentations and wanting to develop his own inoculums, he eventually ran into Trinchero Family Estates, which had similar interests. A partnership was born.

Soon thereafter, Aimee Baker joined Trinchero Family Estates as its director of luxury winemaking. Aimee, who had previously worked at Opus One, partnered with Derek to formalize his trials.

"While at Opus One, I had the opportunity to see one of the first native yeast isolation projects in the Napa Valley; and when I met Derek, I was happy that I'd get to continue down this path. Not only do yeasts isolated from our own vineyards better showcase terroir, these yeasts also often perform better during fermentation," Baker said. "I knew that the process is scalable and could be set up to serve the seasonal nature of winemaking when all the fruit comes in within three months. I see this as a winning proposition for wine quality, sustainability and the bottom line of the winery."

Once these yeasts are isolated in cell banks, when needed, they are propagated from slant to inoculum with a seed train methodology that requires process equipment, like the Vivelys Ecolys or the LEV 2050 system, now distributed by VA Filtration.

In 2021, Derek and Aimee asked Ferminkasi to lead the microbiological sampling effort at Wiley Vineyard, and they began to screen out the duds. With Ferminkasi's help, they identified what they thought were between 10 and 15 different strains of yeast by doing small-lot fermentations. Several of these yeasts were eliminated because they did not finish fermenting or were just too funky. By the time the 2022 vintage came, they had narrowed down their yeast selections to five. From 2022 and 2023, these five *Saccharomyces cerevisiae* yeasts and a non-*Saccharomyces* strain, *Torulaspora delbrueckii* (TD), were prepared for the trials highlighted in this article.

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TRIAL DESCRIPTION:

In a two-part process, field sampling and isolation (Phase 1) included collection of samples from multiple sources and a multitude of locations across different vineyard blocks. Then these isolates were identified and screened (Phase 2) prior to undergoing DNA sequencing to be confirmed by Ferminkasi as unique strains.

Following intensive screening, five native strains advanced to the 2022 and 2023 trials to demonstrate practical viability at the bucket fermentation stage. To pass this bar, fermentations needed to achieve dryness (<0.25% residual sugar), complete fermentation within a reasonable time span versus commercial yeast control and demonstrate consistent performance across multiple trials.

The 2022 and 2023 vintage trials set out to identify the yeast isolate that would merit the next stage of development and, ultimately, become the primary Bravium inoculum(s). Candidates for these trials and their sensory attributes are described below next to their identification code:

TRIAL I:

- W8n: (2022 only) Floral profile (eliminated due to sulfur production in 2023 trials)
- W9n: Pear/apple blossoms with mild acetic acid (eliminated due to sulfur production)
- W10n: Apple/pear profile, robust fermentation characteristics
- W11n: Caramel/candied apple, tendency to form clumps
- W12n: Pear characteristics, consistent performance
- FWY160 (2023 only): Non-*Saccharomyces*: *Torulaspora delbrueckii* (used in conjunction with a *Saccharomyces* strain)
- CONTROL/Commercial Strain EC1118

TRIAL II:

Chardonnay

- TREATMENT: TD23 + w10n
- CONTROL: TD23 + EC 1118

Pinot Noir

- TREATMENT: TD23 + w10
- CONTROL: TD23 + EC 1118

CONTROL: COMMERCIAL YEAST STRAIN EC 1118

Using brewing industry cell banking and propagation technology, each of the *Saccharomyces* strains was prepared to be evaluated versus the commercial strain for the speed at which they complete the fermentation, their flavor profile, presence of off-aromas, and cell viability and morphology using a Muse cell analyzer. All fermentations within the same variety were prepared using the same juice with a nutrient baseline of 200 mg/L Yeast Assimilable Nitrogen (YAN) to minimize variability for comparison.

TD23, a non-*Saccharomyces* strain, does not have the capability to complete the fermentation on its own, so it was added sequentially with the EC 1118 commercial strain, as well as the highest ranked native isolate W10n.

CONCLUSIONS:

Saccharomyces Results

For the sake of brevity, since the 2022 results were similar, only the 2023 chart is shown here. According to Derek, the following three key pieces of data that demonstrated their success are as follows:

- All five native *Saccharomyces* strains achieved dryness with less than 0.25% residual sugar, and a number of these strains produced less ethanol than the control yeast.
- While slower than EC 1118, all strains completed fermentation within three days of the commercial control.
- Each strain produced unique sensory characteristics without any major defective aromas.

Below, **FIGURE 1** shows the 2023 fermentation curve in terms of grams of carbon dioxide (CO_2) weight loss from the fermenters. It shows that the native fermentation kinetics lag slightly yet are closely matched to the commercial control strain.

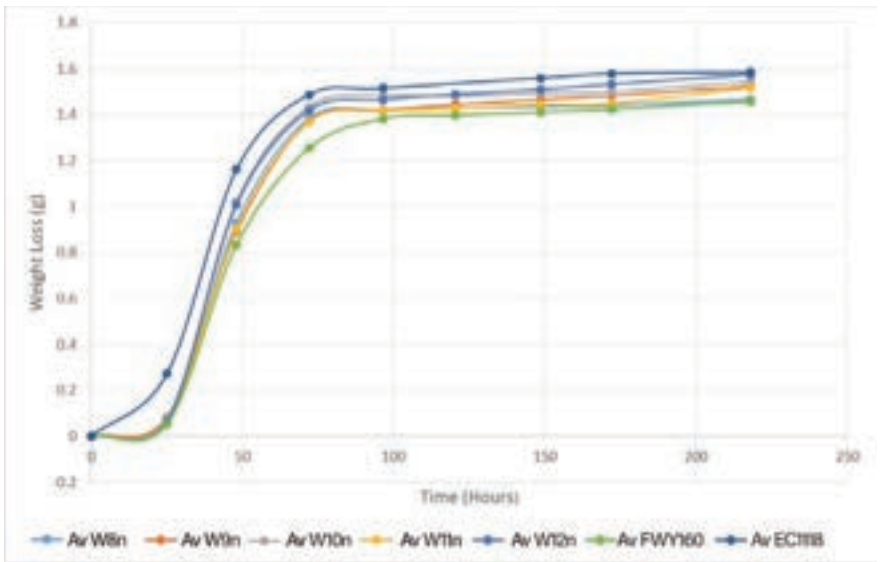

FIGURE 1 2023 study with improved methodology (duplicates, 22° Brix, pH 3.5, YAN 200 mg/L). Note tight fermentation curves and inclusion of FWY160.

Non-Saccharomyces Results.

The non-*Saccharomyces* trial was relatively straightforward. It fermented Chardonnay and Pinot Noir lots by combining the TD23 non-Saccharomyces yeast, known for its bioprotective qualities during the initial fermentation stage, with the preferred native isolate W10n and the commercial control strain EC 1118.

The Bravium study selected three native strains—W10n, W11n and TD23—for 2024 commercial-scale validation, which was conducted on 500-gallon

FIGURE 2 Chardonnay: TD23 + W10n

FIGURE 3 Chardonnay: TD23 + EC 1118

FIGURE 4 Pinot Noir: TD23 + W10n

FIGURE 5 Pinot Noir: TD23 + EC 1118

lots. During the 2024 trials, isolate W10n was confirmed as highest ranked, along with TD23, thereby providing Bravium with both native *Saccharomyces* and non-*Saccharomyces* strains for their minimalist, terroir-based fermentations going forward.

Post-Mort Q&A with Derek Rohlffs

What was the motivation to experiment with autochthonous yeasts over commercially-available Active Dry Yeasts (ADY)?

Rohlffs: These microscopic fungi colonize grape skins, vineyard flora and cellar environments, creating a unique microbial terroir that's as distinctive to a place as are soil and climate. After years of making wine from grapes that I grow in Anderson Valley and Russian River Valley vineyards in multiple wineries, I noticed that spontaneous fermentations produced wines with more complex aromatics and a stronger sense of place than those made with commercial yeasts. I wanted to capture and harness the positive attributes of our native yeast populations and maintain the consistency needed for commercial production while also being sure that the yeast doing the heavy lifting were from the vineyards themselves rather than endemic to the wineries.

When I was trialing native versus commercial yeasts 15 years ago, I found a Lallemand non-*Saccharomyces* product called Biodiva, which was a packaged *Torulaspora delbrueckii* strain, and I liked the results. It staved off the bad bacteria in the early phase of fermentation so that my native ferments could get started cleanly. I was inspired and explored it deeper to craft more terroir-driven wines, leading me to also seek to capture my own non-*Saccharomyces* yeasts from the vineyards.

How did you design your experiment?

Rohlffs: We designed a comprehensive, multi-phase approach to yeast capture, isolation and characterization. Our methodology evolved to sophisticated strain evaluation:

Phase 1: Field Sampling and Isolation - We collected samples from multiple sources: Damaged grape clusters from shaded areas across different vineyard blocks, locations near water bodies and active native fermentations at early to mid-points. After our initial berry sampling yielded no *Saccharomyces*, our partners at Ferminkasi recommended spring sampling from vineyard wildflowers and trees as they've had success finding *Saccharomyces* during that season in vineyard environments. Samples were enriched in sterile grape juice for up to two weeks to select for fermentative microbes and plated on selective WLN media after one week and again after two weeks.

Phase 2: Identification and Initial Screening - All isolates underwent 26s rDNA sequencing and were confirmed by Ferminkasi as unique strains never before identified. From our initial sampling in fall 2021, we made a surprising discovery: berry samples yielded only non-*Saccharomyces* species (*Torulaspora Delbrueckii*, *Metschnikowia pulcherrima*, *Hanseniaspora uvarum* and *Lachancea thermotolerans*) while samples collected from surrounding flowers, fruit and perennial trees, as well as our native Pinot Noir fermentations, contained five distinct *Saccharomyces* strains. We delayed fingerprinting to determine if strains were native, hybrid or escaped commercial yeasts until after sensory evaluation as this expensive testing was only worthwhile for promising strains.

Phase 3: Comprehensive Characterization - For our *Saccharomyces* strains, we evaluated them for their fermentation characteristics:

- Small-scale fermentation trials (five-gallon buckets) using our own juice
- Fermentation kinetics (CO_2 weight loss over time)
- Sugar utilization (glucose vs. fructose preference)
- Nitrogen requirements (tested at 200 mg/L YAN)
- Temperature tolerance (20-30°C range)
- Alcohol tolerance (22° Brix fermentations)
- SO_2 tolerance (0-100 ppm)
- Trialed versus control yeast, EC 1118, a dominant and clean-fermenting yeast
- Sensory profiles through tasting panels with Ferminkasi and winery team

Who else worked with you on this trial? What were your initial hypotheses?

Rohlffs: My colleague Aimee Baker, director of luxury winemaking at Trinchero Family Estates, was instrumental in supporting this research initiative. She had experience collaborating with Ferminkasi and shed light on the use of this technology.

Lucy Joseph and Dr. Vidhya Ramakrishnan from Ferminkasi brought crucial expertise in microbial isolation and identification. They specialize in fermentation solutions, making them ideal partners for this project.

Our initial hypotheses were:

- Native yeasts from different vineyard locations would show distinct strain diversity.
- Berry samples would contain Saccharomyces that could be isolated for winemaking.
- Native strains would complete fermentations but at slower rates than commercial yeasts, and some native strains would have lower alcohol conversion rates.
- Each strain would produce unique aromatic profiles, reflecting their origin.
- We could identify strains suitable for commercial-scale production.

Did the results present themselves as predicted?

Rohlffs: The results were both confirming and surprising. Our most unexpected finding, in November 2021, was that none of the berry samples contained *Saccharomyces cerevisiae* and rather only non-*Saccharomyces* species. However, samples collected from trees and flowers on the vineyard property, as well as native fermentations, yielded five distinct *Saccharomyces* strains and, eventually, also a *Torulaspora delbrueckii* strain, suggesting these yeasts are present in vineyard ecosystems and dominate once fermentation begins, despite not being captured on berries earlier in the process.

Each strain that we found was comprehensively evaluated with the following results:

- Complete fermentation by all strains: All five native *Saccharomyces* strains (W8n, W9n, W10n, W11n and W12n) achieved dryness with less than 0.25% residual sugar, proving their commercial viability, and a number of these strains produced less ethanol than the control yeast.
- Manageable fermentation kinetics: While slower than EC 1118, all strains completed fermentation within three days of the commercial control—an acceptable timeframe for production.
- Distinct aromatic profiles without defects: Each strain produced unique sensory characteristics.

Did you experience any difficulties during the trial?

Rohlffs: We encountered several interesting challenges that provided valuable learning opportunities:

- Absence of *Saccharomyces* on berries: Despite sampling nine different locations, we found only non-*Saccharomyces* species on the grape berries. This challenged our assumptions about where to find fermentation-capable yeasts. We addressed this by focusing on surrounding flora and isolating strains from active native fermentations.
- Strain-specific behaviors: W11n showed a tendency to form clumps and stick to vessel sides, which could affect fermentation monitoring

and racking. We adapted our protocols to account for this behavior in future trials.

- Sulfur production in some strains: Our 2023 follow-up characterization revealed that W8n and W9n produced slight sulfur notes, making them unsuitable for commercial production. This led us to narrow our selection, though we expanded testing to include new isolates (ETS0943, ETS0944) and a *Torulaspora delbrueckii* strain.
- Shipping and handling logistics: During one critical shipment of yeast cultures for trials, over half arrived damaged with significant volume loss. We problem-solved with Ferminkasi to rebuild cell populations. By October 2023, we had refined protocols: cultures shipped on ice via UPS, stored refrigerated until use, temperature-acclimated for one hour before inoculation and carefully opened to release CO_2 pressure.
- Coordination complexity: Managing over 10 different inoculations (each strain in duplicate) required careful coordination among team members Maria Cortez, Jourdan Carter, Franco Fresentese Batiz, Justin Butler and Rodrigo Leytes, plus detailed labeling systems.
- Discovery of new strains: Our continued sampling yielded additional candidates, including coded strains ETS0943 and ETS0944, plus our first *Torulaspora delbrueckii* isolate, showing the importance of persistent exploration.

Did you make trial wines? What was your impression of them?

Rohlffs: You never know exactly what you're going to get when you isolate microbes from the wild and use them to ferment in a completely different habitat. Between 2022 and 2023, we tested many different isolates versus the EC 1118 control. Each year, the winemaking team blind-tasted the finished trial wines, and paired with our fermentation data, the sensory trials led us to select the W10n *Saccharomyces* yeast. Our 2024 commercial trials demonstrated that W10n produced rose petal, red berry aromas and cherry cola flavors with great structure and persistence in our Pinot Noir wines. The TD23 + EC 1118 strain expressed herbal, sanguine, sous bois and berry patch aromas with prototypical fruit flavors. For the Chardonnay trials, w10n was again the preferred *Saccharomyces* strain. It showed apple and floral aromas with tree and tropical fruit flavors. The TD23 + EC 1118 expressed candied apple and pear with a generous mid-palate followed by a saline/mineral finish. (**FIGURES 2, 3, 4 & 5** sensory word clouds.)

What was the most important outcome?

Rohlffs: The most important takeaway is that capturing and characterizing site-specific yeasts is both scientifically achievable and economically viable with clear cost structures for implementation. Our work demonstrates that an approximately $5,000 to $10,000 initial lab and operational investment, followed by $250 per year per strain for maintenance, allows a winery to develop a proprietary yeast bank that delivers the complexity of native fermentation with the reliability of inoculated fermentation.

Key practical insights for winemakers:

- Budget realistically: Initial trials cost ~$1,750 for testing 4-5 strains; annual storage is $250 per strain; inoculum preparation runs $100 per strain.
- Sample strategically: Focus on flora surrounding vineyards and active native fermentations for *Saccharomyces*; consider spring sampling from flowers and trees if fall berry sampling fails.
- Stage your investment: Delay expensive fingerprinting until after sensory trials—only characterize strains you actually want to use.
- Start with small-scale trials: Test in five-gallon food-grade buckets before scaling up; use your own juice for relevant results.
- Plan for production scaling: Ferminkasi partners with Imperial Yeast in Portland for larger volume propagation (10L+ of high-density cells).

- Consider non-*Saccharomyces* options: We isolated a *Torulaspora delbruckii* strain which offers bioprotection, weightless concentration and aromatic complexity to the finished wines.
- Maintain frozen stocks: Long-term storage ensures strain preservation across vintages with annual viability checks.
- Allow 10-day flexibility: Prepared inoculum remains viable refrigerated for 10 days, providing harvest timing flexibility.
- This approach bridges traditional and modern winemaking—using scientific tools to capture, identify and harness what nature provides, creating wines that are both authentic to their origins and consistently excellent.

Do you plan to conduct a follow-up trial to re-test these results? What would be the focus?

Rohlffs: We're actively continuing this research with several focused objectives. Our October 2023 Pinot Noir and Chardonnay bucket trials demonstrated our commitment to systematic evaluation across varieties. After successful small-scale trials, we continued to test our four finalist *Saccharomyces* strains, comparing them against *Torulaspora Delbrueckii* yeast. We are also capturing yeast this year in our estate Plumis Vineyard, a 90-acre Chardonnay planting on the northern banks of the Russian River near Forestville.

Future research priorities include:

1. Cross-variety performance: Evaluating how our strains perform across our full portfolio—we've proven success in Pinot Noir and are continuing our testing in Chardonnay.
2. Production scale-up: Moving from five-gallon bucket trials to larger ferments (500- to 11,000-gallon lots) while maintaining strain purity and performance.
3. Multi-vintage strain stability: Testing whether our isolated strains maintain their fermentation characteristics and sensory profiles across different vintages and varying harvest conditions.
4. Temperature optimization: Our trials at 55°F set temps showed promise; we're exploring optimal temperature ranges for each strain.
5. Advanced characterization of selected strains: Following the latest trials, we will be conducting detailed studies on nutritional requirements, stress tolerance and metabolite production.
6. Blending trials: Investigating co-inoculation strategies using multiple native strains to increase complexity while maintaining fermentation security.
7. Vineyard ecology mapping: Expanding sampling to include spring collection from flowers and trees as recommended by Ferminkasi.
8. Climate adaptation studies: As conditions change, monitoring how our native yeast populations evolve and whether new strains emerge.

Our indigenous yeast program has evolved from experimental research to large-scale commercial implementation, with 2025 representing a transformative production milestone. Bravium will inoculate 20,034 gallons across two varietals—Pinot Noir and Chardonnay—using the proven W10n strain while advancing TD23 development through targeted characterization.

What do you think/hope is the future of this practice?

Rohlffs: I envision a future where the debate about "terroir yeasts" evolves beyond semantic arguments about *Saccharomyces* prevalence in vineyards into practical approaches and applications.

The future I see includes:

1. Regional yeast banks: Wineries maintaining libraries of their successful native strains, preserving microbial diversity as carefully as we preserve clonal selections.
2. Scientific collaboration: Bridging the gap between skepticism and enthusiasm by focusing on measurable outcomes rather than theoretical debates.
3. Climate adaptation: As conditions change, understanding our microbial partners becomes crucial; native yeasts that have adapted to specific sites may offer resilience.
4. Democratization of technology: As costs decrease and knowledge spreads, more small producers can access these techniques.
5. Integration with sustainable practices: Native yeasts as part of a holistic approach to terroir expression and minimal intervention winemaking.

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REFERENCES

1. PNAS (2013) – Microbial biogeography of wine grapes is conditioned by cultivar, vintage, and climate (Bokulich, Thorngate, Richardson & Mills).