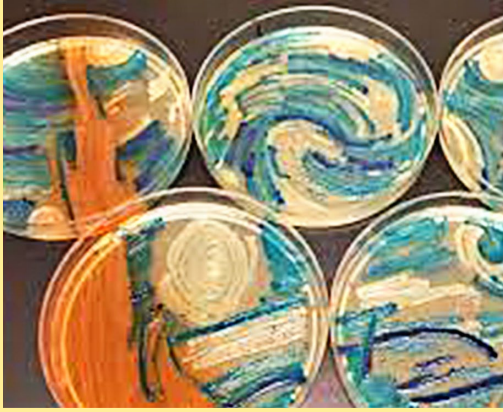
BAKER WINE & GRAPE ANALYSIS

MICROBIOLOGY



Baker Wine & Grape Analysis Newsletter — Microbiology

From vineyard to winery, and even in bottle, microbes are influencing wine. Welcome to BWGA's Microbiology issue where you can learn about the good, the bad, and of course, the chemistry.

Chemistry Influences Microbes

The physical parameters of wine, beer and spirits are instrumental in determining the risk factors for microbial growth. For example, most spoilage microbes do not thrive in environments with high alcohol, low pH and adequate molecular FSO_2. The conversion of malic acid to lactic acid influences both the sensory aspects as well as the stability. Without yeast we wouldn't have these enjoyable beverages! Microbes can both negatively and positively impact the aromatics and mouthfeel and are an important aspect of the nuances and complexities we experience while tasting.

Fast Brett Analysis

Brettanomyces bruxellensis (Brett) is a hardy yeast species that produces compounds with strong odors: band-aid, barnyard and metallic are a few descriptors. Too much of these strong odors (4-EP and 4-EG) are considered unpleasant. Brett can withstand high levels of Free SO_2 and resists sanitation procedures, especially in wood. Plus, it can feed on other nutrients, even if there is no sugar left in the wine.

FUN FACT

"Ferment" comes from the Latin "fervere", to boil, a pretty good description from ancient winemakers observing the process of juice becoming wine.

One Brett cell can multiply into millions, but knowing if you have a Brett infection early on gives you time to mitigate potential problems. BWGA uses a semi-quantitative PCR technology assay to detect Brett DNA with results usually back same day as drop off.

Some important times to test your wine for Brett:

- Before racking wines together to make blends
- Before topping barrels; start with clean, Brett-free topping wine to prevent spreading
- Before purchasing new barrels test older barrels and cull infected barrels
- Before bottling, especially if you plan to bottle unfiltered

***Important note regarding sampling for Brett: Thoroughly mix/stir wines before pulling samples or pull from the bottom of the vessel. Brett can sink to the bottom of storage vessels and potentially lead to inaccurate results in wines that are not thoroughly homogenized.*

Bottle Sterility

What's growing in your sterile-filtered bottled wine? Hopefully nothing. 300 mL of a bottled wine can be cultured to detect yeast, bacteria or mold — thus ensuring that your bottling has been clean. Bottle sterility is a simple and cost-effective way to verify a successful sterile filtration. Suggested times to pull a bottle sample off the line for testing are:

- From the first case bottled
- After a work break or if the bottling line stops for over 30 minutes for repairs/adjustments
- From the final case bottled

Keep in mind that results will take one week to allow for colony growth to present itself.

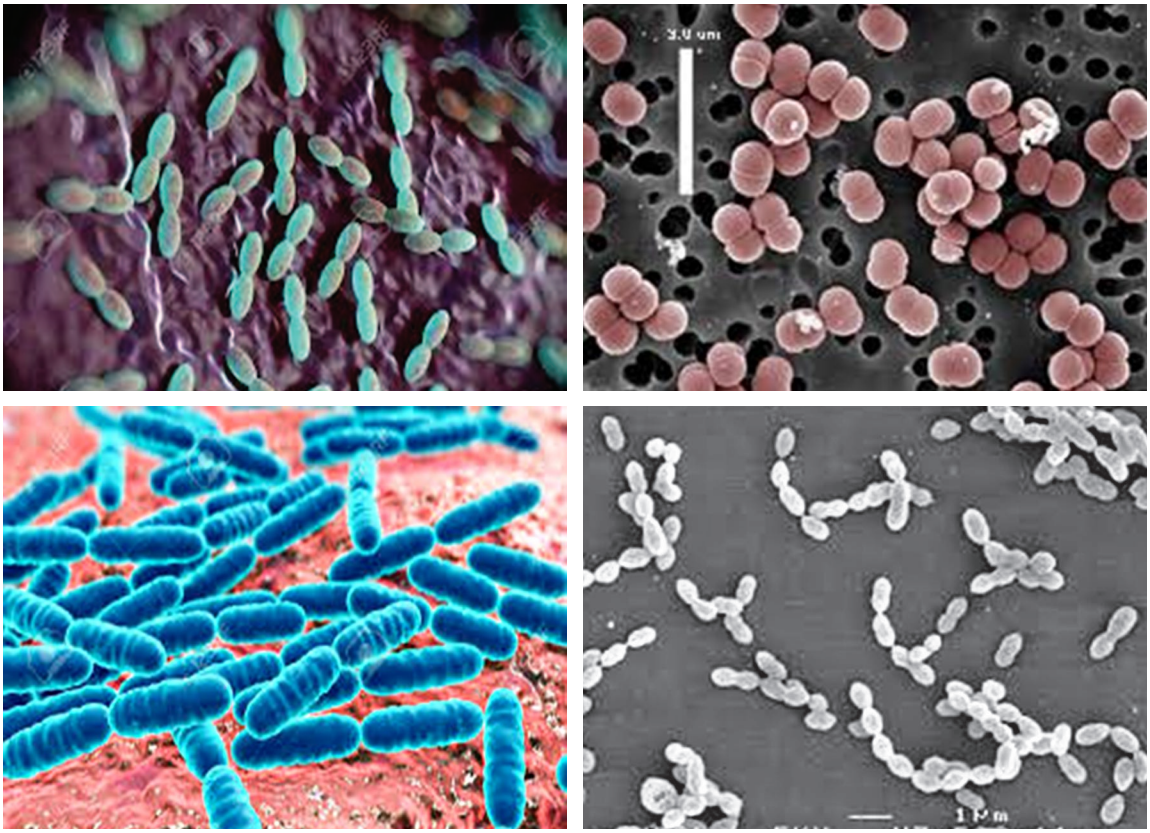
results@bwga.net *www.bwga.net* *(805) 226-8386*

Topping Time

Paying close attention to the quality of your topping wine is an integral step in maintaining wine health. Avoiding an accidental spread of detrimental wine microbes to a clean wine is preventable. Before topping any vessel, here are some suggested tests we can run to check the quality of your topping wine:

1. General Microscan: We will take a look under the scope and identify any unwelcome yeast, bacteria or mold. We specifically look for *Acetobacter*, *Pediococcus*, *Lactobacillus*, and *Oenococcus* as well as yeast.

***Acetobacter*:** Produces acetic acid and ethyl acetate, actually oxidizing ethanol into acetic acid. Increases in *Acetobacter* can occur during racking due to oxygen exposure.
***Pediococcus*:** These bacteria are acid tolerant, difficult to filter, produce lactic acid, diacetyl, and slimy polysaccharides. These slimy polysaccharides appear as a ropiness in wine and can protect the bacteria, allowing it to be tolerant to higher alcohol.
***Lactobacillus*:** An acid tolerant microbe that produces lactic acid and diacetyl. Some *Lactobacillus* is associated with Tourne Disease, a tartaric acid fermentation that increases VA and lowers TA, leaving wine carbonated and flabby.
***Oenococcus*:** Produces lactic acid and diacetyl. Some species produce a mousy sensory effect.



Clockwise from top left: Acetobacter; Pediococcus; Oenococcus; Lactobacillus.

2. Glucose Fructose: This serves as a double check to make sure the topping wine is dry, which means less food for spoilage microbes.

3. Malic: Again, another way to double check dryness.

4. Volatile Acidity: It is always a good idea to know your VA baseline and is an easy way to identify trouble.

5. Free/Total Sulfur: SO_2 levels protect the wine against microbes and oxidation.

6. Brett DNA Testing: If you are concerned about Brett, we can positively identify Brett through DNA testing and give you a general idea of how severe the count may be.

Ochratoxin A

Some countries ask for Ochratoxin A (OTA) analysis for imported wine.

OTA is a toxin produced by mold and fungus. It is found on grape skins that have been infected by *Aspergillus* or *Penicillium*. OTA can inhibit alcoholic fermentation and can cause spikes in volatile acidity. For humans, OTA can cause irreversible damage to kidneys and liver in high doses. The maximum limit for countries requiring testing is 2 micrograms per kilogram, or 2 parts per billion (ppb).

There are quick, easy, and relatively inexpensive tests available for testing OTA. Unfortunately, they do not have the precision to measure down to the 2 ppb level. At BWGA we use the ELISA (Enzyme Linked Immuno Sorbant Assay) method to measure OTA down to low levels. However, it is a costly, labor and time intensive method. The OTA test can be added to our standard export panel or done on its own.

Micro Vocab

Viable: Live, healthy cells

Nonviable: Dead cells that cannot develop or reproduce

Viable, Non-Culturable: Live cells that cannot grow or divide, meaning they can respire or ferment through active metabolism but cannot reproduce and do not form colonies on media; caused by stressful conditions (such as high levels of SO_2)

CFU: Colony forming units; a population of cells visible to the naked eye used as an estimate of the total number of cells (common wine yeasts, LAB, AAB, molds, biofilms)

Culture/media options: Wallerstein nutrient broth supports yeast, mold, acetobacter and lactic acid bacteria

***Saccharomyces*:** Species of yeast known for its fermentation capability to convert carbohydrates to carbon dioxide and alcohol; most common type of yeast used in winemaking and brewing

Non-*Saccharomyes* wine yeast: Yeast species other than *Saccharomyces* with differing fermentative capabilities and resulting in varying organoleptic effects

Lactic acid bacteria: Gram-positive bacteria; *Lactobacillus*, *Oenococcus* and *Pediococcus* are the main genera of lactic acid bacteria (LAB) found on grapes and in wine. While some LAB are necessary for completion of malolactic fermentation, others are responsible for a myriad of wine faults.

Acetic acid bacteria: Gram-negative bacteria; *Gluconobacter oxydans*, *Acetobacter aceti* and *Acetobacter pasteurianus* are examples of acetic acid bacteria (AAB). AAB oxidize ethanol through acetaldyhyde into acetic acid, leading to spoilage of wine.

Mold: Fungi can form mold, which can be harmless or toxic, depending on the species of fungus involved, Mycotoxins formed by fungus species such as *Aspergillus* and *Penicillium*.

Biofilm: Community of microbial cells that adheres to an inert or living surface; requires physical force (scrubbing) to remove, is not removed by rinsing with water or sanitizer

Faults Matching Game

Answer key at the bottom of this page

A. Acetaldehyde
B. Acrolein Taint
C. Ethyl Acetate
D. Geraniol
E. Haze
F. Mousiness
G. Ropiness/Grasse
H. Sulfides/Mercaptans/Thiols
I. TCA
J. Tourne Disease
K. Volatile Acidity
L. Volatile Phenols

____1. growth of yeast/bacteria in the bottle; usually if GF> 2g/L; a potential safety concern due to CO_2

____2. breakdown of glycerol; metallic, bitter on the palate; associated with high pH from immature or damaged grapes; associated with strains of *Lactobacillus* and *Pediococcus*

____3. sum of distillable fatty acids consisting of acetic acid, carbonic acid, sulfurous acid, lactic acid, formic acid, butyric acid, propionic acid, and sorbic acid; vinegar smell

___ 4. volatile sulfide compounds; products of primary fermentation produced by yeast; rotten egg, fecal, garlic

____5. what happens when Brett cells die and lyse open; 4EP & 4EG; barnyard, band aid

___ 6. ethanol + acetaldehyde + iron + saliva; associated with *Lactobacillus*, *Oenococcus* species, and Brett; smells like corn nuts

____7. formation of exocellular polysaccharides from glucose after yeast formation; associated with some *Pediococcus* strains; may appear like raw egg whites

____8. produced by lactic acid bacteria and sorbic acid, smells of geranium

___ 9. tartaric acid fermentation where lactic acid bacteria metabolize tartaric acid; increases VA and decreases TA; fat, flabby, pre-mature browning

____10. caused when yeast oxidizes ethanol; over-ripe bruised apple, nutty

____11. major ester produced by yeast; associated with wild yeast strains — *Hansenula* and *Kloeckera* — during early fermentation; also produced by acetic acid bacteria and dissolved oxygen; fingernail polish remover

____12. compound from plant phenols + mold + chlorine; cardboard, musty

Filterability Index

Are you sterile filtering your wine at bottling? We offer filterability testing to determine your filterability index (FI). FI is an indicator measurement to determine if your wine needs additional clarification, needs a pre-filtration step, or if your wine can be filtered at 0.45 microns. By measuring a specific volume of wine through a filter under a constant pressure, two time points are measured and plugged into an equation to determine the wine's FI. This test can be used to indicate potential problems with filtration on the bottling line.

Yeast Cell Count and Viability

How happy are those little winemakers? Are you prepping your liqueur de tirage for sparkling wine? BWGA can help with monitoring yeast cell count and viability through the use of flow cytometry. Strong live cell counts between 10^7–10^8 are preferred, while a live cell count less than 10^6 indicates little to no fermentation activity is occurring. Viability within 90-100% and yeast counts between 5-6 x 10^7 are ideal for sparkling wine production.

FAULTS MATCHING GAME ANSWER KEY:

1-E 2-B 3-K 4-H 5-L 6-F 7-G 8-D 9-J 10-A 11-C 12-I

results@bwga.net *www.bwga.net* *(805) 226-8386*

BAKER WINE &
GRAPE ANALYSIS
825 RIVERSIDE AVENUE #3
PASO ROBLES, CA 93446
805-226-8386

MICROBIOLOGY

Drop Box, Free Shipping & Courier!

DROP BOX

Leave your samples in the **BWGA drop box** anytime outside of our normal business hours! To access the drop box just open the utility closet at the left of the main doors.

FREE SHIPPING

We offer free shipping through UPS and GLS. Simply log in to your **Client Portal** and select the **Shipping** tab. Fill in the required fields and check your email for a prepaid shipping label.

COURIER

We are pleased to offer a free courier service for our customers in San Luis Obispo and Santa Barbara Counties. Request a courier pickup by logging into your **Client Portal** and selecting the **Courier** tab on the left. Requests must be made by 4pm the day before pickup and can be requested up to 5 days in advance.

Client Portal