Detection of Yeast and Bacterial Contamination in Beer: Methods used in a craft brewery

Christopher Reilly
QA/QC Manager
Barrel Program Manager

Weyerbacher

MBAA Philadelphia Technical Meeting September 24, 2016

Product Contamination Standpoint:

- **What**- are we looking for
- **Where**- Where to start/ where do they reside
- **When** to test
- **How**: to do it
  - Collect samples
  - Select media
  - Get results
  - Initial ID
BEER: PRODUCT OF AGRICULTURE... AND AGRICULTURE IS DIRTY, DUSTY AND MUDDY.

- Estimated 10,000 TO 50,000 SPECIES PER GRAM OF SOIL! -* Roesch, L. F. et al. ISME J. 1, 283–290 (2007)
- Seed to Harvest: billions of bugs looking for a free lunch - crop loss - Bacteria, Yeast, Molds, Mildews
- Farmers battle crop loss / spoilage
- Fusarium blight
  - Not just spoilage but beer issues
  - Fusarium- vomitoxin- DON - Yeast inhibition during Ferm.
  - Flocculation issues (PYF)
  - Hydrophobins- gushing
Battle Spoilage with BioWarfare:

Farmers applying lactic acid bacteria cultures to their barley fields to control Fusarium.


Maltsters similarly dose lactobacillus to steeping liquid reduce competition of aerobic bugs and molds.*

Faster and More Efficient germination


Hop farmers using Integrated Pest Management and BioFungicides

- Spraying Bacillus sp. on hops protects hops from powdery mildews, insects and crop loss.
**Water**

Municipal and private Well:
- Home to E. Coli and Fecal coliforms—water treatment facility usually takes them out.
- Can be present in your post cip rinse water

**Yeast**

- Propagating in house? Culture something from outside?
- Otherwise likely using pure culture from a professional lab service.
  - After that first pitch, things can change
- If you’re not testing testing for contamination/mutations, how do you really know you’re still using a pure pitch?

Heritage Bred Rustic Yeast?
Microbiota of your Brewery affected by geographic area as well as layout/design of brewery itself:

- Cleaning Regiments/regularity
- Cleanability of surfaces/porosity
- Foot/Forklift traffic
- Bay doors opened for deliveries/taking out spent grain
- Airflow/ventilation/Wind
- Water leaks
- Animals (pest control or brewery cat?)
- Sour program/barrel program
- Brewer Hygiene—brewing is a dirty job.

Beer is a product of your environment.
BEER IS A PRODUCT OF MICROBIOLOGY

Before you make any beer, your base ingredients have already seen significant microbial action.

- You may be applying all the conditions needed for the specialized critters to succeed in their slow devastation enroute to lambic land.

If you’re not keeping an eye out, it’s just a matter of time before they find a way into your beer—It WILL happen.
- Likely through the hands of the people making it—Just saying.

Dr. Ian Malcolm knows what I’m talking about.
**What are we looking for?**

- **Organisms that produce or have the potential to produce an unintentional change in our beer**
  - Increased: Acids Lactic/Acetic, Organic Acids/Esters off flavors, Phenolics (POF+/PAD+), DMS H2S, Attenuation=ABV/Carbonation
  - Decreased: mouthfeel, Body, Balance, alcohol (oxidation to acetic acid)
  - Filtration issues, Haze, sediments, Flocculation (PYF), yeast viability

**Wort Spoilers:** hot side, early ferm, oxygenated wort

1. **Bacteria**
   - Enterobacteriacea/Acetic Acid Bacteria:
     - Sluggish ferm/ increased attenuation, DMS, decreased flocculation of yeast, Organic acids, off flavors. H2S,
   - Obesumbacterium/Zymophilus/Selomonas-
     - Pitching yeast
2. **Wild Yeast**
   - Hyper-attenuation: alpha/beta glucosidases Phenolic off flavor POF+
   - Killer toxin potential- reduced house yeast viability
   - Off flavors/ Haze

**Beer Spoilers:** cold side, late ferm, reduced oxygen

1. **Bacteria**
   - Lactic Acid Bacteria-
     - Lactic Acid
     - Diacetyl
     - Ropiness, ExoPolysaccharides (EPO)
   - Megasphaera, pectinatus
     - H2S, Butyric, propionic acids
2. **Wild Yeast**
   - POF+, (Phenolic acid Decarboxylase+)
   - Hyper-attenuation, Excess Carbonation
   - Off flavors / Organic Acids (Horse Blanket)
   - Acetic acid
   - Body/Balance
Gram Positive Bacteria
Bacillus
Enterococcus
Lactobacillus
Leuconostoc
Micrococcus
Pediococcus
Streptococcus

Gram Negative Bacteria
Enterobacteriaceae
Acetobacter
Gluconobacter
Acidomonas
Megasphaera
Pectinatus

Wild Yeasts
-Saccharomyces type: potentially indistinguishable from house strains.
-Non-Saccharomyces type: Brettanomyces, Candida, Cryptococcus, Debaryomyces, Dekkera, Hansenula, Kloeckera, Kluyveromycetes, Pichia, Rhodotorula, Torulopsis, Zygosaccharomyces

Microbiota of malting and brewing.

Bacteria

Fungi

**Step One: Sample Collection**

**Surface Swab Collection:**
Manways FVs, opened equipment, fillers, Drains, Environmental surface audits.
1. Puritan or other cotton tipped sterile swab.
2. Testing on dry surface: dip swab into sterile dH2O in test tube
3. Take swab and rub against 10x10cm surface making a square, while rotating swab between fingers.
4. Wet location surface you don’t need to dip swab into dH2O,
5. Place Swab back into Test tube and cap.

**Aseptic collection of effluent:**
Drainage off FVs, BBTs, Centrifuges, Fillers, Hoses.
1. Spray ETOH around port or drain valve opening
2. Flame off ETOH
3. Open valve
4. Collect sample cleanly and cap.
5. Return to lab and test or Fridge within 2 hours

**Aseptic Collection of Beer (tank)**
1. Spray mouth of valve with ETOH min 70%
2. Open valve to assure no clogs
3. Respray and use cotton swab to wipe interior of valve, careful not to snap tip off.
4. Flame with torch minimum 30 seconds-
5. Open Valve let beer flow to cool mouth
6. Collect sample with minimal disruption or overflow or tube
7. Test within 2 hours, or Refrigerate and test with 24 hours.
How do we find them?

Step Two: Testing- using aseptic technique

Conventional testing-
- Traditional Microbiological plating on Nutrient Agar/Media which allows observation of Phenotypic indicators and as well as detection/enumeration/proliferation.

Colony counting- cfu’s/colony forming units
- Pour plate- molten agar- kills bugs if too hot, serial dilution required
- Spread plate- Dispense sample onto solid media plate, spread with “hockey stick”
  - serial dilutions Req’d to achieve countable number of cells (30-300 vs 10000)
  - quantifiable results *CFU/mL dosed
  - Both Pour and Spread plate recommended for samples with potential high cell count: Yeast, Fermenting Beer.
How do we find them?

Step Two: Testing - Conventional

**Direct Swab**—take sample swab directly to plate

**Media Tubes/Broth**: Reduced Oxygen environment

- **Stab tube**—stab swab into media tube,
- **Add volume of specimen directly tube**
  - Can be selective/differential method
  - **Oxygen Requirements**
  - Nutrient requirements
- **MPN**—Most Probable Number—Lots of tubes
  - Determines the concentration of viable microorganisms in a sample
  - Ten-fold dilutions replicated in triplicate—growth in liquid broth.
  - Solid statistical method—underutilized

**Membrane filtration**: Concentrates expected low organism counts

- Packaged beer, Kegs
- Filtered BBTs
- Diluent, Effluent, rinse water,
- Grow on various plates for selection or differentiation of bugs

**Membrane Filtration Setup** For Bottled Beer

**Various Media Plates**

**Tubes Showing Acid Production**
Classification of Media types for organism growth:

- **Nutritive medium**: Contains various nutrients that are known to support growth of numerous microorganisms. (UBA)
- **Differential medium**: Shows particular change in medium when a specific biochemical reaction occurs. (Acid Production)
- **Enriched medium**: Contains special nutrients that encourages growth of a particular organism that may be low in concentration. (Tween 80 for Lacto)
- **Selective medium**: Encourages growth of select organisms while suppressing others. (Wild yeast)
- **Inhibitory medium**: Inhibits particular microorganisms—addition of antifungal or antibiotics (Cycloheximide/Nystatin/alcohol/Iso alpha Acids)
Overview of Bug Growth Requirements: “FAT TOM”

1. **Food** - Carbon (Residual Plato), Nitrogen (FAN), Micronutrients
2. **Acidity** - optimum pH for each organism
3. **Time** - 2-10 Days typically
4. **Temperature** - 5-57°C (40-135°F)
5. **Oxygen** - Aerobe or Anaerobe, - Incubate accordingly
6. **Moisture** - unbound available water (90-95% of what’s in your beer.)

Give the bugs what they need
Different nutrients/inhibitors depending on what you’re looking for.

Make according to Manufacturer’s inst.-
  ○ typically autoclave 15 Psi- 15 min

All the Bugs- Non-selective Brewery Media
  ○ YPD (yeast extract peptide dextrose) (yeast prop)
  ○ Wort+Agar: literally just that
  ○ UBA- Universal Beer Agar, Media+beer
  ○ WLN/WLD- Wallerstein Labs Nutrient media/ Differential with Cyclo
  ○ SDA/LMDA-Lee’s Multi-Differential Agar/Schwarz Differential Agar
    ■ Without Cyclo Non-specific-potential to pick up most everything, Beer related.
General Beer Bacteria/AAB:
- UBA/WLN/WLD- Ae/AN
- SDA/LMDA- Ae/AN
- BMB- Barney Miller Broth- (Miller Brewing)
- NBB- Nocive Brewers Bacteria agar

LAB:
- Raka Ray-AN, addition of Fructose
- MRS- de Man, Rogosa and Sharpe
- HLP- Hsu Lacto Pedio (tube)

Enterics:
- MAC-MacConkey-Enteric bacteria

Mega/Pect
- SMMP- Selective Media Megasphaera and Pectinatus-AN

All spoilers:
- ABD- Advanced Beer Spoiler Detection- Ae/AN/Tube
  - MRS- Nutrient reduced inhibitory/Differential media for select spoiler detection.

Misc. Plates in Homemade Uncubator

BD Anaerobic Chamber/ GasPak

Lacto On RakaRay w/ bromo green

Anaerobic Chamber

ABD Tubes
**Wild Yeasts- Selective/Differential Media’s**

- **LWYM** (Sacc type), Selective/Differential- Crystal Violet
  - House sometimes
- **LCSM** (Non Sacc Wild types), Selective/Differential- Cupric Sulphate
  - House sometimes
- **Lysine** (Non Sacc Wild type), Lysine sole nitrogen source
  - House sometimes
- **ABD**- reduced nutrient (cycloheximide added min 4ppm,)
- **CLEN**: (cadaverine, lysine, ethylamine, and nitrate)
- **XMACS**- (xylose, mannitol, adonitol, cellobiose, sorbitol)

Need to use a combo of these for total coverage
**How do we find them?**

**Step Two: Testing- Advanced methods**

**Rapid testing:**
- Bioluminescence / enzymatic- ATP swabs/meters-
  - CIP confirmation only- Surface hygiene monitoring
- Biomolecular - Looks at Genetic markers/Genotype-
  - PCR, DNA Fingerprinting
  - HorA, HorC genes, Genus/Species ID kits
  - Lactic Acid development, POF+ (PAD+), Others

*(Invisible Sentinel, Pall-GeneDisc, Hybriscan, Pika, others)*

**Well funded research level labs:**
- Immunoassays, Flow Cytometry, Fluorescence, ELISA, Monoclonal, latex agglutination-
  - Not commercially available for brewing
**Conventional testing “Old School”**

<table>
<thead>
<tr>
<th><strong>Pros</strong></th>
<th><strong>Cons</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>- Generally the most affordable option-&lt;br&gt;- Selective to a degree&lt;br&gt;- Some phenotype related and often species ID information generated via metabolic testing.</td>
<td>- Time consuming-&lt;br&gt;- Media Prep&lt;br&gt;- Incubation time&lt;br&gt;- Not definitive&lt;br&gt;- Trained personnel&lt;br&gt;- Dreaded viable but not culturable (VNBCs)-not cool.</td>
</tr>
</tbody>
</table>

**Rapid Testing “too cool for school”**

<table>
<thead>
<tr>
<th><strong>Pros</strong></th>
<th><strong>Cons</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>- Fast results&lt;br&gt;  ○ Same day&lt;br&gt;- Small sample size&lt;br&gt;- Highly sensitive and selective&lt;br&gt;- May be cost prohibitive-&lt;br&gt;  (Great option for sending out colonies for ID to outside lab.)</td>
<td>- Can be cost prohibitive&lt;br&gt;- Trained personnel&lt;br&gt;- Not necessarily definitive if it’ll spoil <strong>YOUR</strong> beer.</td>
</tr>
</tbody>
</table>

**Combining traditional methods with new technologies will provide for a robust well rounded micro program.**

---

*NEED TO EXPERIMENT WITH WHAT MEDIA WORK BEST FOR YOU, YOUR HOUSE YEAST STRAINS AND YOUR TESTING DESIGN.*

~OUR BELGIAN STRAIN GROWS ON A FEW WILD YEAST PLATES.*
Essentially any and all SCCPs (Spoilage Critical Control Point)-
- Contamination risk to product

Typically any tank change or process affect to product.
- Pitching Yeast
- Brewhouse Casting - Post HE and Pre FV (hose) Sample jars -
  - Confirms clean wort to FV, No issues with HE or Cast Hose.
- Wort Manifolds post chilling.
- FV Tank Full - 24-72 hours
- Post Downstream Processes - Dryhop/Filtration/centrifugation
- BBT-dependent on tank turnover time - could be last chance for decision making
- Package - Last Chance to detect problems before consumer gets it.
  - Bottle, Can, 750's, Keg
- Beer in Oak Barrels.
- Extra Audits post Bug usage - Sour/Barrel program - Bio Acid Tanks
  - Process lines
  - Centrifuge
  - Fillers
  - Hoses, tanks, equipment utilized.
**Example: comprehensive coverage, all bugs**

- **Casting jars** - Plate if signs of Ferm 2-3 days - WLN/WLD
- **FV full** - 24-72 post KO -
  - WLD/SDA(ae), BMB/RAKA(an)+LCSM/LYS+LWYM, ABD,+ Tube+ PCR
- **Post Centrifuge/Filtration** -
  - WLD/SDA(ae), BMB/RAKA(an)+LCSM/LYS+LWYM, ABD,+ Tube+ PCR
- **Post Pasteurization/Sterile Filtration** -
  - Looking for anything that grows to confirm PUs - UBA/WLN no inhibitors.
- **BBT** - membrane filtration - typically retroactive since tank turnover can be same day
  - WLD/SDA(ae), BMB/RAKA(an)+LCSM/LYS+LWYM, ABD,+ Tube+ PCR
- **Package** - Membrane Filtration
  - WLD/SDA(ae), BMB/RAKA(an)+LCSM/LYS+LWYM, ABD,+ Tube+ PCR

**Statistics will aid in sampling volume/frequency but generally want to duplicate testing for robustness and 95% or greater confidence.**

- **Every brewery and lab runs differently**
  - How comfortable you are with your results and depth of testing.
# How to ID Bugs

**First Steps: Reculture for Isolation-triple streak**

<table>
<thead>
<tr>
<th>Triple streak: for distinct isolated colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td>● Flame loop in alcohol lamp, touch media to cool</td>
</tr>
<tr>
<td>● Use Inoculating loop to “pick” colony from spread plate/pour plate or other source.</td>
</tr>
<tr>
<td>● Touch loop to new plate and pass back and forth on small portion of plate</td>
</tr>
<tr>
<td>● Flame loop, cool loop, turn plate 20 degrees &amp; drag loop across last area streaked. Pass back and forth without recrossing last area.</td>
</tr>
<tr>
<td>● Repeat 2 times, results: isolated bugs- distinct colony</td>
</tr>
</tbody>
</table>

**Next: Gather info from bugs growth patterns**

**Phenotype:**
- ● Relationship to Oxygen- Where was sample taken from?
- ● Gram Stain- morphology
- ● Catalase- Ability to neutralize toxic oxygen species- very important to reduce cellular damage- Reason O3 Ozone works as a disinfectant.
- ● Oxidase- Tests for cytochrome C oxidase enzyme, Aerobes use O2 for energy production.
- ● Carbon source assimilation, Metabolic capabilities/nutritional requirements
- ● Growth on plates
  - ○ Acid and gas production? Durham Tubes/ bubbles in agar tube.

**Genotype:**
- ● Genetic Makeup- helps differentiate between species of lactobacillus, PCR, DNA fingerprinting
Beer Bacteria Growth Patterns in Relation to Oxygen Tolerance

**Obligate Aerobe** - needs O2
  SOD+ Cat+
  Acetobacter, Gluconobacter, Micrococcus, Bacillus (wort spoiler)

**Obligate Anaerobe** - O2 Harmful
  SOD Neg CatNeg
  Pectinatus, Megasphaera, Selenomonas, Zymophilus

**Facultative Anaerobes** with/wo O2
  SOD+ Cat+
  Wort spoilers: Enterobacteriaceae-Citrobacter, Enterobacter, Hafnia, Klebsiella, Oresum, Proteus, Rahnella, Serratia, Most wild yeasts

**Microaerophiles** - Little O2
  Zymomonas

**Aerotolerant Anaerobes** - SOD+
  Don’t need O2 but not harmful either
  Lactobacillus, Pediococcus, Zymomonas

Higher [Conc.] Oxygen

Lower [Conc.] Oxygen
Gram Staining protocol:

- 60 seconds then rinse with H2O
- 60 seconds then rinse with H2O
- This is where you wash off your sharpie labels from the slide
- 60 seconds then rinse with H2O
Gram Staining Pattern and Morphology

If it looks like this then you're doing something very very wrong - B. Anthracis
Catalase

Tests for Organisms ability breakdown toxic Oxygen Species. Anaerobes typically Cat Neg Aerobes typically Cat Pos
Oxidase

Tests for cytochrome C oxidase enzyme

Aerobes use O2 for energy production

Positive Org- Pseudomonas sp.
Biochemical testing: Carbon/nitrogen Utilization

- **API Strip**
- **Biolog Microplate**

Each well contains specific carbon/nitrogen nutrient
- After incubation can be graded and coded based on reactions
- Looked up code in database for Genus/often species ID of organisms.

PCR/DNA fingerprinting

- **Pall GeneDisc Kits**
- **BrewPAL, BrewMAP, BrewBrux/Dekk, BrewLAP**

Various PCR kits for Genus/Species ID
**Potential Microorganism Growth From Beer**

- **Molds**
  - Non spoilers
  - Aerobic: Fuzzy, Spreading "Lawn"
  - Anaerobic: atypical

- **Yeast**
  - Growth on UBA, LYS, LCSM, LWYM, CLEN, XMACS, ABD or Growth at 37C
  - No/Minimal growth on Cyclo Plates or at 37C on UBA
  - **Wild Yeast** (potential spoiler)
    - Morphology, Sporulation, Metabolic Testing, PCR
  - **Brewing Yeast** (know your yeast)
    - Lager strains: no growth at 37C
    - Ale strains: Growth at 37C

- **Bacteria**
  - Gram Negative
    - Koh Pos- Stringy
  - Gram Positive
    - Koh Negative
  - POF+ Phenols? Acids?

**Ale strains:** Growth at 37C

**Lager Strains:** No growth at 37C
Bacilli

Catalase

POS

Bacillus Sp. (spores) Aerobic

NEG

Lactobacillus Sp. (Anaerobic)

Acid or Gas +

L.Brevis (35%), L.Lindneri (25%), L.Brevisimilis (3%), L.Frigidus (2%)

Gas -

L.Corniyformis (3%), L.Casei, L.Plantarum (1%)

Pedio

Catalase

POS

Micrococcus-Staph (aerobic) non-spoiler

NEG

Tetrads= M.Kristinae (rare-depends)

Chains= Leuco- Lactococcus

Gas +

Diacetyl +

Gas -

Lactococcus lactis (1%)

Leuconostoc mesenteroides/ para (1%- rare)

Lactobacillus (Anaerobic)

Acid/Gas

P. Damnosus (17%)

P. Inopinatus (1%)

Beer SPOILERS

Potential but rare SPOILERS

G+ Flowchart

GRAM POSITIVE BACTERIA
Gram Negative Bacteria

Bacilli

- Oxidase: POS
- Catalase: NEG
  - Pseudomonas (Aerobe)
  - Acidomonas
    - Acetic Acid: POS
      - Acetobacter/Gluconobacter (Motile) high ABV and low pH tolerance
    - Acetobacter/Gluconobacter (Motile) high ABV and low pH tolerance
  - Zymomonas-(ales) no maltose (high ABV) Acetaldehyde, H2S Fruit beers, bottle Cond.
  - Zymophilus (Motile) pitching yeast >4.6 pH <5%ABV

Cocci

- Catalase: NEG
  - Megasphaera (strict anaerobe) >4.1 pH <3.5 %ABV
  - Enterobacteriacea (Wort Spoilers) Facultative Anaerobes
    - Pectinatus (Strict Anaerobe) >4.5 pH <6%ABV
    - Zymophillus (Motile) pitching yeast >4.3 pH <5%ABV
    - Selemonas (Ob Anaerobe) >4.3 pH yeast/biofilms

POS: Positive
NEG: Negative
<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>SHAPE</th>
<th>GRAM</th>
<th>O2</th>
<th>TEMP (°C)</th>
<th>FERMENT</th>
<th>SP. IN BREWERY</th>
<th>SPOIL</th>
<th>NOTES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactobacillus</td>
<td>Rod</td>
<td>Pos</td>
<td>Aerotolerant Anaerobe</td>
<td>30°C</td>
<td>Hetero/ Homol</td>
<td>BREVE, BREVIPILLIUS, LINDENI</td>
<td>Yes</td>
<td>P. damosiae, P. parvulus, SCM510-NT, SCM510-265, SCM510-266 promote growth, calcium phosphate necessary for growth</td>
</tr>
<tr>
<td>Pediococcus</td>
<td>Rod</td>
<td>Pos</td>
<td>Aerotolerant Anaerobe</td>
<td>25°C</td>
<td>Homo</td>
<td>DAMO 6/9</td>
<td>No</td>
<td>Hetero, INDICATOR OENOCACTUS OENOCOCCUS ENEOS, NO REPORTS OF GROWTH IN BEER, CAN GROW ON MEDIA FOR LAB, DIFFER FROM PEDIO IN THAT IT GROWS IN CHAINS, DIFFER FROM L. SODA DUE TO ABILITY FOR SPORE FORMATION, RAW MILK/Dairy</td>
</tr>
<tr>
<td>Leuconostoc</td>
<td>Rod</td>
<td>Pos</td>
<td>Obligate</td>
<td>25°C</td>
<td>Hetero</td>
<td>NOSERODENOSIS OENOCACTUS ENEOS, NO REPORTS OF GROWTH IN BEER, CAN GROW ON MEDIA FOR LAB, DIFFER FROM PEDIO IN THAT IT GROWS IN CHAINS, DIFFER FROM L. SODA DUE TO ABILITY FOR SPORE FORMATION, RAW MILK/Dairy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactococcus</td>
<td>Rod</td>
<td>Pos</td>
<td>Obligate</td>
<td>25°C</td>
<td>Hetero</td>
<td>LACTI</td>
<td>No</td>
<td>Hetero, INDICATOR OENOCACTUS OENOCACTUS ENEOS, NO REPORTS OF GROWTH IN BEER, CAN GROW ON MEDIA FOR LAB, DIFFER FROM PEDIO IN THAT IT GROWS IN CHAINS, DIFFER FROM L. SODA DUE TO ABILITY FOR SPORE FORMATION, RAW MILK/Dairy</td>
</tr>
<tr>
<td>Micrococcus/ Kocuria</td>
<td>Rod</td>
<td>Pos</td>
<td>Facultative Anaerobe</td>
<td>5°C-70°C</td>
<td>Homo</td>
<td>Aerobe</td>
<td>No</td>
<td>Hetero, INDICATOR OENOCACTUS OENOCACTUS ENEOS, NO REPORTS OF GROWTH IN BEER, CAN GROW ON MEDIA FOR LAB, DIFFER FROM PEDIO IN THAT IT GROWS IN CHAINS, DIFFER FROM L. SODA DUE TO ABILITY FOR SPORE FORMATION, RAW MILK/Dairy</td>
</tr>
<tr>
<td>Staphylococcus</td>
<td>Rod</td>
<td>Pos</td>
<td>Facultative Anaerobe</td>
<td>5°C-70°C</td>
<td>Homo</td>
<td>Aerobe</td>
<td>No</td>
<td>Hetero, INDICATOR OENOCACTUS OENOCACTUS ENEOS, NO REPORTS OF GROWTH IN BEER, CAN GROW ON MEDIA FOR LAB, DIFFER FROM PEDIO IN THAT IT GROWS IN CHAINS, DIFFER FROM L. SODA DUE TO ABILITY FOR SPORE FORMATION, RAW MILK/Dairy</td>
</tr>
<tr>
<td>Bacillus</td>
<td>Rod</td>
<td>Pos</td>
<td>Aerobe</td>
<td>5°C-70°C</td>
<td>Homo</td>
<td>Aerobe</td>
<td>No</td>
<td>Hetero, INDICATOR OENOCACTUS OENOCACTUS ENEOS, NO REPORTS OF GROWTH IN BEER, CAN GROW ON MEDIA FOR LAB, DIFFER FROM PEDIO IN THAT IT GROWS IN CHAINS, DIFFER FROM L. SODA DUE TO ABILITY FOR SPORE FORMATION, RAW MILK/Dairy</td>
</tr>
<tr>
<td>Enterococcus</td>
<td>Rod</td>
<td>Pos</td>
<td>Facultative Anaerobe</td>
<td>5°C-70°C</td>
<td>Homo</td>
<td>Aerobe</td>
<td>No</td>
<td>Hetero, INDICATOR OENOCACTUS OENOCACTUS ENEOS, NO REPORTS OF GROWTH IN BEER, CAN GROW ON MEDIA FOR LAB, DIFFER FROM PEDIO IN THAT IT GROWS IN CHAINS, DIFFER FROM L. SODA DUE TO ABILITY FOR SPORE FORMATION, RAW MILK/Dairy</td>
</tr>
<tr>
<td>Streptococcus</td>
<td>Rod</td>
<td>Pos</td>
<td>Facultative Anaerobe</td>
<td>5°C-70°C</td>
<td>Homo</td>
<td>Aerobe</td>
<td>No</td>
<td>Hetero, INDICATOR OENOCACTUS OENOCACTUS ENEOS, NO REPORTS OF GROWTH IN BEER, CAN GROW ON MEDIA FOR LAB, DIFFER FROM PEDIO IN THAT IT GROWS IN CHAINS, DIFFER FROM L. SODA DUE TO ABILITY FOR SPORE FORMATION, RAW MILK/Dairy</td>
</tr>
<tr>
<td>Vagococcus</td>
<td>Rod</td>
<td>Pos</td>
<td>Facultative Anaerobe</td>
<td>5°C-70°C</td>
<td>Homo</td>
<td>Aerobe</td>
<td>No</td>
<td>Hetero, INDICATOR OENOCACTUS OENOCACTUS ENEOS, NO REPORTS OF GROWTH IN BEER, CAN GROW ON MEDIA FOR LAB, DIFFER FROM PEDIO IN THAT IT GROWS IN CHAINS, DIFFER FROM L. SODA DUE TO ABILITY FOR SPORE FORMATION, RAW MILK/Dairy</td>
</tr>
<tr>
<td>Acetobacter</td>
<td>Rod</td>
<td>Neg/ Variable</td>
<td>Obligate</td>
<td>30°C</td>
<td>Hetero</td>
<td>DOX DIPHTHANT</td>
<td>No</td>
<td>Acetobacter, INDICATOR OENOCACTUS OENOCACTUS ENEOS, NO REPORTS OF GROWTH IN BEER, CAN GROW ON MEDIA FOR LAB, DIFFER FROM PEDIO IN THAT IT GROWS IN CHAINS, DIFFER FROM L. SODA DUE TO ABILITY FOR SPORE FORMATION, RAW MILK/Dairy</td>
</tr>
<tr>
<td>Gluconobacter</td>
<td>Rod</td>
<td>Neg/ Variable</td>
<td>Obligate</td>
<td>30°C</td>
<td>Hetero</td>
<td>DOX DIPHTHANT</td>
<td>No</td>
<td>Acetobacter, INDICATOR OENOCACTUS OENOCACTUS ENEOS, NO REPORTS OF GROWTH IN BEER, CAN GROW ON MEDIA FOR LAB, DIFFER FROM PEDIO IN THAT IT GROWS IN CHAINS, DIFFER FROM L. SODA DUE TO ABILITY FOR SPORE FORMATION, RAW MILK/Dairy</td>
</tr>
<tr>
<td>Enterococcus/Enterobacter</td>
<td>Rod</td>
<td>Neg/ Variable</td>
<td>Obligate</td>
<td>30°C</td>
<td>Hetero</td>
<td>DOX DIPHTHANT</td>
<td>No</td>
<td>Acetobacter, INDICATOR OENOCACTUS OENOCACTUS ENEOS, NO REPORTS OF GROWTH IN BEER, CAN GROW ON MEDIA FOR LAB, DIFFER FROM PEDIO IN THAT IT GROWS IN CHAINS, DIFFER FROM L. SODA DUE TO ABILITY FOR SPORE FORMATION, RAW MILK/Dairy</td>
</tr>
<tr>
<td>Oenococcus/ Enterococcus/ Enterobacter</td>
<td>Rod</td>
<td>Neg/ Variable</td>
<td>Obligate</td>
<td>30°C</td>
<td>Hetero</td>
<td>DOX DIPHTHANT</td>
<td>No</td>
<td>Acetobacter, INDICATOR OENOCACTUS OENOCACTUS ENEOS, NO REPORTS OF GROWTH IN BEER, CAN GROW ON MEDIA FOR LAB, DIFFER FROM PEDIO IN THAT IT GROWS IN CHAINS, DIFFER FROM L. SODA DUE TO ABILITY FOR SPORE FORMATION, RAW MILK/Dairy</td>
</tr>
<tr>
<td>Clostridium/ Enterococcus/ Enterobacter</td>
<td>Rod</td>
<td>Neg/ Variable</td>
<td>Obligate</td>
<td>30°C</td>
<td>Hetero</td>
<td>DOX DIPHTHANT</td>
<td>No</td>
<td>Acetobacter, INDICATOR OENOCACTUS OENOCACTUS ENEOS, NO REPORTS OF GROWTH IN BEER, CAN GROW ON MEDIA FOR LAB, DIFFER FROM PEDIO IN THAT IT GROWS IN CHAINS, DIFFER FROM L. SODA DUE TO ABILITY FOR SPORE FORMATION, RAW MILK/Dairy</td>
</tr>
<tr>
<td>Citrobacter</td>
<td>Rod</td>
<td>Neg/ Variable</td>
<td>Obligate</td>
<td>30°C</td>
<td>Hetero</td>
<td>DOX DIPHTHANT</td>
<td>No</td>
<td>Acetobacter, INDICATOR OENOCACTUS OENOCACTUS ENEOS, NO REPORTS OF GROWTH IN BEER, CAN GROW ON MEDIA FOR LAB, DIFFER FROM PEDIO IN THAT IT GROWS IN CHAINS, DIFFER FROM L. SODA DUE TO ABILITY FOR SPORE FORMATION, RAW MILK/Dairy</td>
</tr>
<tr>
<td>Klebsiella</td>
<td>Rod</td>
<td>Neg/ Variable</td>
<td>Obligate</td>
<td>30°C</td>
<td>Hetero</td>
<td>DOX DIPHTHANT</td>
<td>No</td>
<td>Acetobacter, INDICATOR OENOCACTUS OENOCACTUS ENEOS, NO REPORTS OF GROWTH IN BEER, CAN GROW ON MEDIA FOR LAB, DIFFER FROM PEDIO IN THAT IT GROWS IN CHAINS, DIFFER FROM L. SODA DUE TO ABILITY FOR SPORE FORMATION, RAW MILK/Dairy</td>
</tr>
<tr>
<td>Zymomonas</td>
<td>Rod</td>
<td>Neg/ Variable</td>
<td>Obligate</td>
<td>30°C</td>
<td>Hetero</td>
<td>DOX DIPHTHANT</td>
<td>No</td>
<td>Acetobacter, INDICATOR OENOCACTUS OENOCACTUS ENEOS, NO REPORTS OF GROWTH IN BEER, CAN GROW ON MEDIA FOR LAB, DIFFER FROM PEDIO IN THAT IT GROWS IN CHAINS, DIFFER FROM L. SODA DUE TO ABILITY FOR SPORE FORMATION, RAW MILK/Dairy</td>
</tr>
<tr>
<td>Pectinatus</td>
<td>Rod</td>
<td>Neg/ Variable</td>
<td>Obligate</td>
<td>30°C</td>
<td>Hetero</td>
<td>DOX DIPHTHANT</td>
<td>No</td>
<td>Acetobacter, INDICATOR OENOCACTUS OENOCACTUS ENEOS, NO REPORTS OF GROWTH IN BEER, CAN GROW ON MEDIA FOR LAB, DIFFER FROM PEDIO IN THAT IT GROWS IN CHAINS, DIFFER FROM L. SODA DUE TO ABILITY FOR SPORE FORMATION, RAW MILK/Dairy</td>
</tr>
<tr>
<td>Selenomonas</td>
<td>Rod</td>
<td>Neg/ Variable</td>
<td>Obligate</td>
<td>30°C</td>
<td>Hetero</td>
<td>DOX DIPHTHANT</td>
<td>No</td>
<td>Acetobacter, INDICATOR OENOCACTUS OENOCACTUS ENEOS, NO REPORTS OF GROWTH IN BEER, CAN GROW ON MEDIA FOR LAB, DIFFER FROM PEDIO IN THAT IT GROWS IN CHAINS, DIFFER FROM L. SODA DUE TO ABILITY FOR SPORE FORMATION, RAW MILK/Dairy</td>
</tr>
<tr>
<td>Zymophilus</td>
<td>Rod</td>
<td>Neg/ Variable</td>
<td>Obligate</td>
<td>30°C</td>
<td>Hetero</td>
<td>DOX DIPHTHANT</td>
<td>No</td>
<td>Acetobacter, INDICATOR OENOCACTUS OENOCACTUS ENEOS, NO REPORTS OF GROWTH IN BEER, CAN GROW ON MEDIA FOR LAB, DIFFER FROM PEDIO IN THAT IT GROWS IN CHAINS, DIFFER FROM L. SODA DUE TO ABILITY FOR SPORE FORMATION, RAW MILK/Dairy</td>
</tr>
<tr>
<td>Megasperma</td>
<td>Rod</td>
<td>Neg/ Variable</td>
<td>Obligate</td>
<td>30°C</td>
<td>Hetero</td>
<td>DOX DIPHTHANT</td>
<td>No</td>
<td>Acetobacter, INDICATOR OENOCACTUS OENOCACTUS ENEOS, NO REPORTS OF GROWTH IN BEER, CAN GROW ON MEDIA FOR LAB, DIFFER FROM PEDIO IN THAT IT GROWS IN CHAINS, DIFFER FROM L. SODA DUE TO ABILITY FOR SPORE FORMATION, RAW MILK/Dairy</td>
</tr>
</tbody>
</table>
- Saccharomyces
  - Might be close relation to house strain, hard to detect on microscope or plates

- Non-Saccharomyces
  - Plate Growth - which plates are they growing on.
  - Cyclo resistant? Wilds yes, house no (typically)
  - Colony morphology
  - Microscopic morphology
  - Sporulation - rare in brewer's strains -
    - Sign of wild yeast, time consuming 2 weeks.
  - Budding patterns
    - Binary - Schizosaccharomyces
    - Polar - Hanseniaspora
    - Multilateral - Debaryomyces, Dekkera, Kluyveromyces, Pichia, Torulaspora, Zygosaccharomyces, Williopsis

- Generally don't worry about genus of wild yeast
  - **Determine if it will grow in beer or not.**
    - All saccharomyces wild yeast will grow in beer
  - Fermentative
    - Saccharomyces, Brettanomyces, Kluyveromyces, Torulaspora, Zygosaccharomyces
  - Non fermentative - require oxygen
    - Pichia, Debaryomyces, Dekkera, Issatchenka,
Wild Yeast Flowchart

Yeast Vegetative Growth:

Binary Fission
- Schizosaccharomyces
  - (H2S, off flavors, Haze)

Multilateral Budding
- Saccharomyces (POF+ Esters)
- Kluyveromyces - Off flavors, Haze
- Torulaspora - Off flavors, Haze
- Zygosaccharomyces - Off flavors, Haze
- Candida (pellicle/haze)

Polar Budding
- Hanseniaspora - Off flavors, Haze
- Saccharomyces (Haze)
- Kloeckera apiculata - Off flavors, Haze

Analysis:
- Glucose Ferment (Strong/POS)
- Acetic Acid (POS)
- Growth Nitrate (POS)
- Rapid: Use API 20C kit / DNA fingerprinting

Simplified ID of Common Yeast Contaminants*

Binary Fission

Multilateral Budding

Polar Budding
More advanced methods:

- **Biochemical Utilization Assays - 2-3 DAYS - self contained multi chambered strips each cell containing various nutrient to metabolic determination- coded database for ID upon reading and grading of each strip.**
  - BioMerieux: Yeast ID: **API ID 32C, API 20C AUX, API 50 CHL**
  - BioMerieux: Bacterial ID: API 32 E, API 20E, API20A, Rapid ID 32A
  - Biolog: PM 1- 2A Microplates for Carbon Substrate Utilization
- **PCR approaches/ ID Kits- VERY specific, way FASTER**
- **Send out**
### Lab Worksheet

#### CASTING SAMPLES

<table>
<thead>
<tr>
<th>Date</th>
<th>Media</th>
<th>Media</th>
<th>Media</th>
<th>Media</th>
</tr>
</thead>
<tbody>
<tr>
<td>HE</td>
<td>12345678901112</td>
<td>12345678901112</td>
<td>12345678901112</td>
<td>12345678901112</td>
</tr>
<tr>
<td>VLD</td>
<td>WLD</td>
<td>WLD</td>
<td>WLD</td>
<td>WLD</td>
</tr>
<tr>
<td>WYP</td>
<td>WYP</td>
<td>WYP</td>
<td>WYP</td>
<td>WYP</td>
</tr>
<tr>
<td>ARO</td>
<td>ARO</td>
<td>ARO</td>
<td>ARO</td>
<td>ARO</td>
</tr>
<tr>
<td>HLP</td>
<td>HLP</td>
<td>HLP</td>
<td>HLP</td>
<td>HLP</td>
</tr>
</tbody>
</table>

#### VDK FORCE TEST:

1000 ml pitched beer, 30 min @ 60°C waterbath, GRAM COMPARABLE TO UNHITENED BEER

#### DAILY:
days 2-10

#### Final Report:

Digital Log

---

**Documentation:** DO IT

**Daily:**

---

**Final Report:**

Digital Log

---

**Notes:**
**Next Steps:**

**Depends on data you collect.**

- **Spoilage potential of beer?**
  - ABV, IBUs, pH, Draft or Bottle
- **Dump it and Cut your losses**
- **Salvage**
  - Sterile Filtration
  - Pasteurization
  - See what happens.
  - Distillery?
- **Root cause analysis**
  - Prevent from happening again.
How to avoid these bugs.

- Get yourself a flash/tunnel pasteurizer?
  - Not necessarily a solution.

- Realistic and Achievable:
  - Good Process Design
  - Confirmation of successful CIPs
  - Good Housekeeping
  - Good SOPs
  - Good yeast handling
  - Robust CCP Monitoring
  - Process and Environmental Audits
  - Hose down your brewers from time to time.
We are all consumers
- Each have expectations of other breweries' beers as well as our own.

Everyone in Brewery has a role in QC
- It's our job to provide beers that meet:
  1. Customer Expectations
  2. Product Stability
  3. Food Safety
  4. Pathogen/Disease free beer.

Lastly: No one likes drinking bad beer.

THANK YOU!