



Basic Microbiology for Small Breweries

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Who are we and why are we here?

- Open fermentation
 - 1 yeast strain
- All yeast is top cropped
- Serial re-pitching





Outline of today's talk

- Why microbiology is important for even the smallest breweries
- Overview of wort and beer spoiling organisms
- Level 1: ATP Testing & The \$500 Lab
- Level 2: HLP & The \$1000 Lab
- Level 3: Advanced methods and additional media for detecting bacteria and wild yeast
- Resources for starting & maintaining a lab

Why should you care about microbes?

- Bacteria & wild yeast are everywhere!
- Typical brewing processes: many opportunities for contamination
- Loss of reputation / consumer confidence

Relative Expenses:

Lab supplies & personnel (<\$15/BBL)

Dumping beer (>\$100/BBL)

RECALL

- Packaging
- Distribution
- Reacquisition



Contaminated Beer: A ticking time bomb

- Once product leaves the brewery, you no longer have control of it!
- Extended storage at warm temps
- Low-level contamination – no off-flavors when it leaves the brewery
- Serious off-flavor production, exploding bottles/cans



Case in point

- Barrel-aged beer
- Shortcomings in training
- Incomplete cleaning, sanitation
- Compounded issues

Wort Spoilers vs. Beer Spoilers

Wort Spoilers

- Bacteria found in grain, soil, etc.
- Potential to ruin wort if held pre-boil for extended periods of time
- Killed off by boil or outcompeted by yeast
- Alcohol sensitive

Beer Spoilers

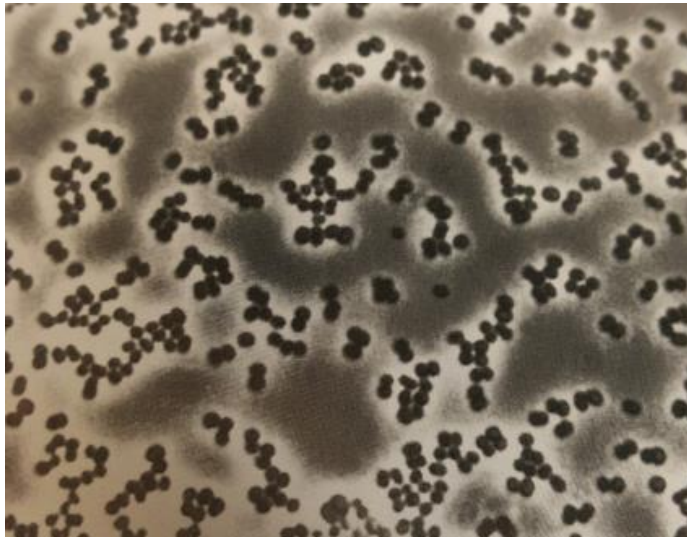
- Lactic acid bacteria (LAB), wild yeast
- Can grow in anaerobic conditions (packaged beer)
- *Somewhat* alcohol tolerant
- *Somewhat* hop tolerant

Typical Micro Test Points

- Incoming water, water treatment
- Cooled wort (heat exchanger)
- Yeast (pre-pitch)
- Fermenter full (multiple batches + yeast)
- After dry hopping, dry spicing
- Crashed fermenter
- Filter
- Bright tanks
- Packaged beer

Pediococcus and *Lactobacillus*

- Two biggest genera of beer spoilers
- *Pediococcus damnosus*: ropiness, diacetyl production, 'gym sock' aromas



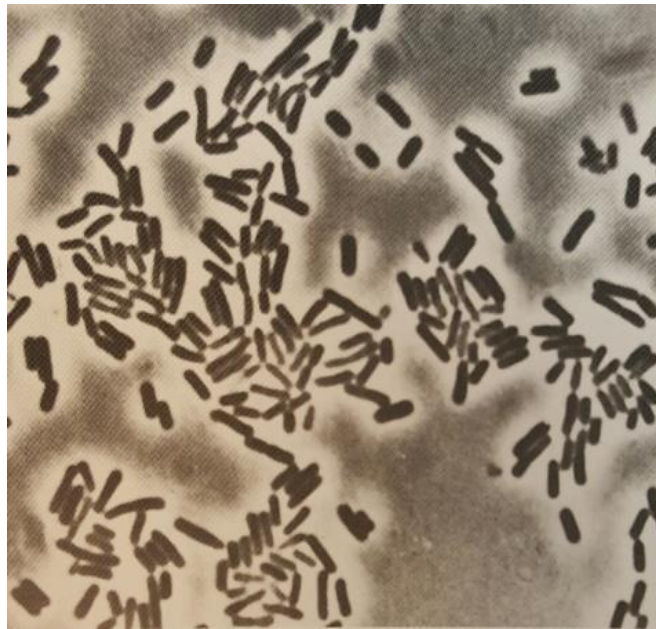
*Colour Atlas and Handbook
of Beverage Biology, 2005*



Brian Lagoe

Pediococcus* and *Lactobacillus

- *Lactobacillus brevis*, *L. brevisimilis*, *L. casei*, etc.:
haze, lactic acid production



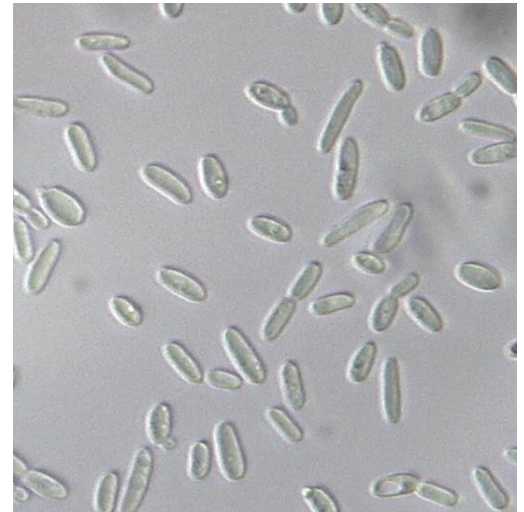
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***Megasphaera, Pectinatus,* and more!**

- *Megasphaera cerevisiae*: large, oval cocci
- *Pectinatus* species: very small rods
- Off-aromas and flavors
 - Butyric acid (vomit)
 - Caproic acid (cheesy, waxy, goaty)
 - Hydrogen sulfide (sulfur, eggs)
 - Acetic acid (vinegar)
- Strict anaerobes with low alcohol tolerance
 - More likely to be found in beers with low D.O.

Wild Yeast

- Any yeast strain not intentionally pitched into your beer
- Can originate from environment or use in another beer produced at your brewery
 - Saccharomyces
 - Schizosaccharomyces
 - Brettanomyces, etc
- You selected your yeast strains for a reason!





You don't need a PhD (or a million dollars) to start a lab!

- Identify the tests that are crucial for your system
- Don't put the cart before the horse – start small
- Science background is helpful
- Spending some time & money to get it right will pay dividends later

Bare Bones Basics

- Hydrometers (\$15 each)
 - Temperature correction
 - Keep them clean!
- pH Meter (\$100)
 - Keep it calibrated!
- A clean space to work
 - Out of the way
 - Low traffic
 - Away from potential contaminants
- Accurate record-keeping system



Level I: ATP Testing (The \$500 lab)

- Adenosine Triphosphate (ATP): energy produced and used by all living organisms
- Presence of ATP in water or on surfaces indicates presence of life
- Molecule has a short half-life



ATP Testing: How it works

- Disposable swabs for surfaces or water
 - Contain firefly luciferase
 - ATP + luciferase = light
- Light produced is measured with a handheld luminometer
- Data presented as Relative Light Units (RLUs)



ATP Testing: Pros & Cons

Pros

- User friendly
- Rapid results (go/no-go)
- Relatively inexpensive
 - ~\$2 / swab
 - Free rental

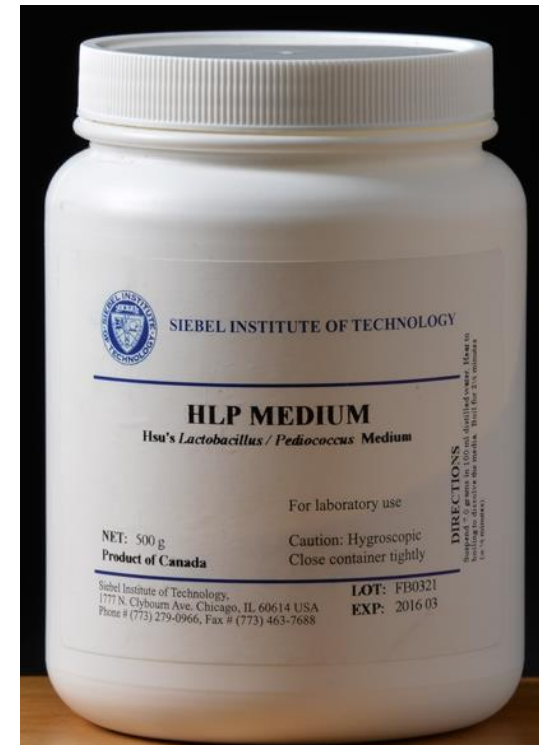
Cons

- Lack of specificity
- Swabs must be kept cold until use, finite shelf life

Best for testing efficacy of CIP/Sanitation processes and cleanliness of surfaces, cannot differentiate between yeast and harmful microorganisms!

Level 2: HLP Testing (The \$1000 lab)

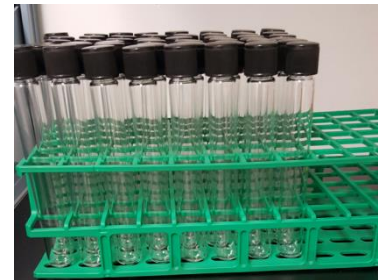
- Hsu's *Lactobacillus* and *Pediococcus* medium
- Semi-solid agar that selectively grows *Lacto* & *Pedio*
 - Cycloheximide inhibits yeast growth



Level 2: HLP Testing (The \$1000 lab)

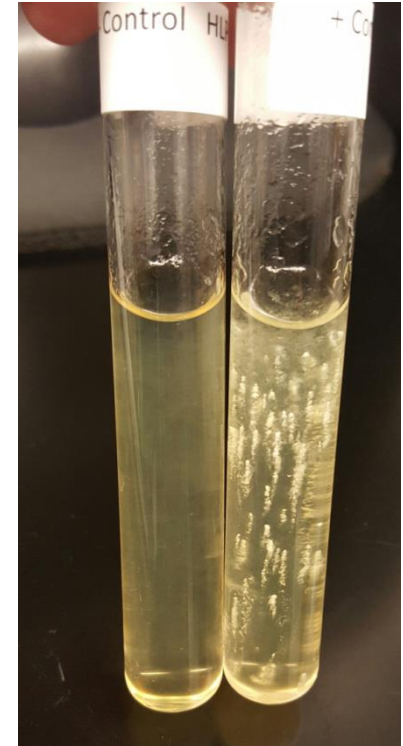
Supplies Required

- Media: \$150 / 500g
- General lab supplies:
 - Balance: \$50
 - Pipettes & Pump: \$30
 - Test tubes & Flasks: \$30
- Heat source: \$150
- Light microscope: \$100+
 - Now you can do LOTS more!



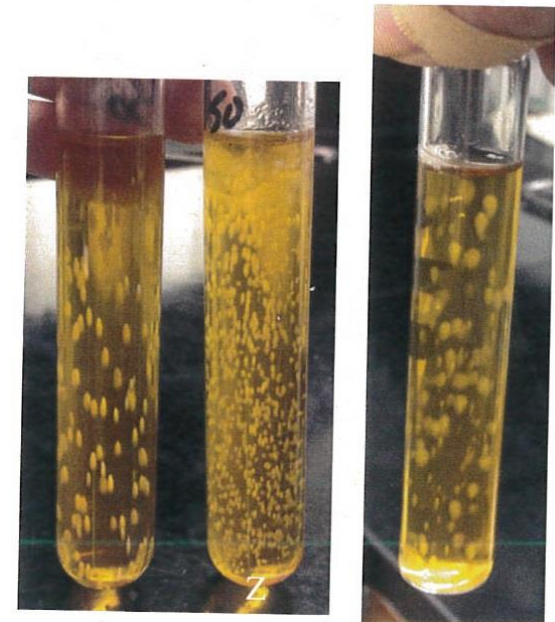
Use and Interpretation of HLP Media

- Heat & mix media to dissolve
- Boil 2 minutes to sterilize
- Distribute 9 mL into sterile tubes
- Cool slightly
- Add 1 mL of sample, invert to mix
- Incubate 5-7 days at 30 °C



Use and Interpretation of HLP Media

- *Lactobacillus* and *Pediococcus* will grow as distinct colonies (thick haze if heavily contaminated)
- Wet mount and observe under 400x to identify
- Brewers' yeast may be present, but is much larger than bacteria



Dr. Terese Barta
University of Wisconsin – Stevens Point

HLP: Pros & Cons

Pros

- Can be used for beer in any phase of process
- Specificity for two major spoilers
- Relatively clear results

Cons

- More labor intensive
- Less user-friendly
- Slow turnaround time

Level 3: Additional Media & PCR-based Methods

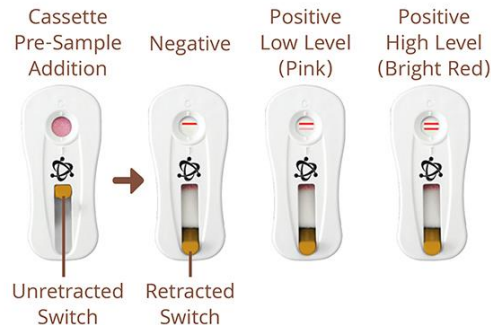
- More technical expertise required
- Prior lab experience beneficial
- More plating = more \$\$ per sample
- Several additional types of media for ID of other bacteria, wild yeast
 - Most are plate-based
 - 60 mm Petri dishes recommended
 - Biological containment hood very useful

Level 3: Additional Media & PCR-based Methods

- General use media:
 - Universal Beer Agar (UBA)
 - Schwarz Differential Agar (SDA/LMDA)
 - Will grow spoilers and non-spoilers alike
 - Further testing to ID & determine spoilage potential
 - Gram-staining
 - Catalase testing
- Selective media:
 - Lin's Wild Yeast Media (LWYM)
 - Lin's Cupric Sulfate Media (LCSM)
 - Barney Miller Brewery Medium
 - NBB Broth
 - Important to know what will/won't grow on each

Level 3: Additional Media & PCR-based Methods

- Several options for turn-key spoilage detection
- Can be expensive to start up and run
- Can be limited in scope
- Generally user-friendly



Invisible Sentinel –
www.invisiblesentinel.com

External Analysis

- Ship samples to an outside lab for ID
 - Send beer or an isolated colony
- Can be expensive
- Helpful to know genus, species to determine spoilage potential

BREWING & DISTILLING
ANALYTICAL SERVICES LLC 



You've found a contaminant: Now what?

- **Releasing contaminated beer to market is a huge gamble.**
 - Date code your packaged beer!
- Incubate samples to determine:
 - If spoiler is growing
 - If off-flavors are being produced
- Determine level, extent of contamination
- Identify source of contamination
- Improve training, cleaning and sanitation procedures



Resources for starting (and maintaining) a lab

- Brewing Science Institute (www.brewingscience.com)
- American Society of Brewing Chemists Methods of Analysis (www.asbcnet.org)
- Quality Management: Essential Planning for Breweries (Mary Pellettieri, 2015)
- Local labs, colleges and universities
- Colour Atlas and Handbook of Beverage Biology (Werner Back, 2005)



Resources for starting (and maintaining) a lab

- Supplies:

- Weber Scientific
- VWR
- Fisher Scientific
- Cynmar
- Thomas Scientific

- Equipment

- Ebay
- Amazon
- Local university property disposition departments



Questions?