Building a Quality Program Based on Micro

The Journey from Tribal Knowledge to Solid Science

ASBC Annual Conference

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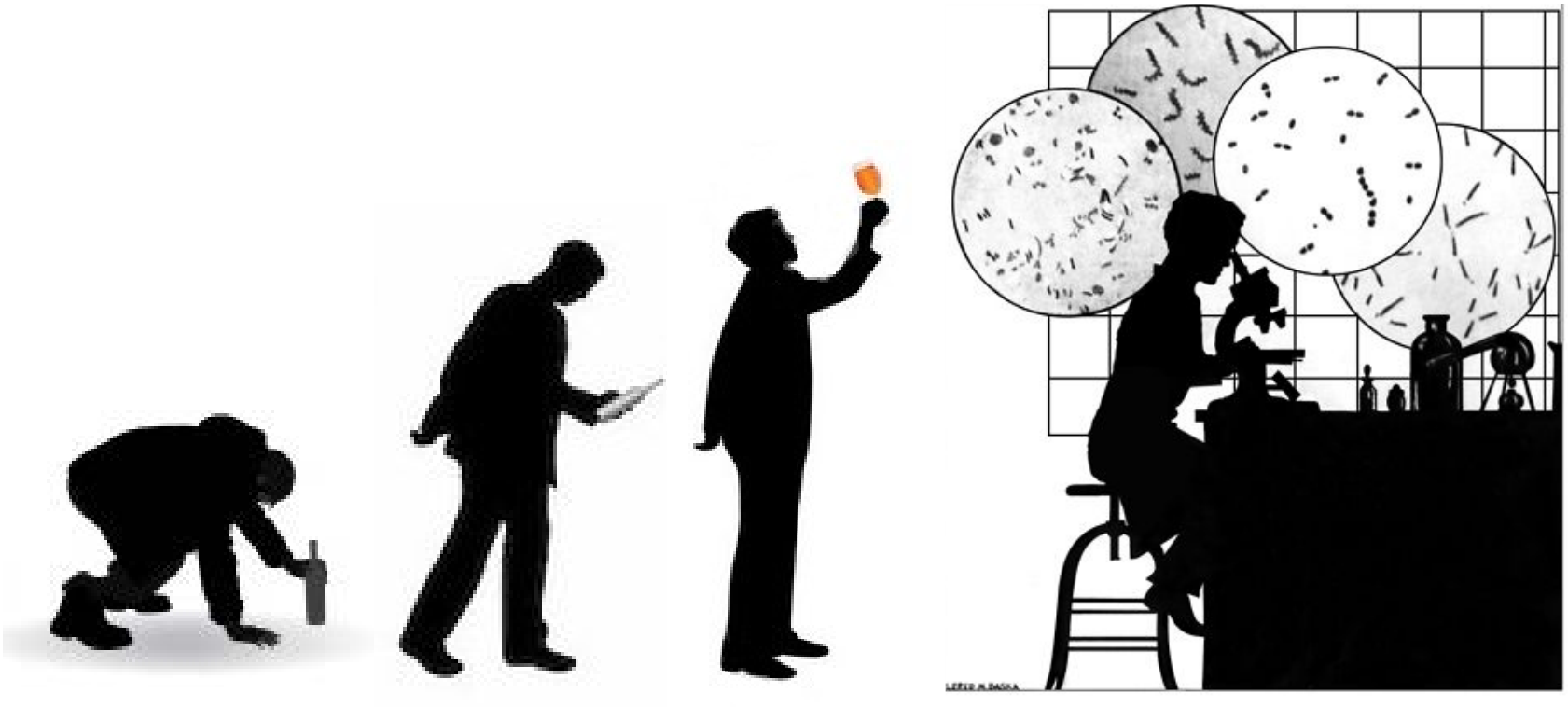
The Science of Beer

The March of Progress



What this workshop is all about

- GMP will take you far. Challenge your assumptions!
- Transition from intuitive decision-making to data-based decision-making
- Objective measurements over instinct
- Always use controls – and trust your data!
- A how-to guide for setting up your brewing quality program

Why have a micro program?

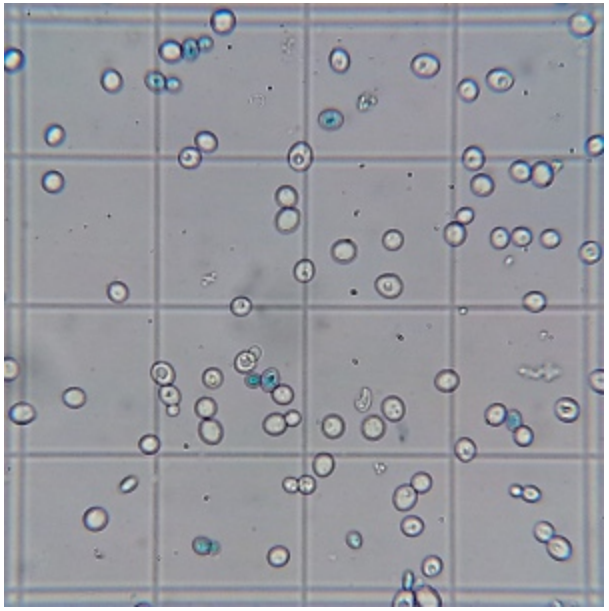
- *All about cleanliness*
- Brewery cleanliness is priority #2
  - (safety is #1)
- Prevent beer spoilage and control fermentation
- Out-of-control microbial contamination can be profoundly damaging to your business
  - Startups: limited short-term fallout, word-of-mouth, reputation for bad beer at cost to future growth
  - Established breweries are talking recall – could cost millions!
  - Dumping beer – time, effort, materials, energy down the drain
  - Hard-stop on production until source found and eliminated

Stepping Up Your Micro Program

- Intro
  - Microscope, cell counts and viability/vitality
- Basic
  - Basic selective media, basic plating, ATP swabs for spot-checking CIP
- Intermediate
  - Membrane filtration
  - Basic regimen of selective media and general growth media
    - Anaerobic
  - Classical microbial ID
  - Robust data analysis
  - In-house yeast propagation
- Expert
  - Robust regimen of selective media and general growth media
    - Anaerobic + aerobic incubation
  - Bioprospecting
  - Modern microbial ID techniques (PCR, sequencing, MALDI-TOF, etc.)
  - In-house cryogenic yeast masters

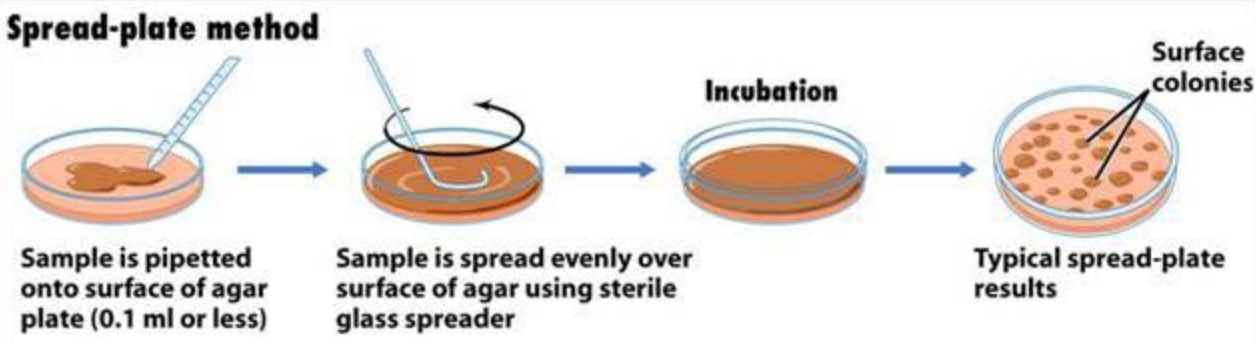
Intro

- Microscope
  - 40x objective
  - Hemocytometer
  - Viability/vitality dye
    - Citrate methylene blue, membrane permeability, industry standard, good consistency
      - Alternative: Trypan blue, membrane permeability, slightly better color distinction
    - Alkaline methylene blue, reduction potential, poor consistency
    - Alkaline methylene violet, reduction potential, best consistency



Basic

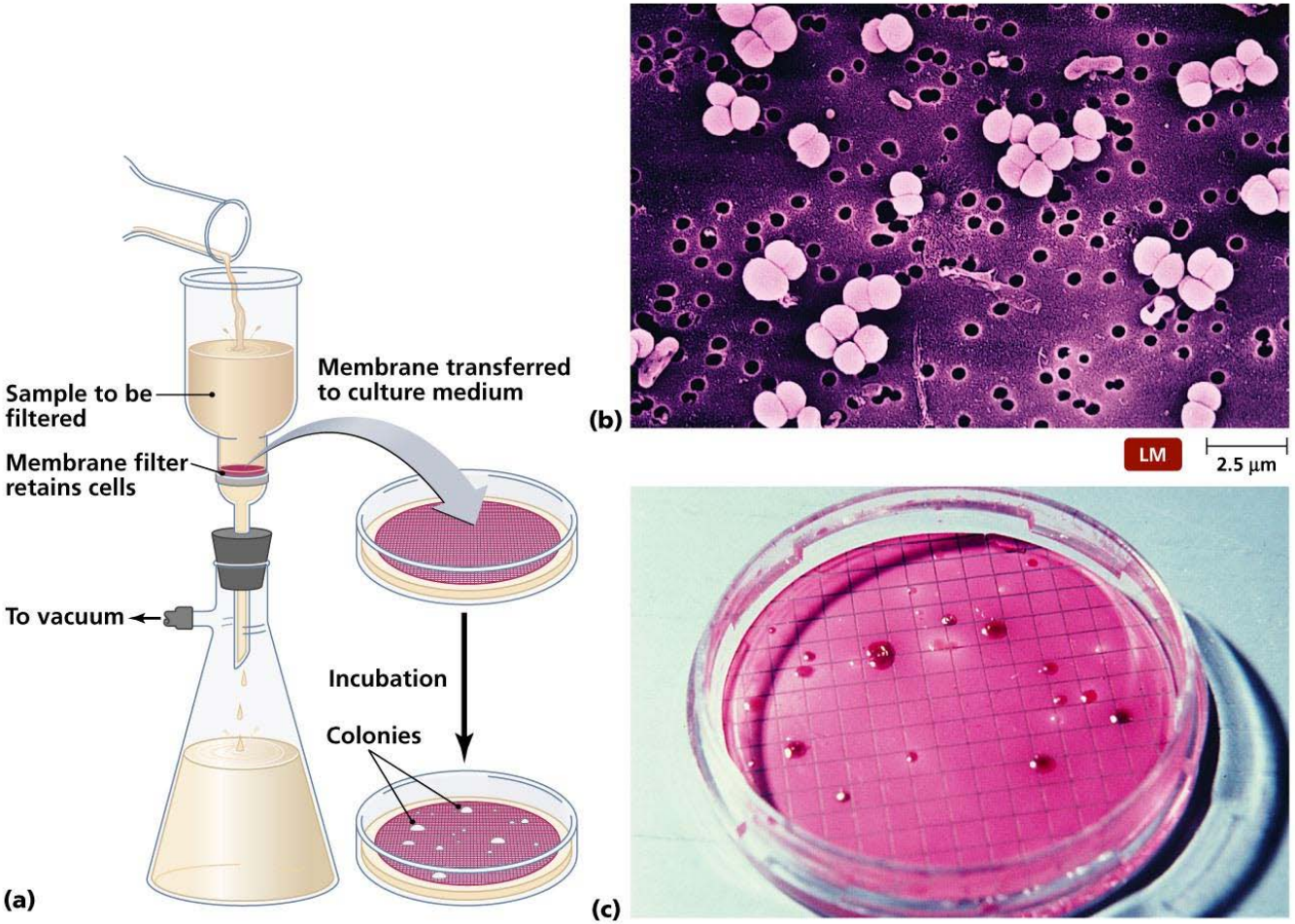
- Start getting into cleanliness testing – see ASBC webinar “introduction to brewing microbiology”
- Simplest, easiest selective media
  - HLP, screens for lactobacillus and pediococcus
- ATP swabs
  - verifies removal of organic material by CIP prior to sanitization
- Basic plating on growth media (spread-plate technique)
  - Very low sensitivity (only 0.1mL sample per plate)



Intermediate

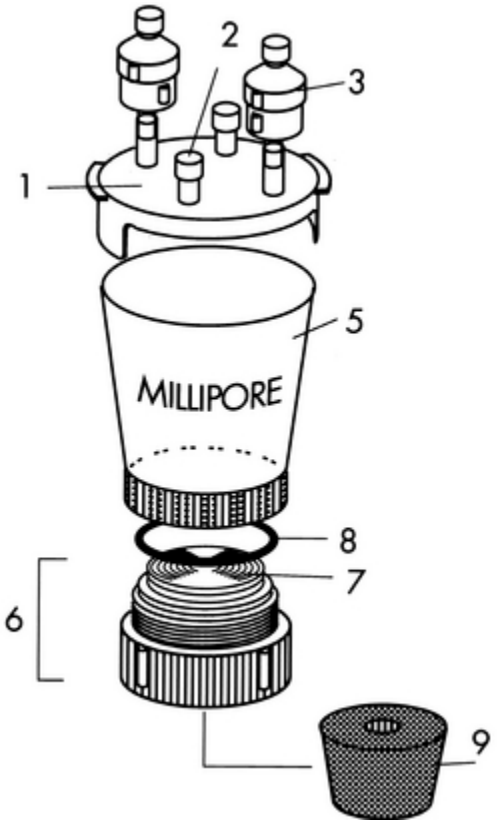
- Microscope with 100x objective and oil immersion lens
  - Can see bacteria
- Membrane filtration
  - High sensitivity (20-250mL sample)
- Basic selective media and general growth media regimen
  - Anaerobic incubation capability
- Classic microbial ID techniques
  - Gram stain
  - Catalase test
  - Oxidase test
- In-house yeast propagation
  - Cold storage of slants

Membrane Filtration



Membrane Filtration

- High sensitivity
  - 20-250mL sample per plate
  - 200-2,500 times more than spread-plate method!
- Millipore Sterifil makes cups with a lid
  - Helps if you don't have laminar flow hood or clean room
  - Currently achieving 95% accurate negative controls in a completely nonsterile environment
  - Relatively cheap. Assemble, autoclave, vacuum-filter.

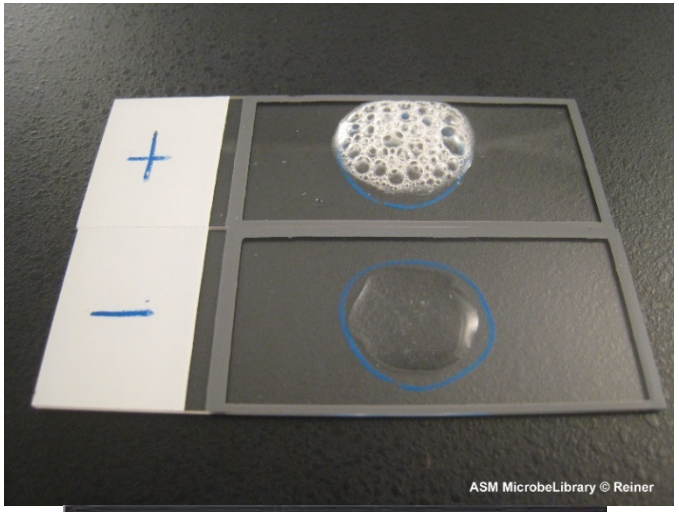


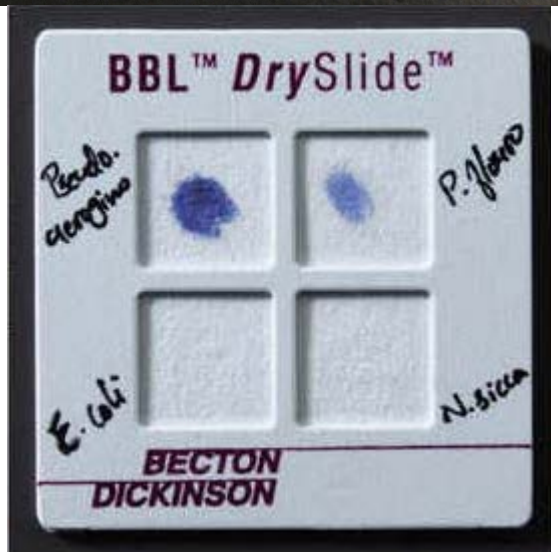
Media

- SDA/LMDA
  - High differential capabilities – easy colony distinction, acid indicator
- UBA
  - Beer-specific general growth media – very limited colony distinction
- WLN
  - Great yeast colony differentiation and acid indicator. Can select against some bacteria, not as good bacterial colony distinction
- HLP
  - Great lacto/pedio selection
- PIKA fast-orange
  - Very versatile spoiler detection
- Modified MRS
  - Great pectinatus selection
- LCSM
  - Great wild yeast selection
- LWYM
  - Doesn't work well
- Lysine
  - Great wild yeast selection
- YMA
  - Great for yeast propagations
- TTC Overlay
  - Great for detecting RD mutants
- SMMP
  - Great megasphaera/pectinatus selection

Classic Microbial ID

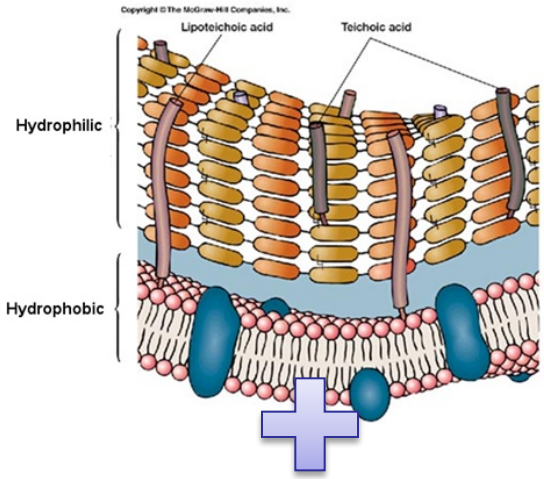
- Catalase test
  - Detects catalase enzyme on cell's surface
  - Protects from oxidative damage
- Oxidase test
  - Detects cytochrome oxidase
  - Key to respiration, aerobic metabolism

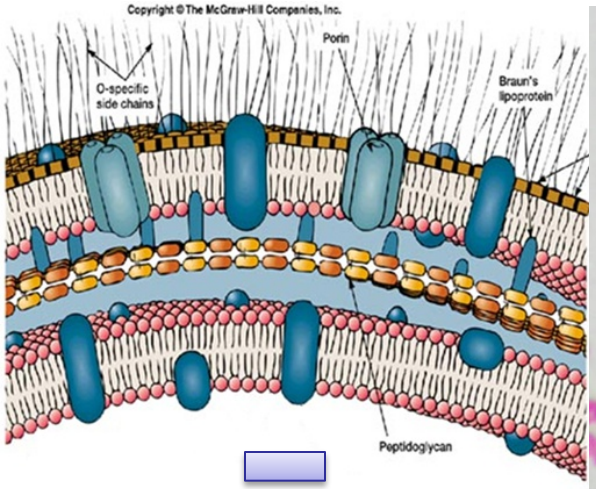


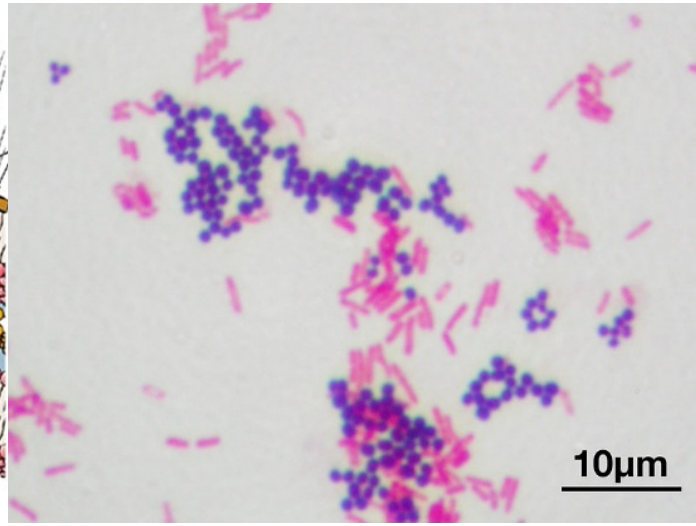


Classic Microbial ID

- Gram stain
  - Differential stain based on structure of cell wall
  - Gram positive – thick peptidoglycan layer
  - Gram negative – thin peptidoglycan layer

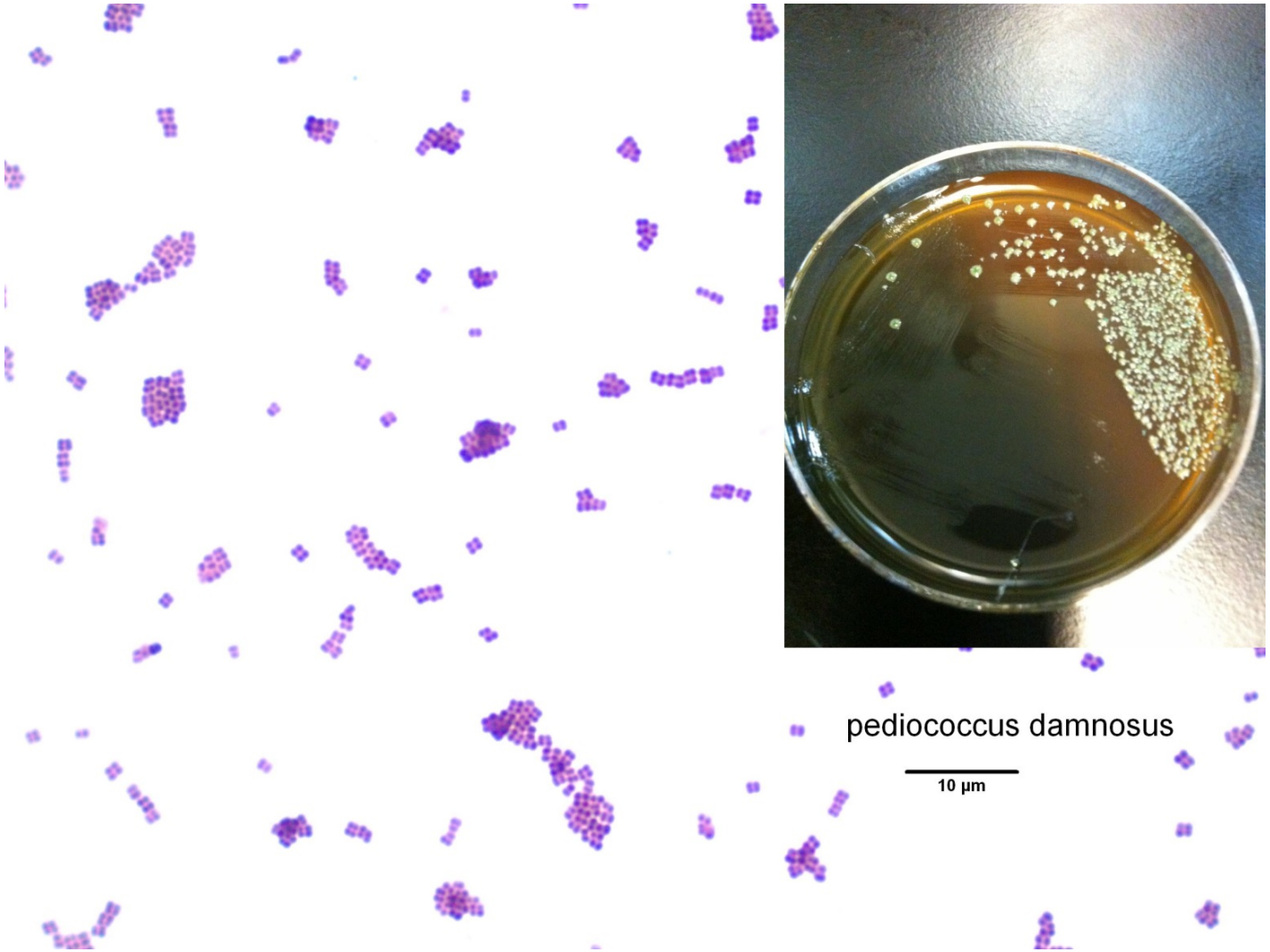


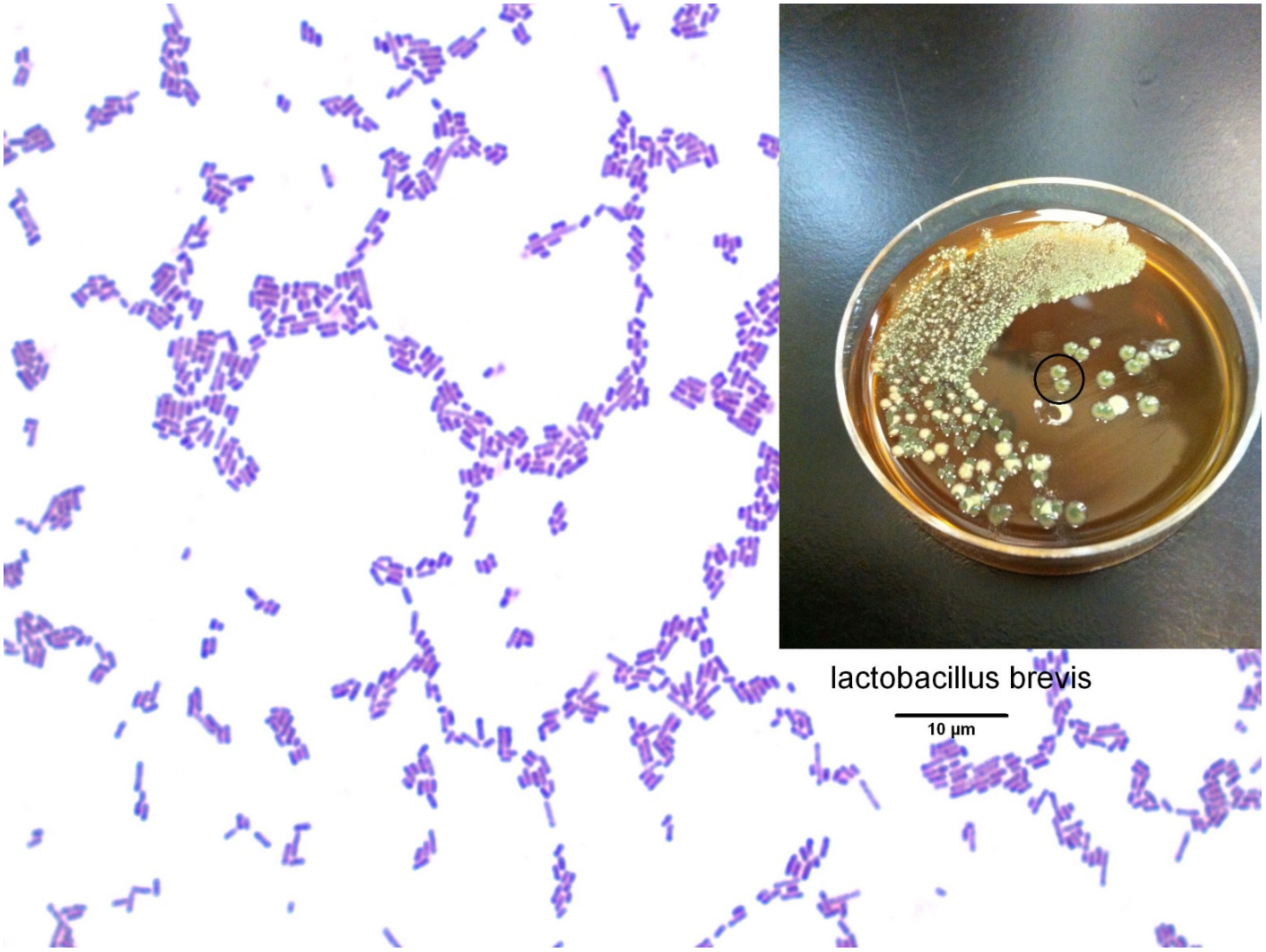




Classic Microbial ID

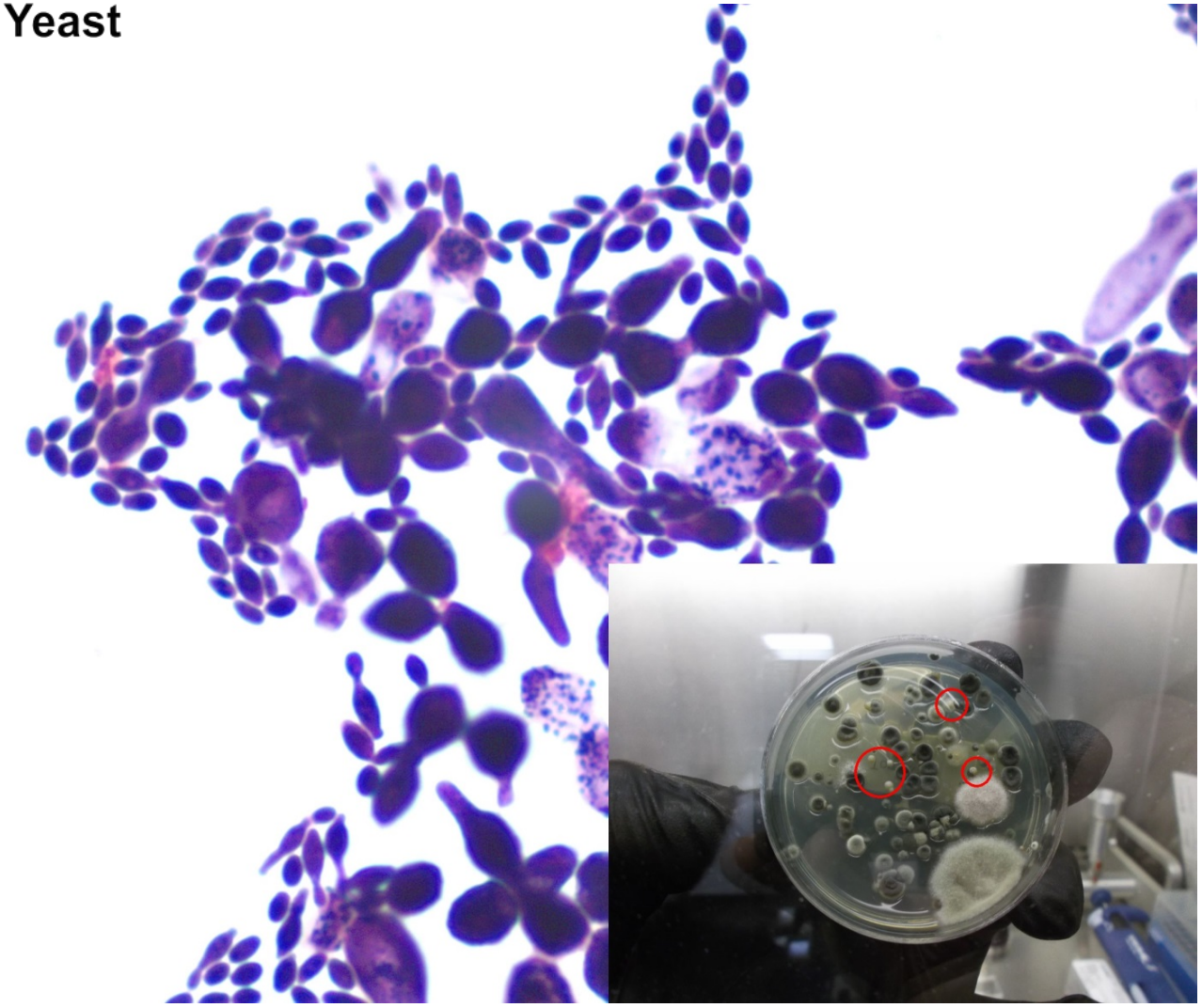
ID	Morphology	Growth Pattern	Gram	Cat	Ox
Acetobacter	rod/ellipsoid	singly, pairs, chains	-	+	-
Enterobacter	rod	pleomorphic	-	+	-
Glucanobacter	rod	pairs, chains	-	+	-
Lactobacillus	rod	Singly, pairs	+	-	-
Lactococcus	cocci	chains	+	-	-
Megasphaera	cocci	pairs, chains	-	-	-
Leuconostoc	cocci	chains	+	-	-
Pectinatus	curved rod	singly, pairs	-	-	-
Pediococcus	cocci	tetrads	+	-	-
Staphylococcus	cocci	clusters	+	+	-
Wild yeast	round, ovoid	pseudo-hyphae	+	+	+
Zymomonas	short, plump rods	pairs	-	+	-
Kocuria	cocci	tetrads, clusters	+	+	+


pediococcus damnosus
10 µm

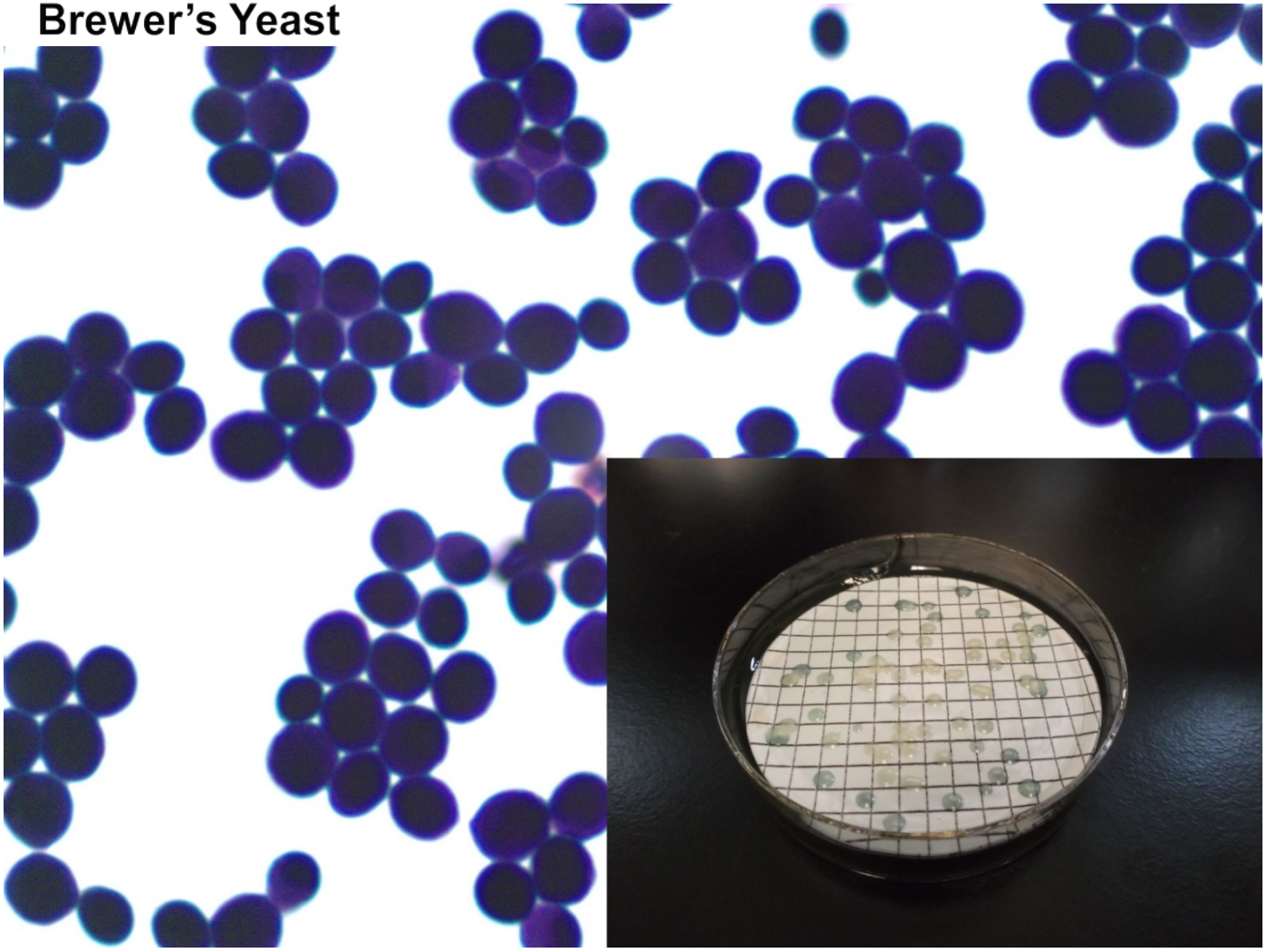

lactobacillus brevis
10 µm


glucanobacter xylinus
10 µm

Wild Yeast



Brewer's Yeast



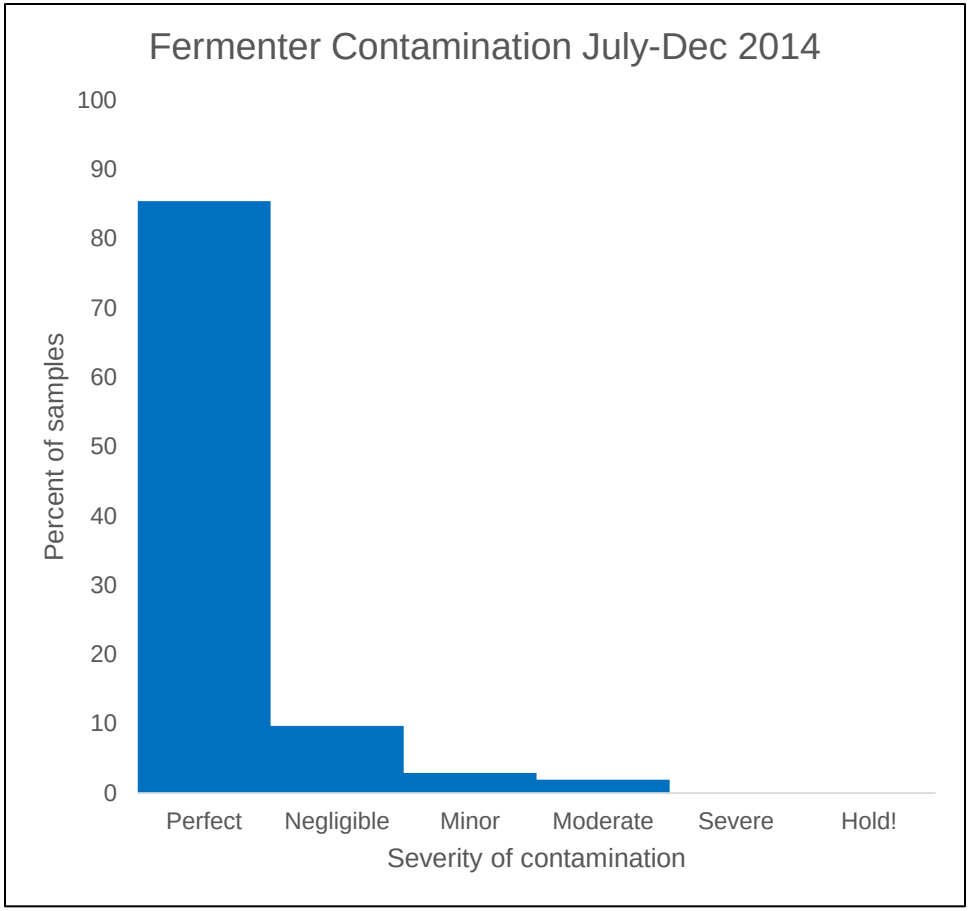
Data Analysis

- Data analysis goal: transition from reactive → proactive
- Reactive: hard to use micro as QC. Incubation time puts you at a disadvantage.
- Spoiler reaction plan: hold, force age, extra analysis (3rd party ID?), destroy/release, assess spread
- Proactive: use other microbes as indicators to identify hotspots; QA/prevention
- Very difficult to quantify an organic system
  - CFU's matter
  - Species matter
  - Variety of microbes matter
  - Acid production, spoiler-type characteristics matter

Data Analysis

- Make a histogram; example categories below
  - 0: Perfect – perfectly clean
  - 1: Negligible – few CFU, single harmless organism
  - 2: Minor – few CFU, several species / handful of CFU, single harmless organism
  - 3: Moderate – minor acid production, significant CFU, or multiple species
  - 4: Severe – significant acid production, significant CFU, and spoiler-esque characteristics
  - 5: Hold – confirmed beer spoiler

Data Analysis



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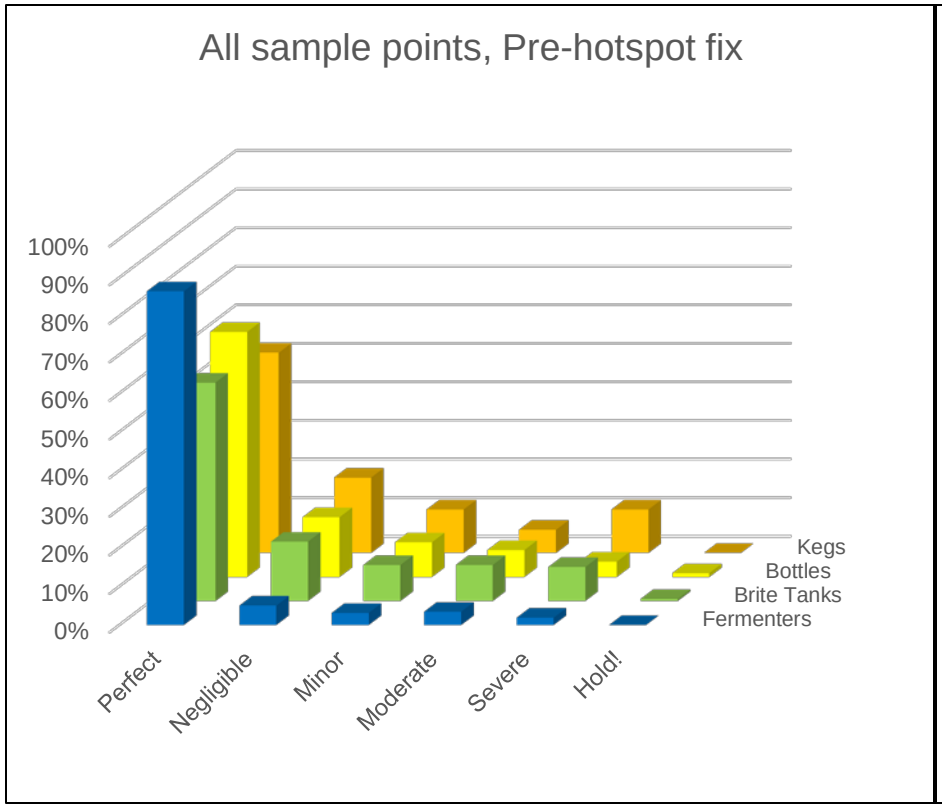
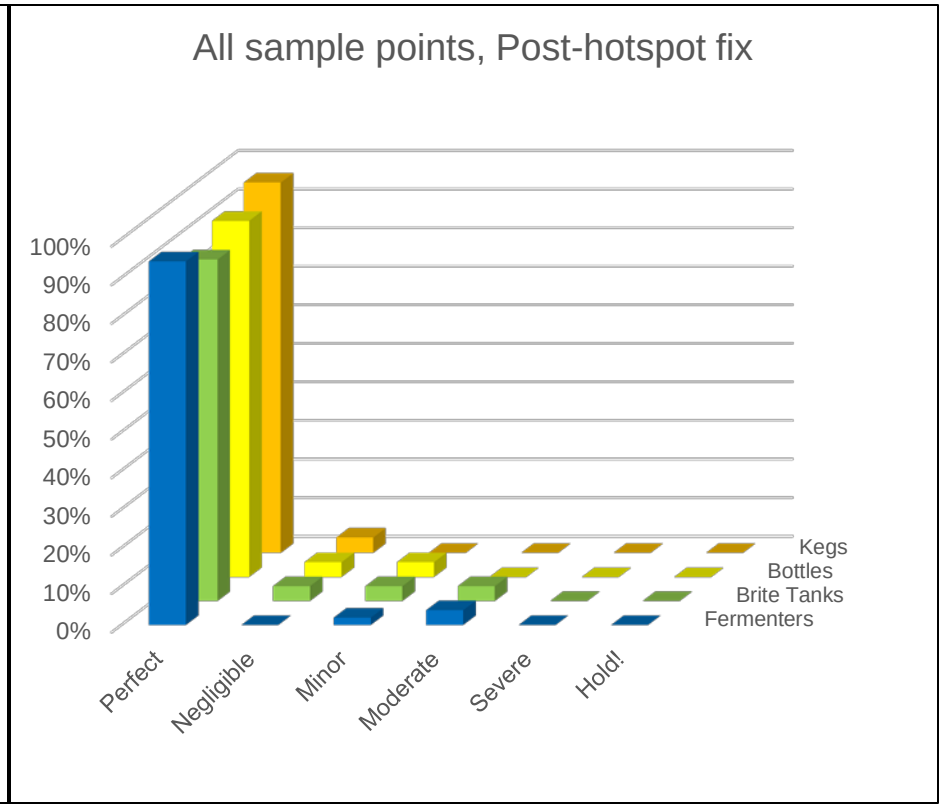
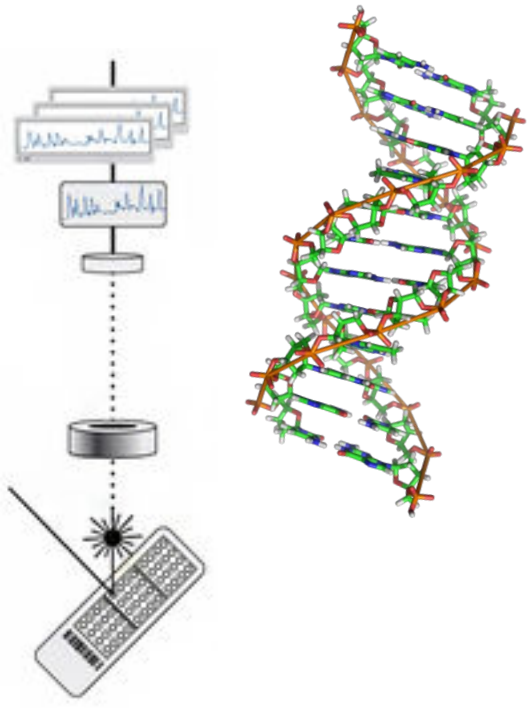




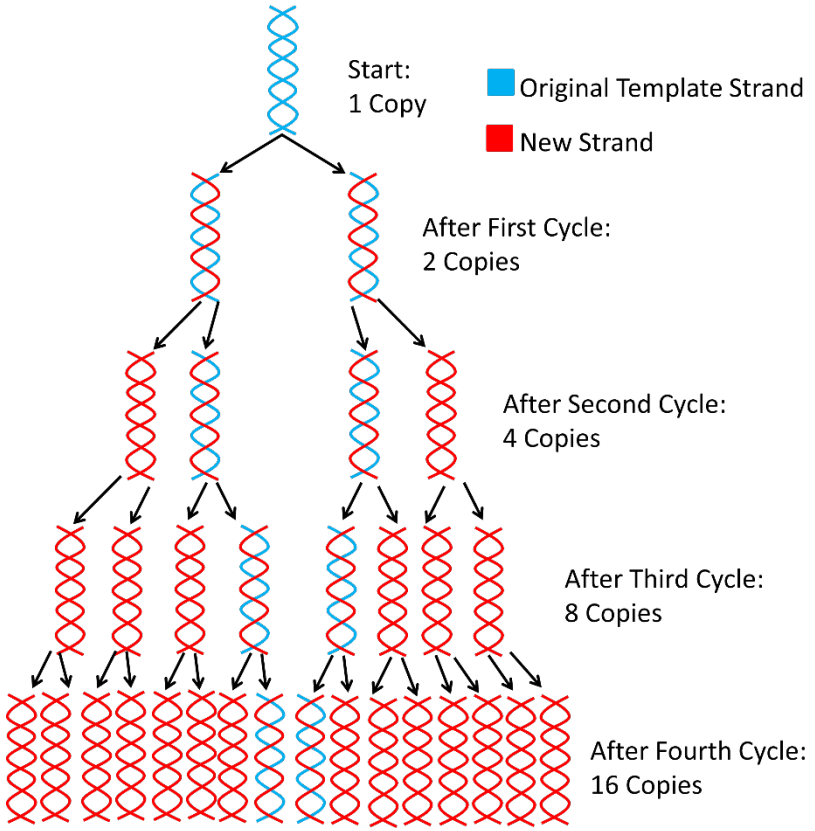
Expert

- Robust regimen of selective media and general growth media
  - Anaerobic + aerobic incubation
  - SDA/SDA+, LCSM/Lysine, modified MRS, HLP/Pika, TTC overlay
- Modern detection and ID
  - PCR
  - Sequencing
  - MALDI-TOF
- Colony isolation/bioprospecting
- In-house cryogenic yeast masters



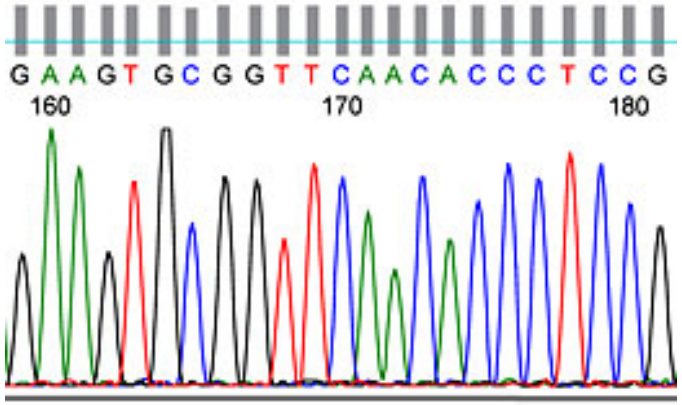
Modern Microbial ID

- PCR
  - Highly sensitive
  - Cannot ID everything, just +/- for what the kit is designed to find
  - Good kits will get you species-level ID for positives
  - Many kits still require incubation
  - Be smart when buying your kit – you need internal controls for each reaction well. Your dilution still matters!
  - Ease vs. functionality
    - Control, melting curves, etc.



Modern Microbial ID

- Sequencing
  - Species- or even strain-level ID
  - Run against genome database for matches



Modern Microbial ID

- MALDI-TOF MS
  - Matrix-assisted laser desorption/ionization-time of flight mass spectrometry
  - Species- or strain-level ID for anything in database

HOW MALDI-TOF works

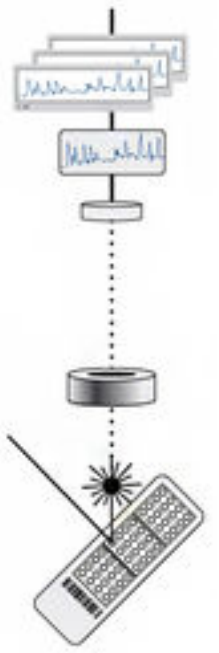
1. The target slide is prepared and introduced to a high-vacuum environment.

2. A precise laser burst ionizes the sample.

3. A “cloud” of proteins is released and accelerated by an electric charge.

4. After passing through the ring electrode, the proteins’ Time of Flight is recorded using a formula from the time recorded.

5. Proteins are detected with a sensor to create a spectrum that represents the protein makeup of each sample.



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- Lee, S.Y. 1994. SMMP – A Medium for Selective Isolation of *Megasphaera* and *Pectinatus* from the Brewery. Journal of the ASBC. 52(3). Golden, CO.

Images

- Evolution of fermentation understanding “march of progress”
  - http://res.freestockphotos.biz/pictures/15/15807-illustration-of-a-silhouette-of-a-laboratory-researcher-pv.jpg
  - http://i.istockimg.com/file_thumbview_approve/18804737/3/stock-illustration-18804737-alcoholic-and-trump-silhouette.jpg
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  - http://static3.depositphotos.com/1004525/180/v/950/depositphotos_1807077-Man-with-a-bottle-drinking-silhouettes.jpg
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  - http://intranet.tdmu.edu.ua/data/kafedra/internal/micbio/classes_stud/en/pharm/prov_pharm/ptn/Microbiology%20with%20basis%20immunology/2/Lesson%203.%20The%20main%20methods,%20principles%20and%20steps%20of%20isolation%20of%20bacteria%E2%80%99s%20pure%20cultures.files/image006.jpg
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  - http://www.ohsu.edu/xd/research/centers-institutes/vollum/images/vollum_dnacore_1.jpg

Thank you!

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- Next up: Karen Fortmann, Ph.D.
  - 3rd party validation

Data Analysis