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Fuel Ethanol Fermentations A Race to the Finish

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Fermentations: An Ecosystem

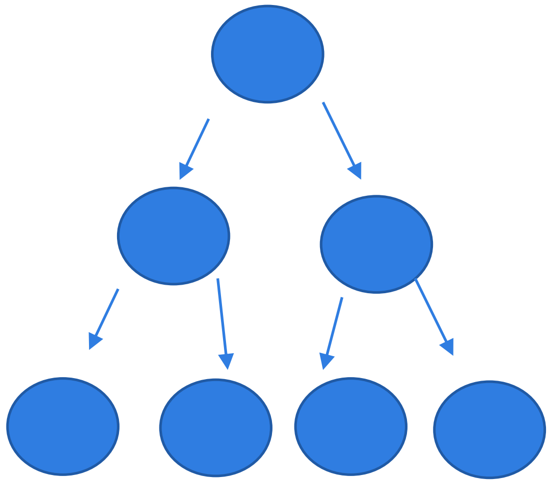
Ecosystems

- Boundaries
- Physical Properties
  - Temperature Range
  - pH range
  - Weather
- Energy flows in & out
- Nutrients
  - Calories
  - Micronutrients
  - Macronutrients
- Occasional disturbances
  - Fire
  - Flood
  - Extreme Temperatures
- Dominant Species
- Species within a system interact with each other and the environment

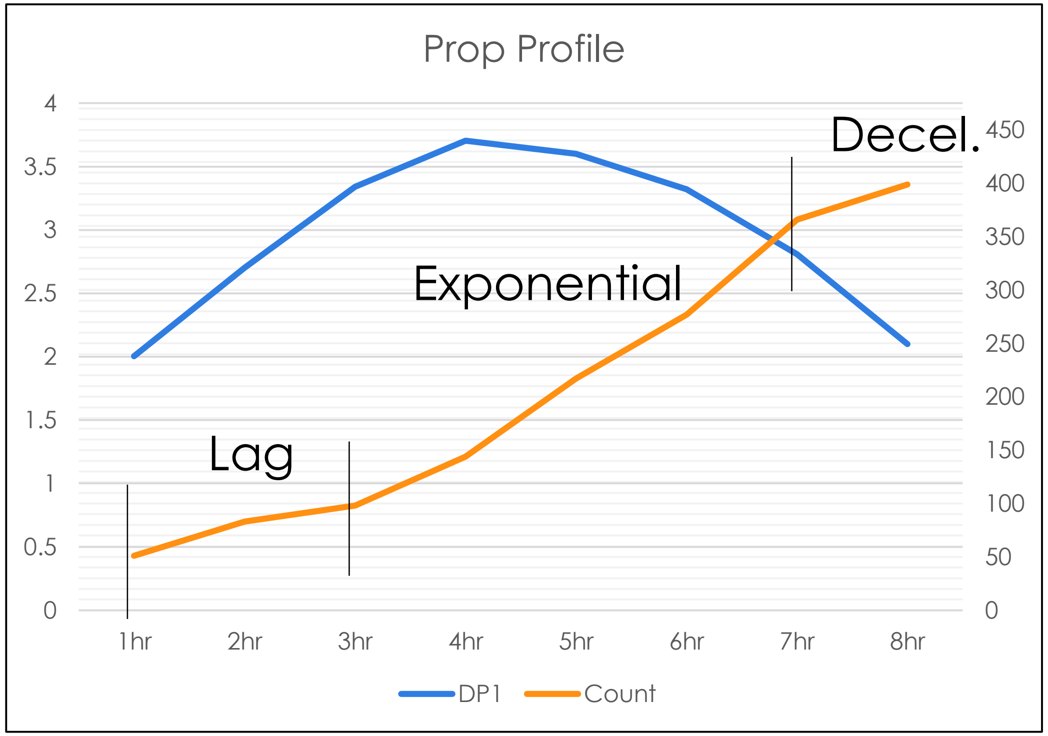
Ethanol Fermentations

- Bound by a Tank Wall
- Specific Physical Properties
  - 88 to 94 F
  - 4.8 to 5.2 pH (dry mill)
  - 3.8 to 4.5 pH (wet mill)
- Energy flows in & out
- Nutrients
  - Glucose
  - Micronutrients
  - Macronutrients
- Occasional disturbances
  - Pitch
  - Contamination
  - Temperature excursions
- *Saccharomyces cerevisiae*
- Species with a reactor interact with each other and the environment

Ethanol Fermentation – Race to the Finish



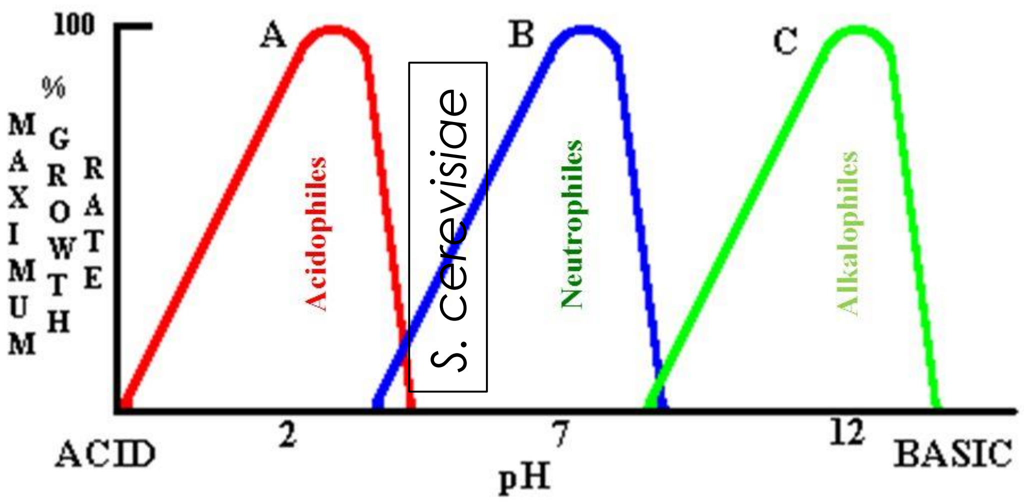
Cell growth is described as a logarithmic (exponential) progression



Growth Limited by Process Conditions

- There are billions of microbial species, why don't they all grow in an ethanol fermenter?

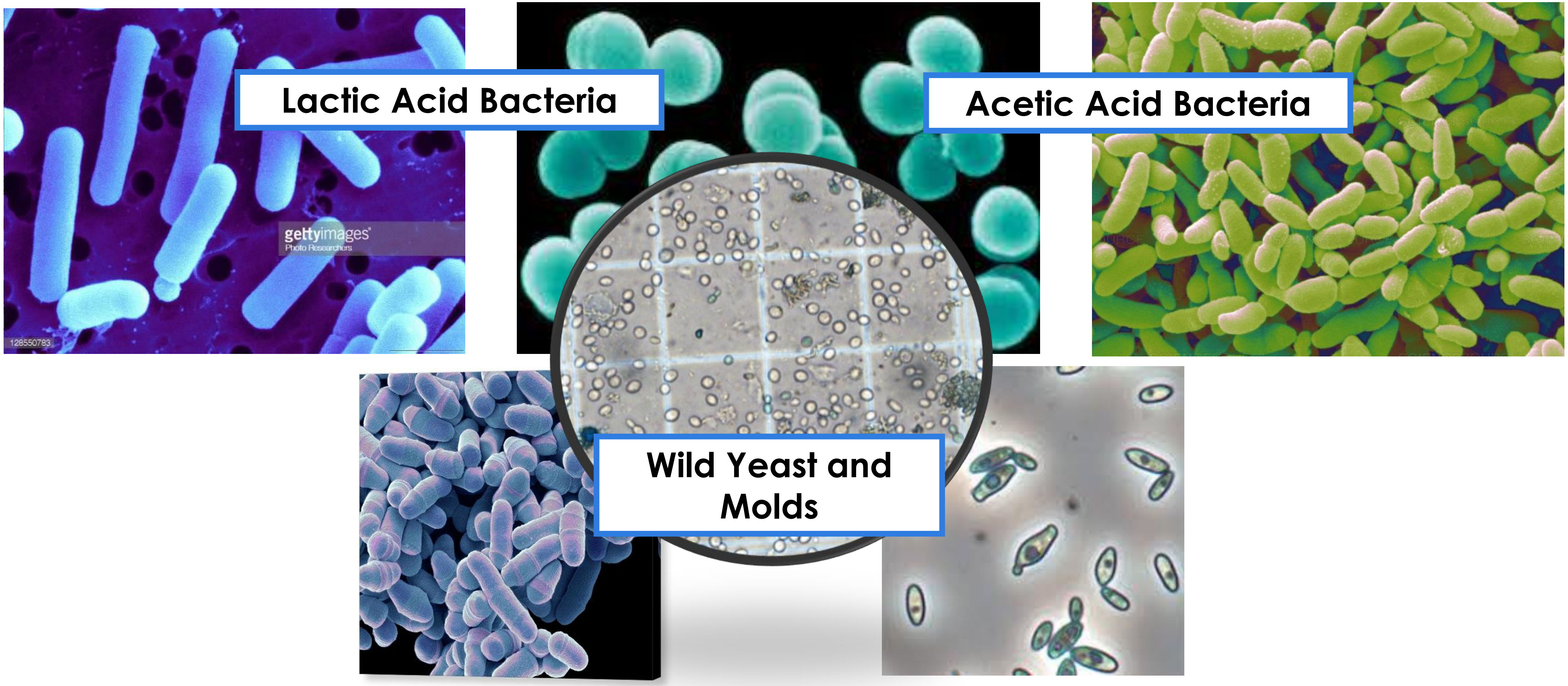
Physical Requirements: pH



- Most bacteria grow between pH 6.5 and 7.5
- Molds and yeasts grow between pH 5 and 6

pH limits
Temperature limits
TOLERANCE TO ETHANOL
Nutrients
Oxygen limitation (production vessel)

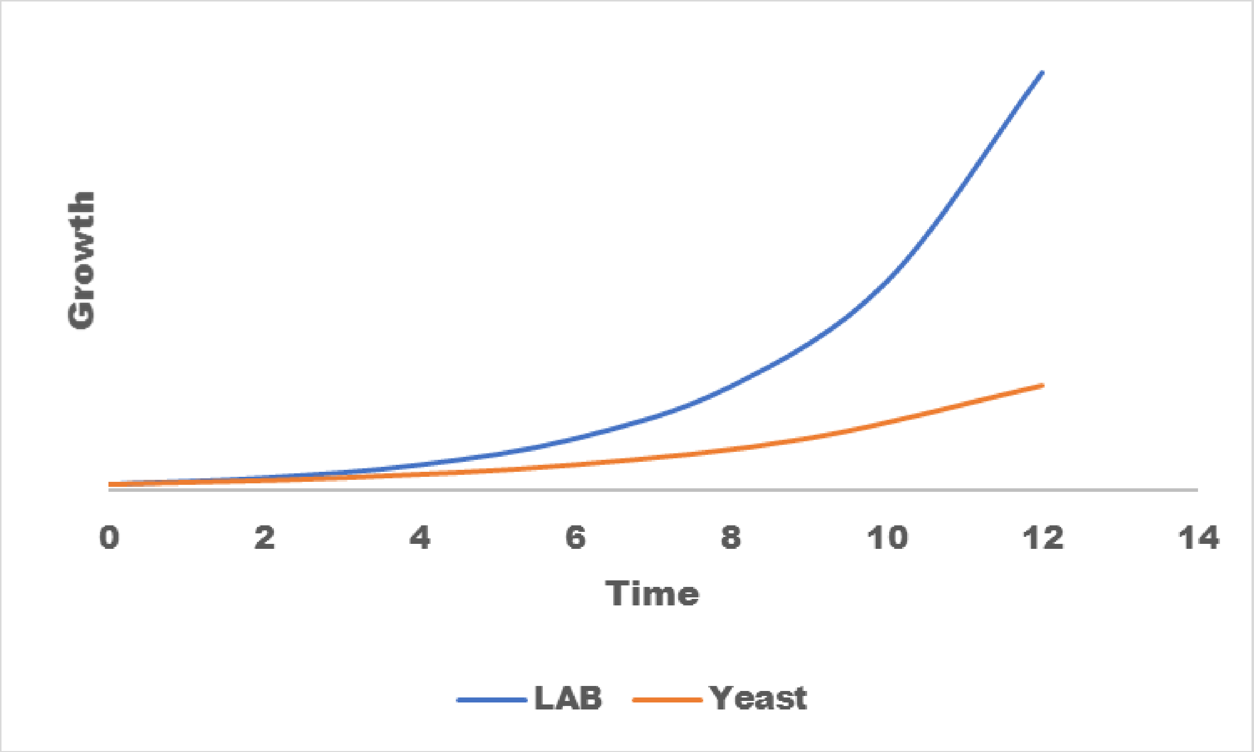
Ethanol Contaminant Challenges



Images courtesy of www.sciencesource.com.

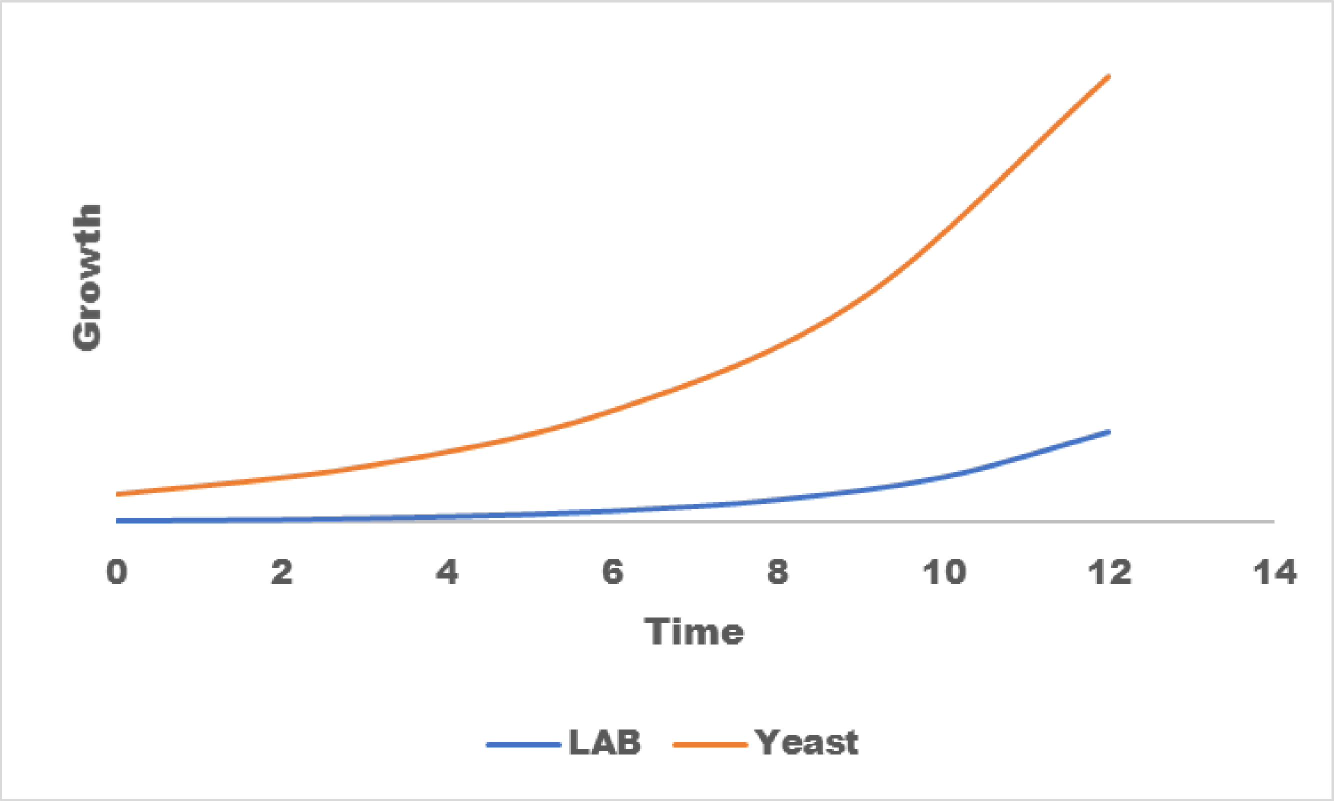
Who wins the race? The Speediest

- Under optimal conditions, the organism that grows the fastest wins
  - Outcompete for nutrients
  - Generate product which may inhibit competitor
- Generation time: time it takes for a population to double its numbers during exponential phase
  - Lactic acid bacteria: 25-45 min
  - *S. cerevisiae*: 75-150 min

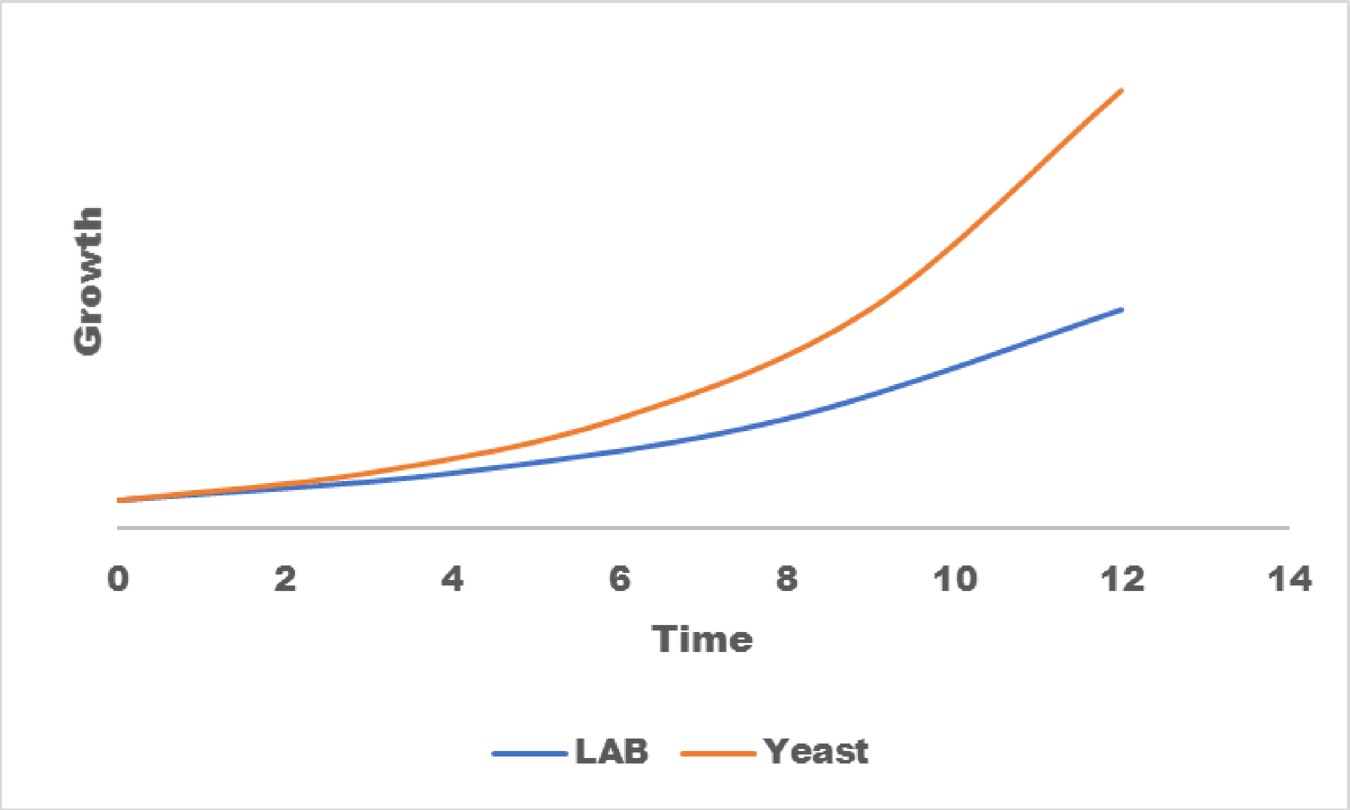


Race to the Finish: Giving the Yeast a Head Start

- A large difference between yeast and bacterial cell count numbers gives the yeast the advantage
- Keep initial bacterial counts low (CIP)
- Keep initial yeast counts high (yeast dose, propagation optimization)



Race to the Finish: Bacterial Hurdles



- pH
- Ethanol
- Temperature
- Antibiotics
- Oxygen limitation

Cell load at start is equal but now yeast grow faster than bacteria

Microbial Control

Antimicrobial

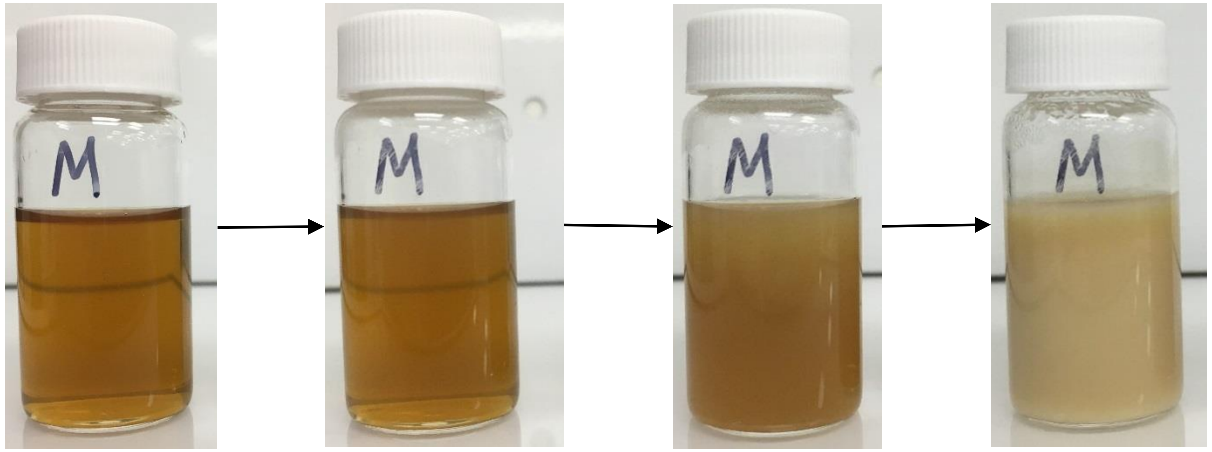
- A chemical agent that kills or inhibits microorganisms
  - Ethanol
  - Chlorine Dioxide
  - Strong Acid
  - Strong Base
  - Antibiotic
  - Heat: Physical not chemical.
- Non-specific (except antibiotics)
  - Effective against all with little resistance; careful dosing

Antibiotic

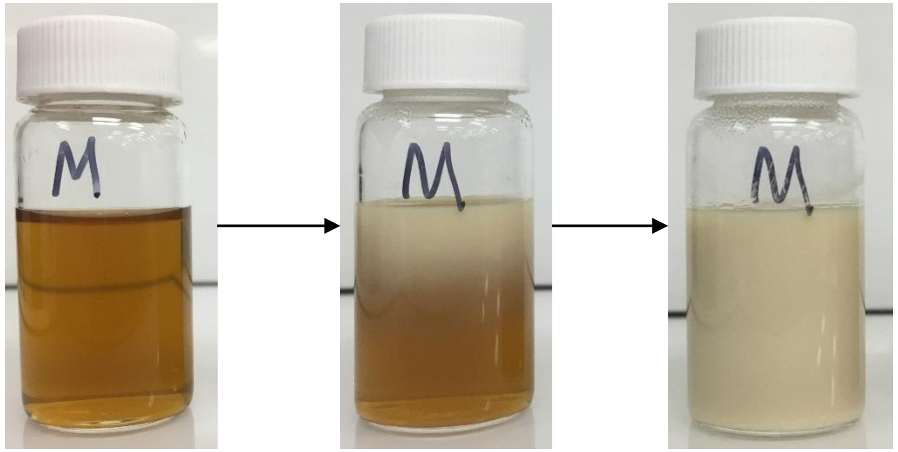
- A chemical substance produced by a microorganism that kills or inhibits other microorganisms
  - Penicillin (Pen-G)
  - Cephalosporins (Keflex)
  - Fluoroquinolones (Cipro)
  - Macrolides (Erythromycin)
  - Aminoglycosides (Neomycin)
- Specific targets
  - Not effective against all microbes, increased chance of resistance, wider dosing ranges

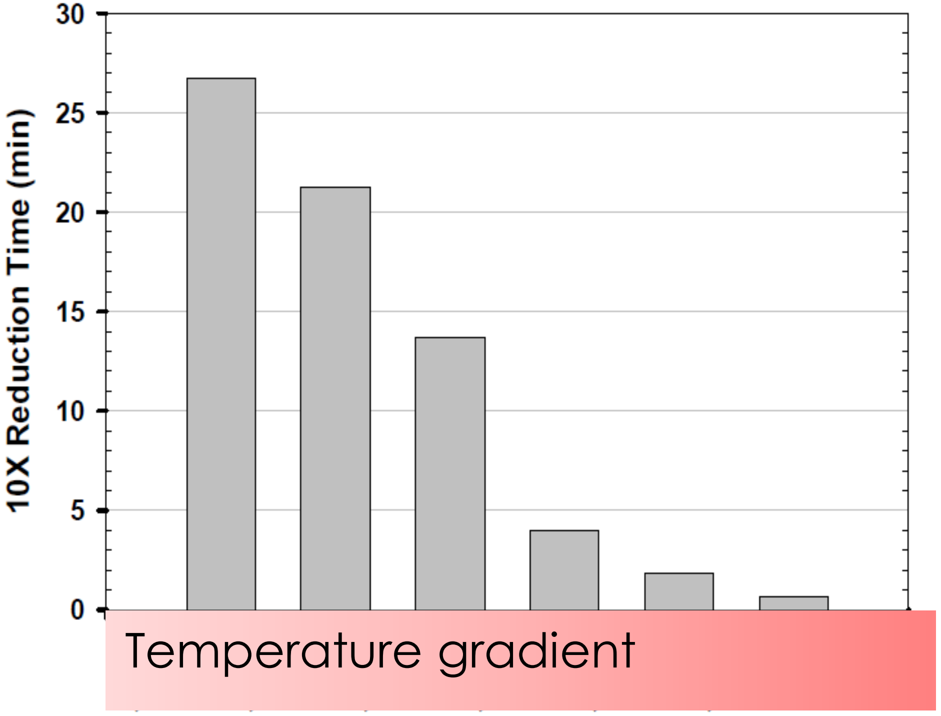
How Heat Kills

	0 min	1 min	2 min	5 min
160F				



	0 min	1 min	2 min
185F			





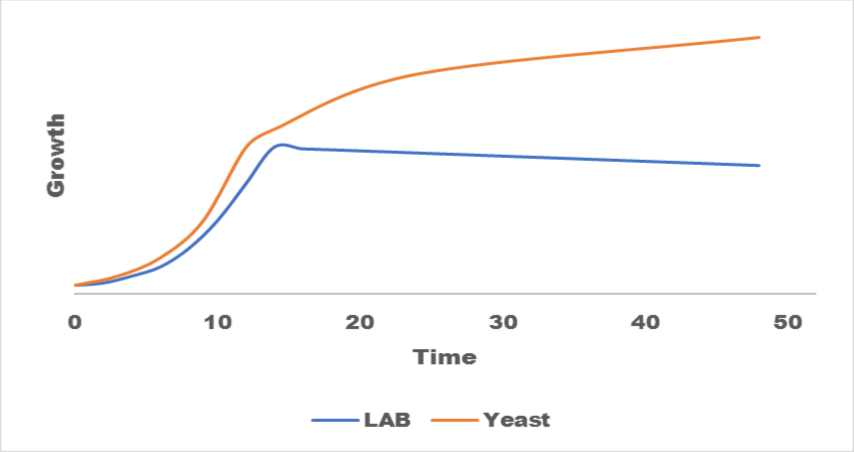
Antibiotics in the Industry

Antibiotic	Class	Mode of Action	Target Organisms
Penicillin	β-lactam	Inhibition of cell wall biosynthesis	Gram positive bacteria
Virginiamycin	Streptogramin	Inhibition of protein biosynthesis	Broad spectrum of bacteria

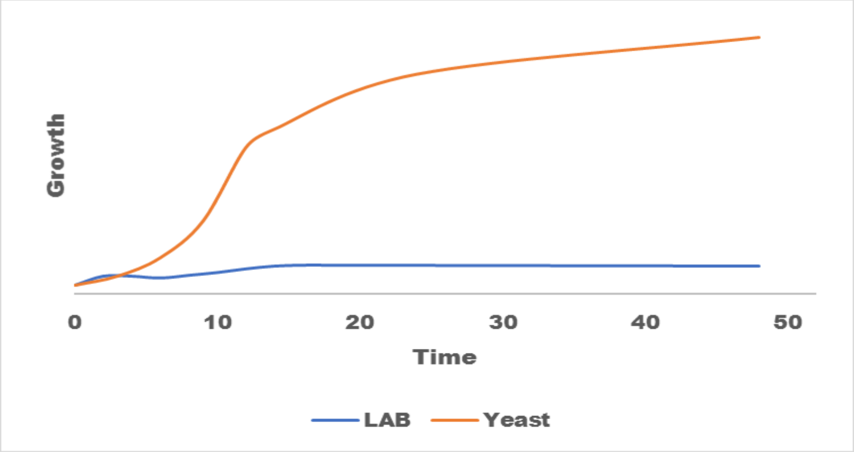
- Penicillin prevents strengthening of the bacterial cell wall
  - Gram positive bacteria (*Lactobacillus, Enterococcus, Pediococcus*) are highly dependent upon their cell wall for structure and reproduction
  - Gram negative bacteria (*Acetobacter, Gluconobacter, Enterobacteriaceae*), possess an outer membrane which reduces dependence upon cell wall strength
- Virginiamycin prevents the production of proteins and enzymes
  - All bacteria need to synthesize proteins and their ribosomes are specifically susceptible
  - Prevents bacteria from growing, but doesn't immediately kill
- Both are only effective on actively growing cells

LAB Contamination Comparison

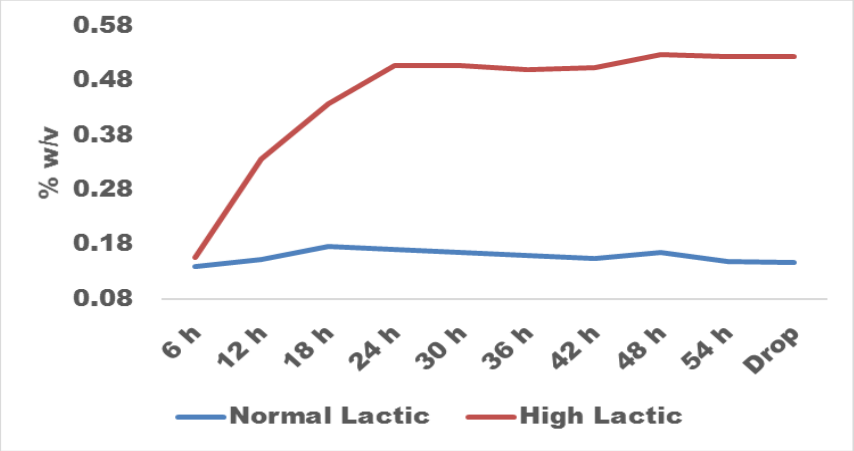
- High Lactic Growth Profile



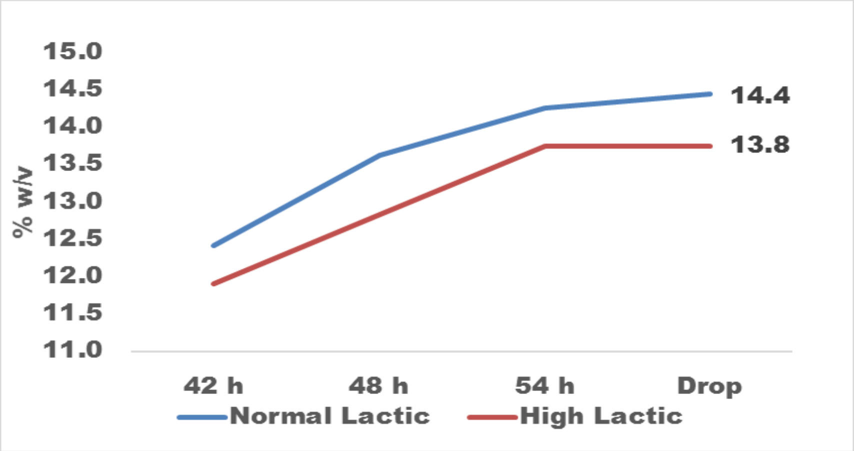
- Normal Lactic Growth Profile



- Lactic acid HPLC Profile



- Ethanol HPLC Profile

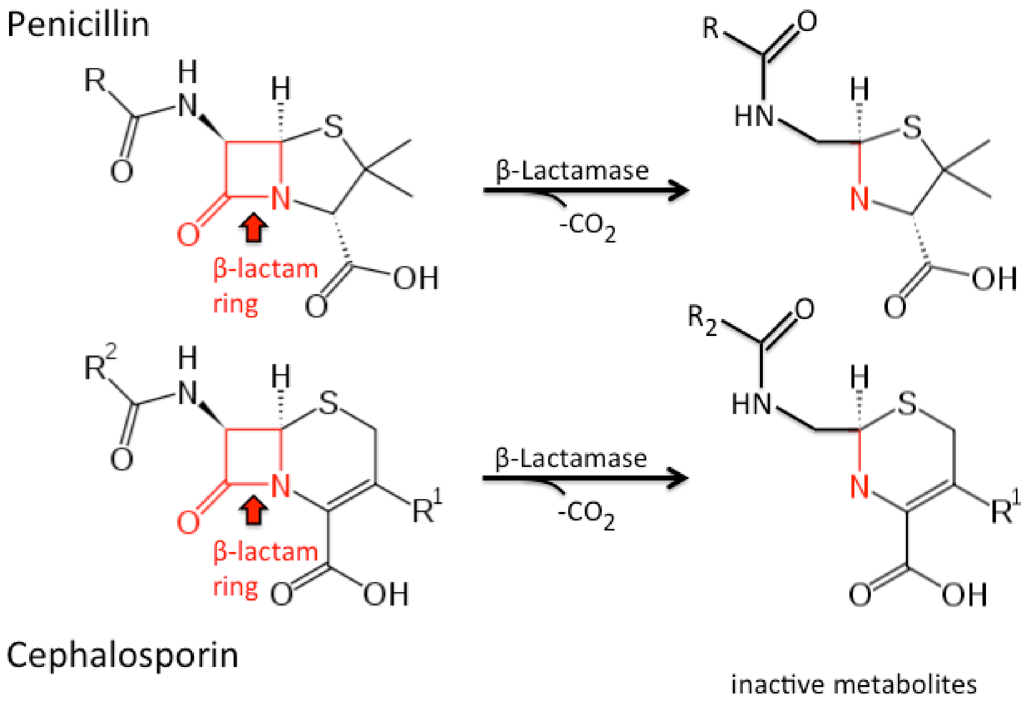


Microbial Resistance to Antibiotics

- Driven by Natural Selection
  - A selective pressure is applied to a population: bacteria growing in the presence of an antibiotic
  - An individual acquires a characteristic that confers resistance (transferred from another organism, random mutation, etc.)
  - New organism is most "fit" and overtakes previous population: resistant bacterial strain reproduces faster than the original population
- Antibiotic resistant bacteria lead to 25,000 deaths/yr
- Wide use of Penicillin in agriculture started in 1958, penicillin resistant bacteria isolated in 1962

Examples of Antibiotic Resistance

- Active efflux
  - *Pseudomonas aeruginosa*
- Enzymatic modification of antibiotic
  - β-lactamase
- Alteration of target site
  - Virginiamycin resistance by altering the ribosome to decrease antibiotic binding



- Usually reversible
  - Once the selective pressure is removed (stop using or change antibiotics), bacteria will lose their resistance over time
  - The non-resistant strain becomes the most "fit" and overtakes the resistant population

Plant Hygiene

Bacteria – Good at Hiding

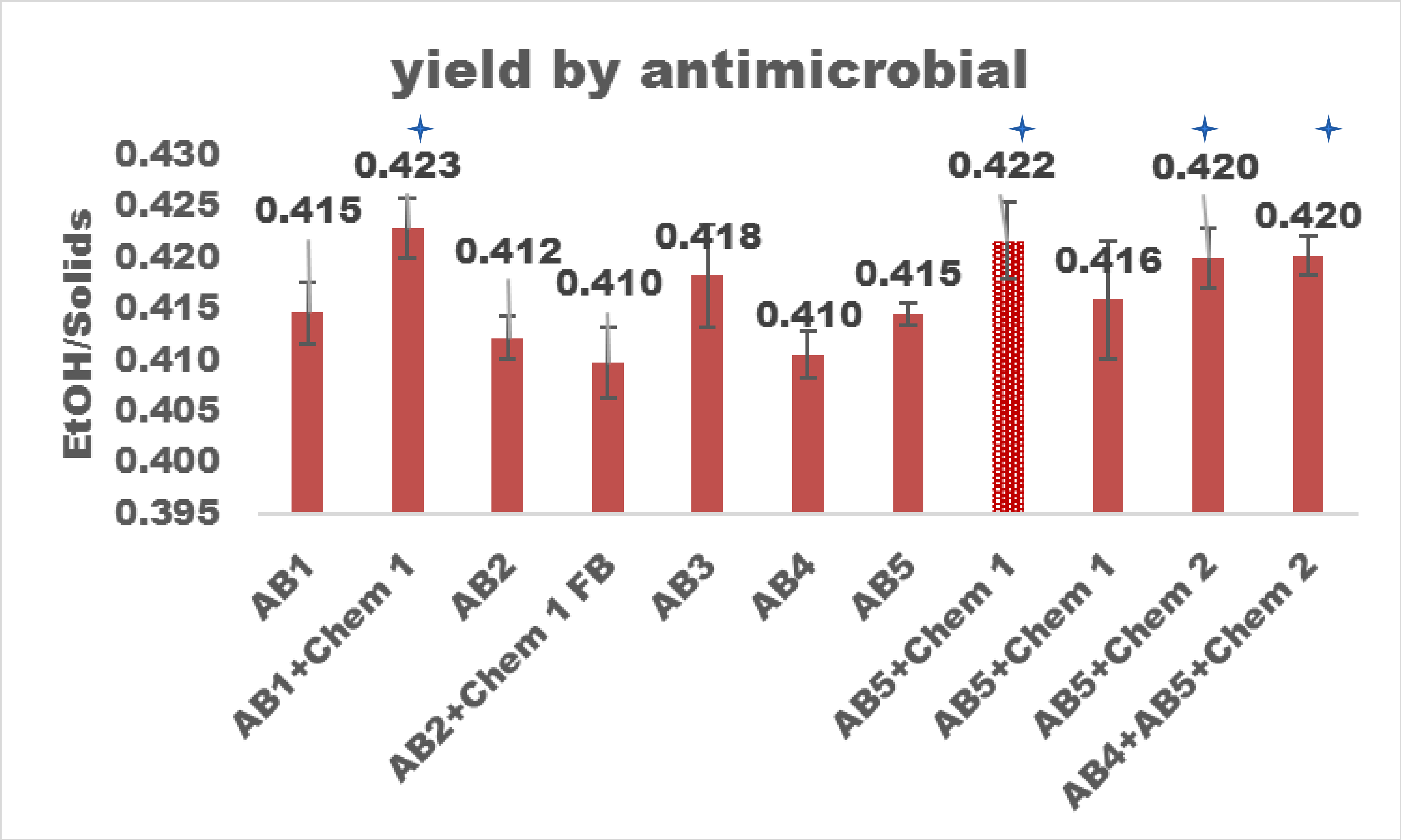
- The real world isn't sterile, but limiting entry points can go a long way
  - Clean surfaces that may act as sources of inoculum; heat exchangers, foamed into CO_2 scrubbers, etc.
  - Just because you can wash a dead bird into your sump doesn't mean you should
- Plants are aging and bacteria hide well
  - Microcracks in vessels, valve seals, etc.
  - Increased surface area
- Once a contaminant has established in a plant, it is very difficult to displace
- "Crud" is very good at protecting microorganisms
  - "You won't believe this, I found bacteria in my caustic tank entombed within some slimy material"
  - Heat penetration is key – run hot and long CIP
- Caustic breaks down organics but can make metallic deposits worse
  - Caustic is not good at breaking down fats
- Some acids are good at breaking down metallic deposits but may make organic deposits worse
- Rotate cleaning agents

Resolve or Control

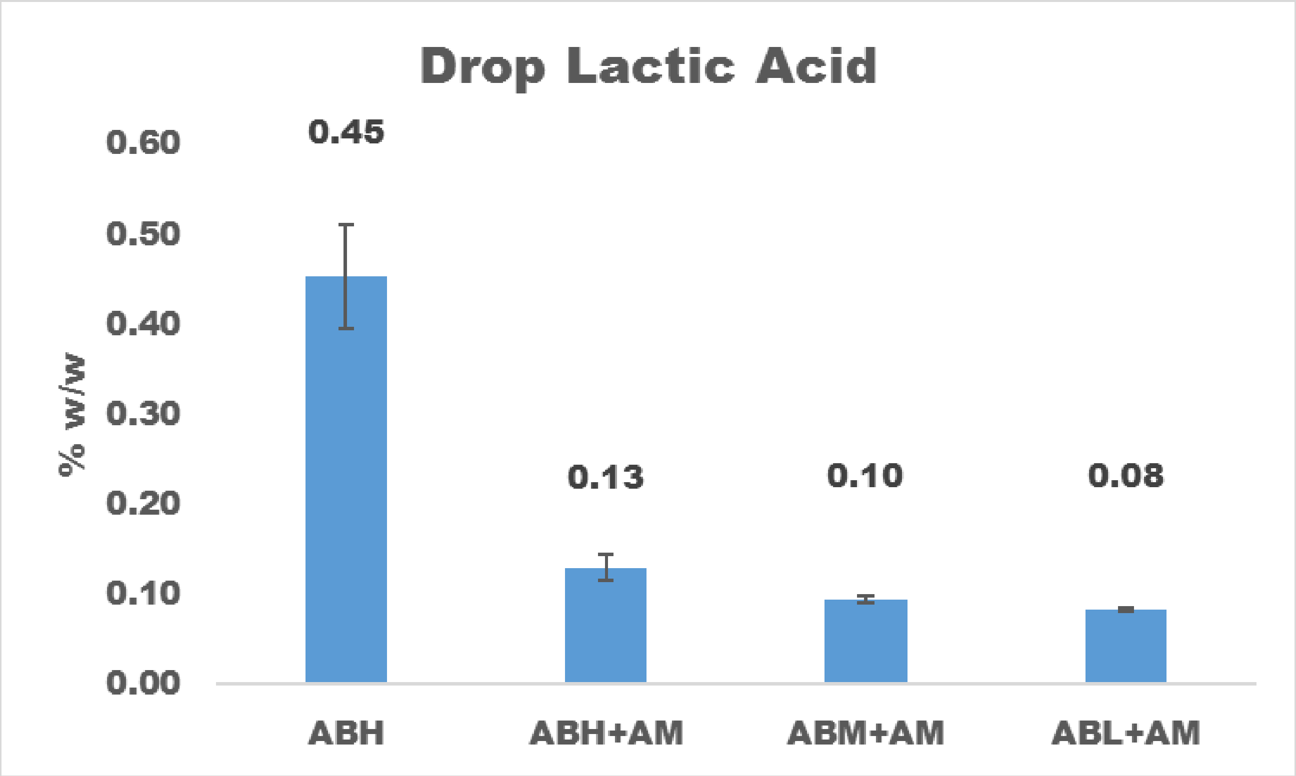
- Talk to your antibiotic vendor and rotate antibiotics – you must rotate into different families of antibiotics
- Sample your plant and plate for antibiotic resistant bacteria
  - Target antibiotics that actually inhibit your infection
- Consider a combination of antibiotics with chemical kill agents
- Track key fermentation metrics by antimicrobial agent
  - Be realistic about your statistics. Small sample size cannot distinguish between “true” and “noise”
    - 11+ drops to be confident in a conclusion

Sample	LAB counts (cfu/g)
Slurry	$<10^5$
Liq out/Exch. in	$<10^5$
Exch. out/Cooler in	$<10^5$
Cooler out	1.15×10^7
Ferm 1 (early)	3.90×10^6
Ferm 2 (early)	7.44×10^6
Ferm 3 (late)	$<10^5$
Ferm 4 (mid)	5.4×10^5
Beerwell	$<10^5$
Propagator	1.42×10^7

Combinations of Antimicrobial Agents: Yield



Combinations of Antimicrobial Agents: Lactic Acid



Summary conclusions

- This is a complex struggle in nature. It won't be a quick win
- Clean thoroughly
- Don't skimp on CIP
- Rotate antibiotics
- Consider other antimicrobial agents
- Track key fermentation metrics vs antimicrobial agent

Acknowledgements

- Stephanie Gleason
- Bradley Plantz

Thank You for Your Time

Questions?