



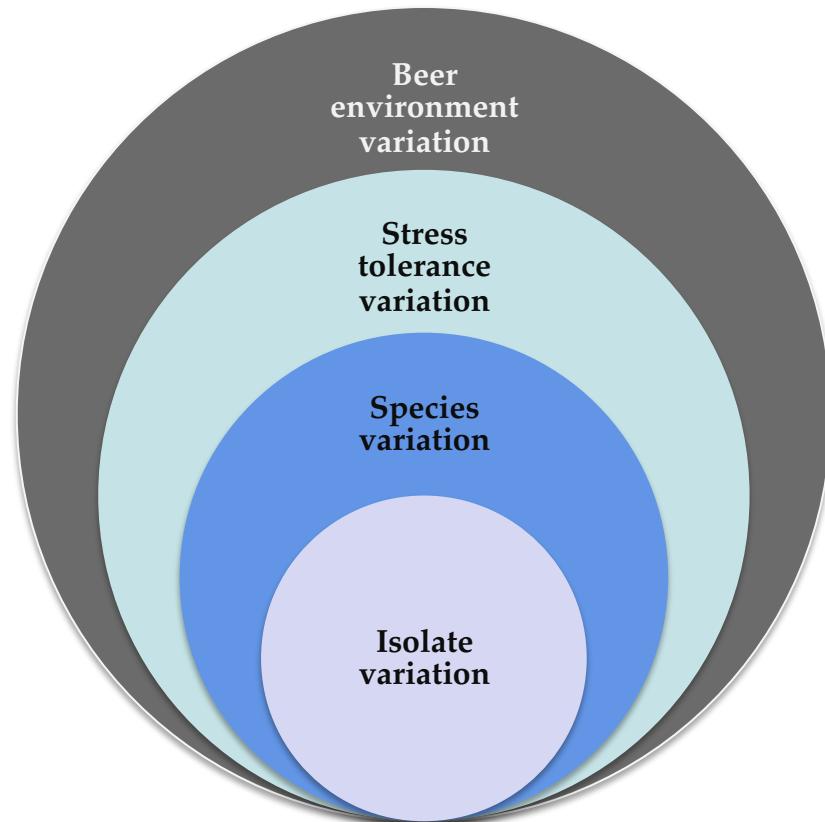
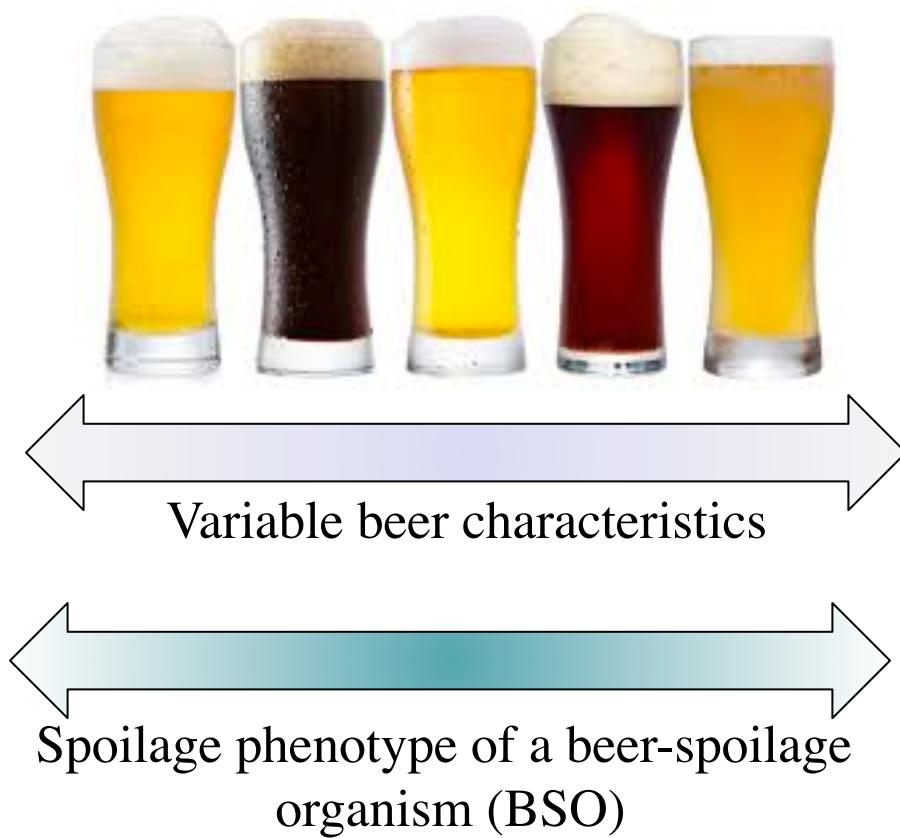
Genetic adaptations for lactic acid bacteria beer-spoilage revealed by RNA sequencing

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April 23rd, 2016

YSS, Chico CA

Beer-Spoilage \neq Binary Phenotype

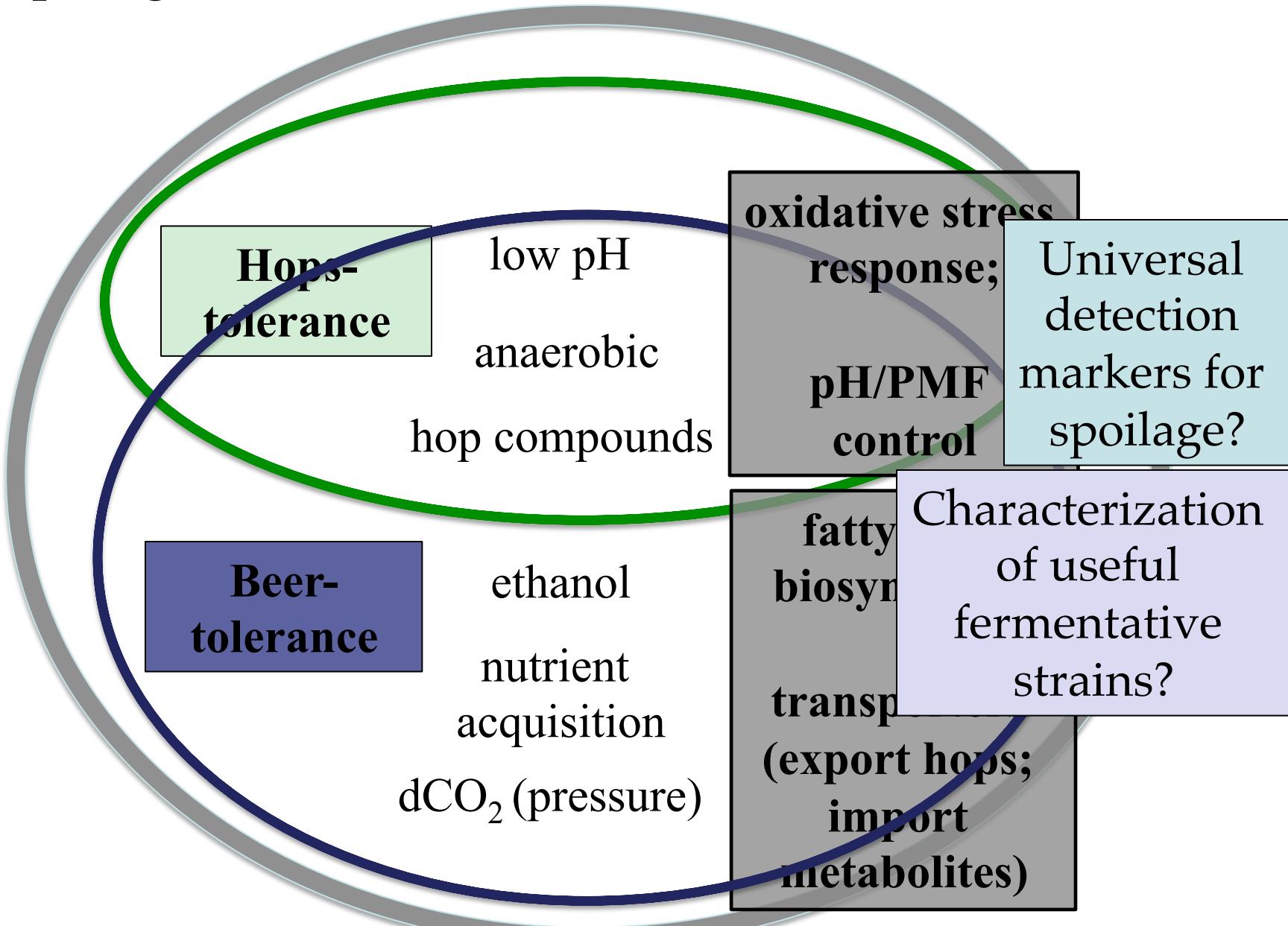


Hop-tolerance ≠ “the Holy Grail”

- *hitA, horA, horB, horC, bsrA* genes
 - not universal – false (+) and false (-)
 - detection does not guarantee function
 - plasmid-harbored...but assumed conservation

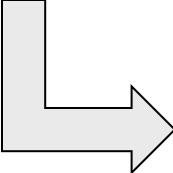
3-4 genes to describe all of LAB BSOs!?

Beer-spoilage LAB cell modifications

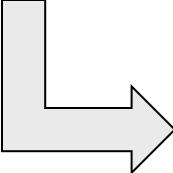


Power of Genetic Sequencing:

LAB and brewery
diversity



DNA:
- genome
- metagenome



RNA:
- transcriptome
- metatranscriptome

Non-BSOs

Targeted sequencing:
Universal Genetic
Markers

Commonalities?
Differences?

BSOs

Beer
Qualities and
Design

Active
Microflora

RNA sequencing (RNAseq)

- Sequence all messenger RNA (mRNA) transcripts present in cell at one time

Gene B 4x > Gene A



- Provides general picture of cell regulation and activities under condition “X”

mMRS media, degassed beer, packaged beer

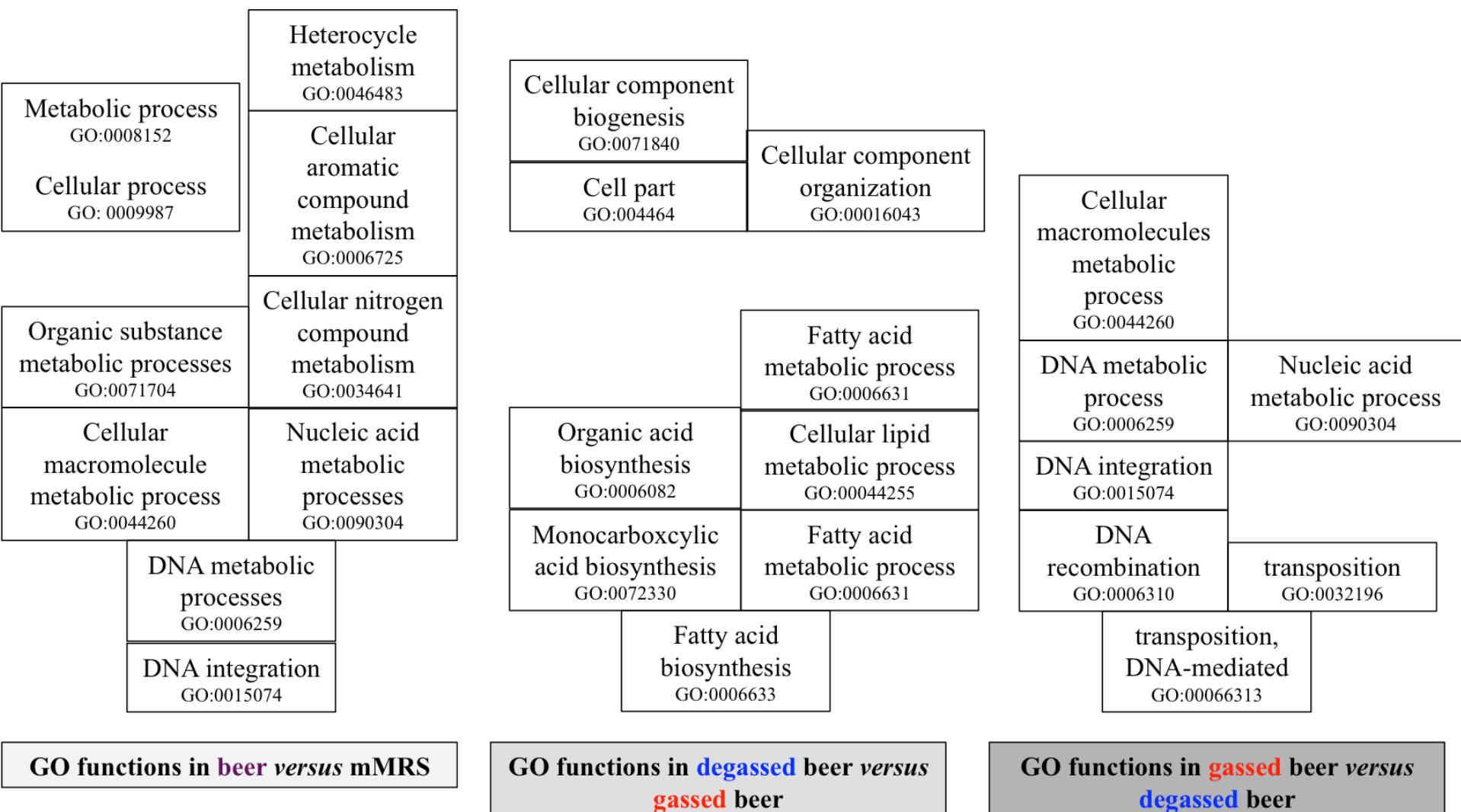
Lactobacillus brevis
BSO 464

Lactobacillus backii
L101

- Genome assembled;
- 8 plasmids
- Tolerant to pressurized beer environment and ~135 BU hops

- Genome sequenced;
- 4-5 plasmids (?)
- Tolerant to pressurized beer environment and ~75 BU hops

“Big Picture” Gene Ontology Terms:



What activities are important during growth in beer?

Acid tolerance

Biogenic amine metabolism

Nutrient scavenging

Yeast “Leftovers” ~ pentoses;
specific to breweries??

Osmoregulation

Hop tolerance

Membrane transport;
metabolite scavenging

Biogenic amine (BA) metabolism

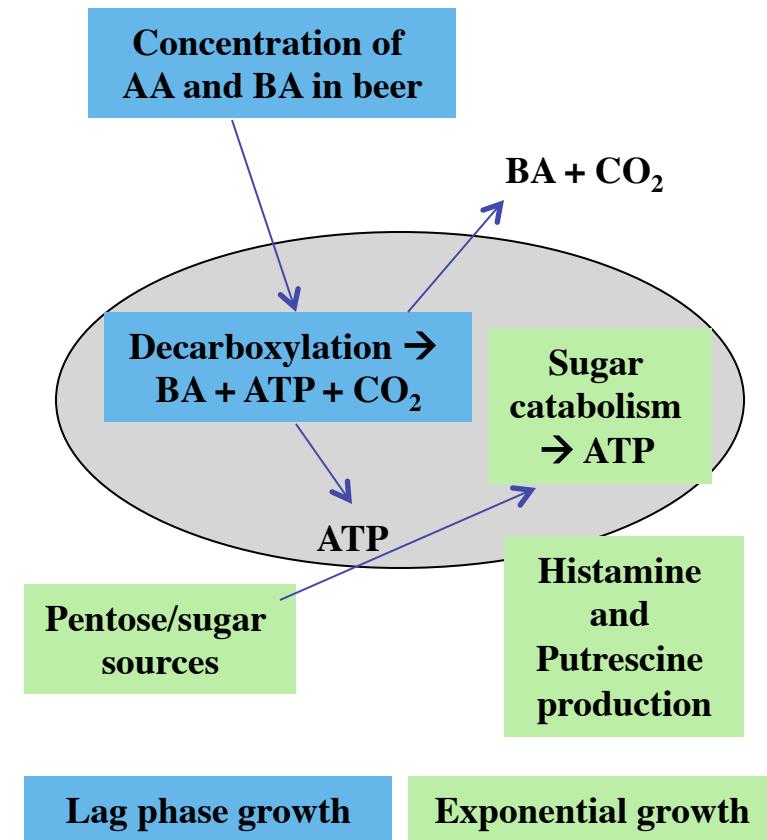
Literature:

Raw materials (Precursor)	Microbial Spoilage (Precursor)
Agmatine (Arginine)	Histamine (Histidine)
Putrescine (Agmatine and Ornithine)	Tyramine (Tyrosine)
Spermidine (Putrescine)	Cadaverine (Lysine)
Spermine (Putrescine)	

Amino acids are transported into the cell, decarboxylated, and BA's secreted back into the environment ~ internal and external pH regulation

What is happening during mid-exponential growth?

Upregulated
Agmatine deiminase pathway (putrescine carbamoyltransferase)
Spermidine/putrescine ABC transporter system
Arginine:ornithine antiporter (**Arginine deiminase pathway NOT upregulated)
Histidine metabolism; imidazolonepropionase
Butanoate metabolism (α -acetolactate decarboxylase) – acetoин production

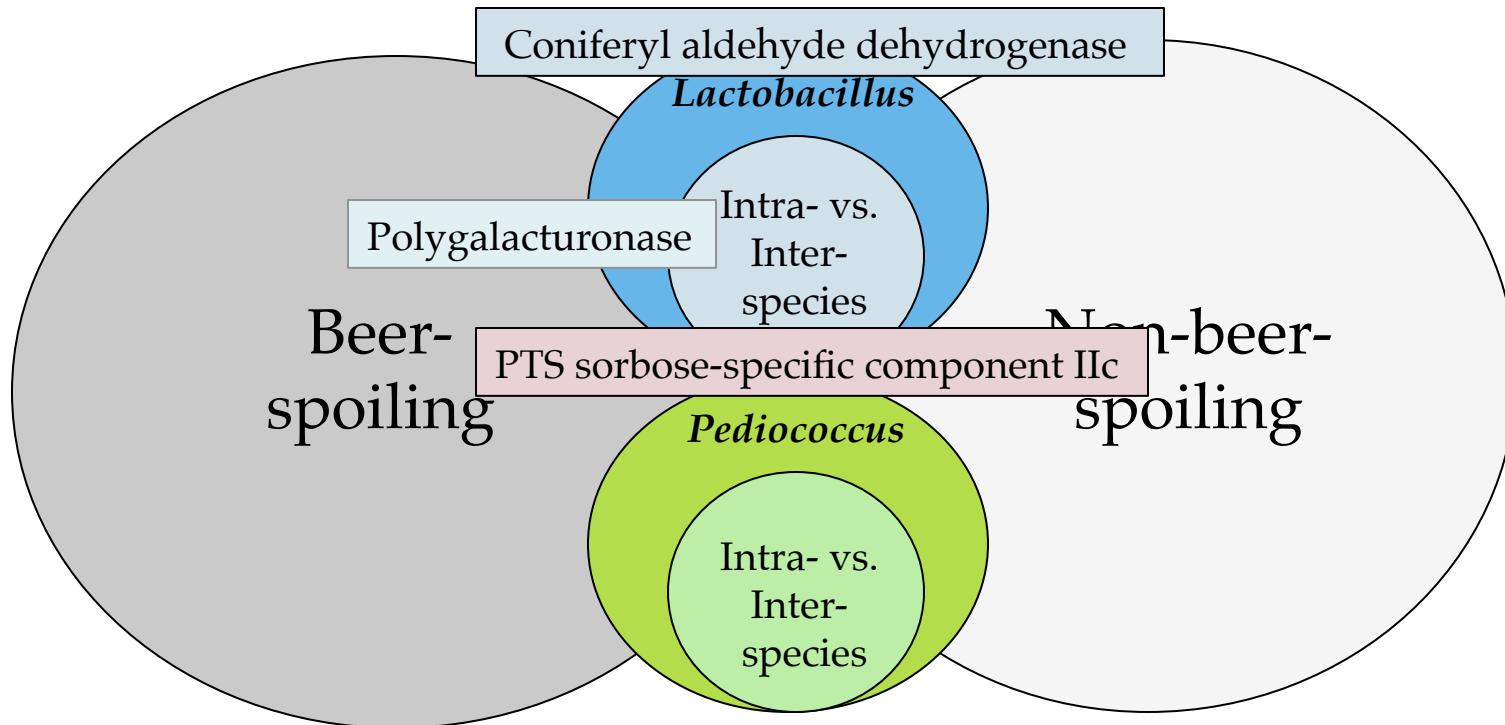


What about those “super”-virulence mechanisms for spoiling packaged beer?

- Plasmid-based regulatory transcripts
- DNA regulation and repair
- Ferric uptake regulator (*Fur*) – manganese uptake; osmoregulation
- Butanoate metabolism

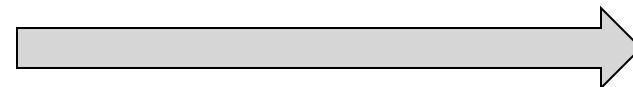
Cell wall and
membrane
modification?

RNAseq informs model of beer-spoilage; but what do we know about genes common to beer-spoilage?



To wrap all this up...

RNAseq reveals common pattern of beer-spoilage metabolism



Still allows for determination of “next steps” and facilitates genome comparisons

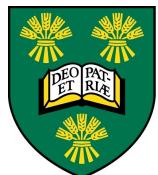
Mechanisms are at once complex, yet common to other BSOs

Despite apparent “core” physiology – response still likely to be influenced by brewery-specific factors

Where does that leave us re: “Genomics” in beer?

- Its all about context...
 - RNAseq viewed as a means to an end (universal genetic markers)
- Application of genomics in itself can be the end:
 - Source tracking
 - Baseline “microflora” establishment
 - Hygiene program development
 - Selection of appropriate strains for fermentation

Acknowledgments



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BARTH-HAAS GROUP
FOR YOUR SUCCESS