



WORLD BREWING CONGRESS

August 13–17, 2016 • Denver, Colorado, U.S.A.

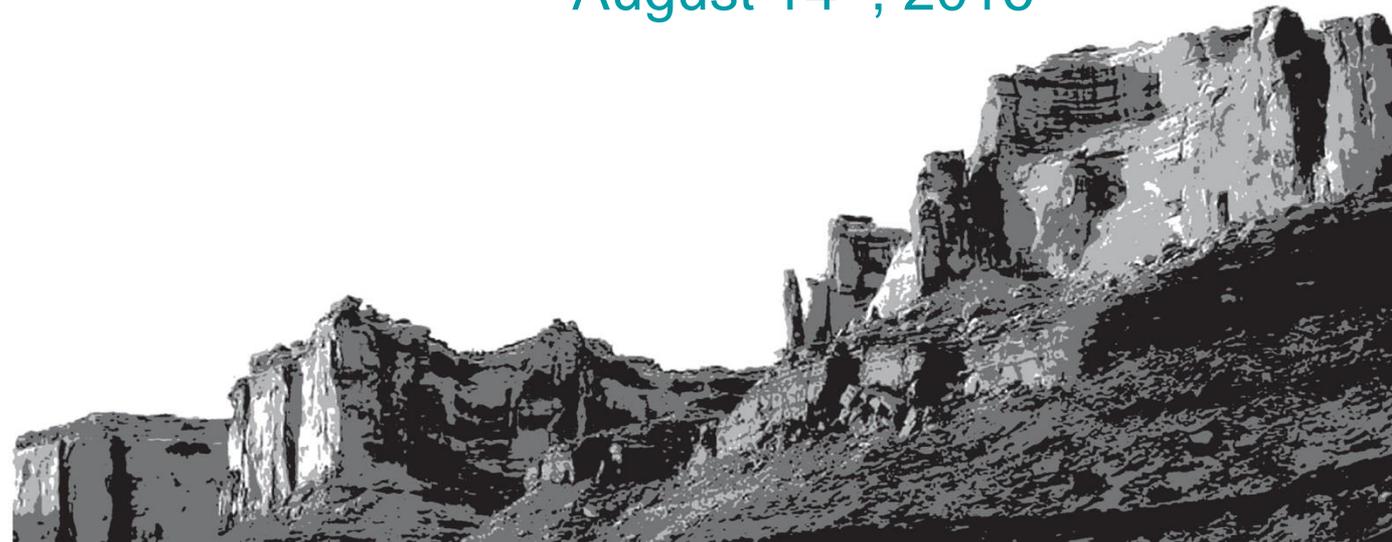
#ElevateBeer



Genome comparisons of *Lactobacillus* and *Pediococcus* reveal genetic markers of brewery adaptation.

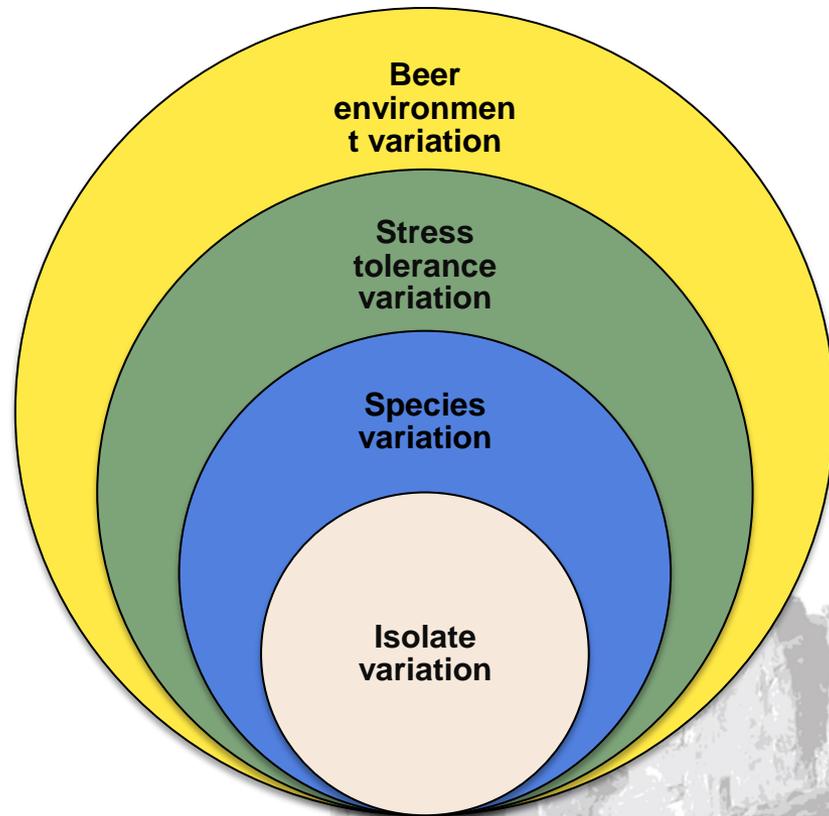
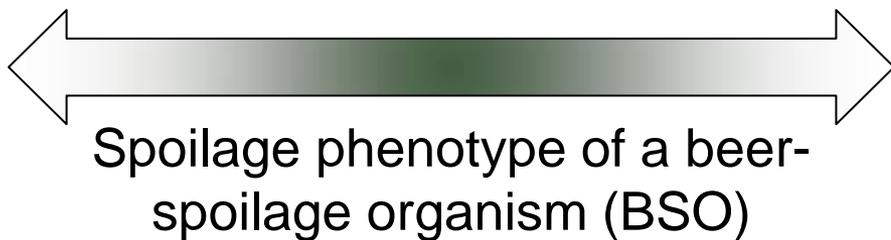
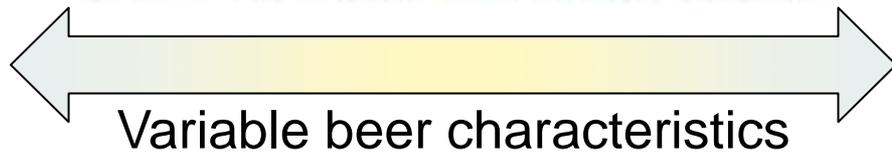
Barry Ziola, Jordyn Bergsveinson, Ilkka Kajala

August 14th, 2016





Beer-Spoilage \neq Binary Phenotype





Hop-tolerance \neq “the Holy Grail”

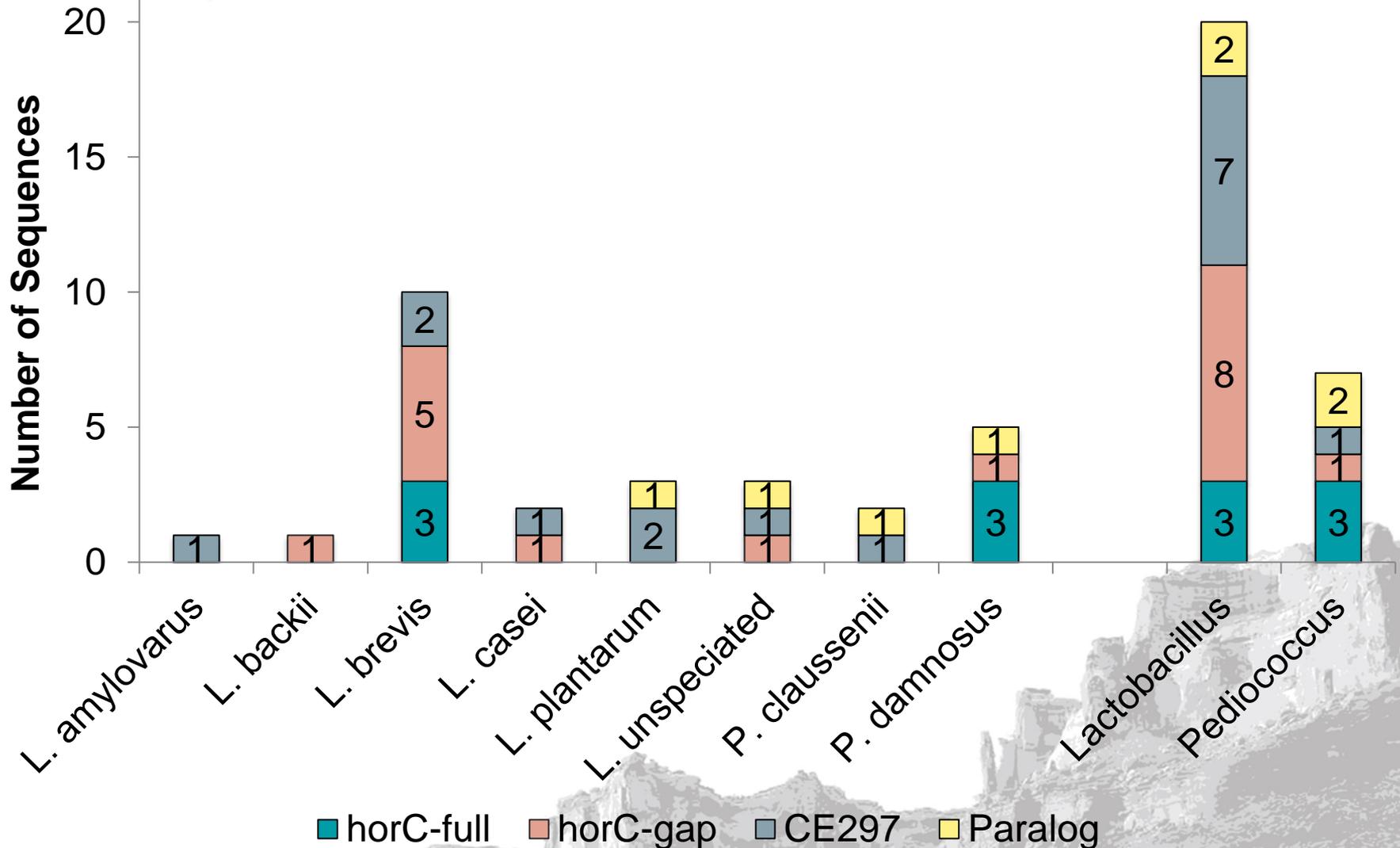
- *hitA*, *horA*, *horB*, *horC*, *bsrA* genes
 - not universal – false (+) and false (-)
 - detection does not guarantee function
 - plasmid-harbored...but assumed
- **3-4 genes to describe all of LAB BSOs!?**





horC variability

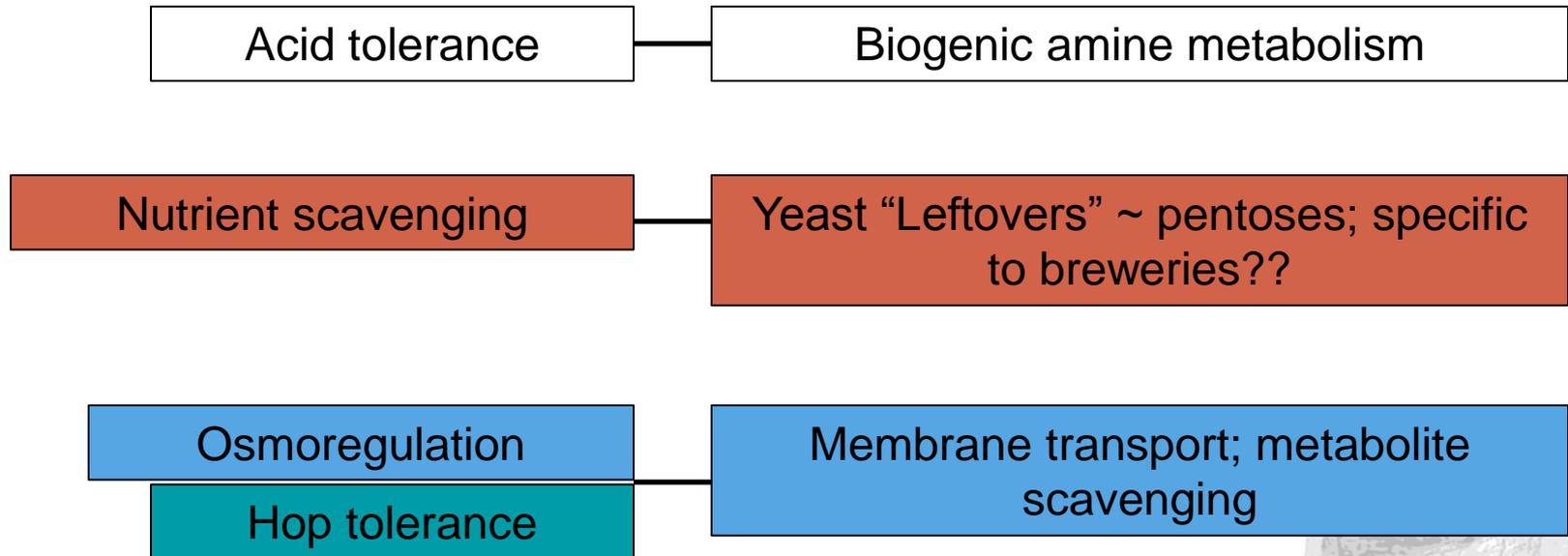
Genetic Variability in the Hop-Tolerance horC Gene of Beer-Spoiling Lactic Acid Bacteria
Bergsveinson et al. (2016) *J Am Soc Brew Chem* 74:173-182





What activities are important during growth in beer?

Transcriptome analysis of *Lactobacillus brevis* BSO 464^{*} and *Pediococcus clausenii* ATCC BAA-344^{T**}



^{*} Transcriptome analysis of beer-spoiling *Lactobacillus brevis* BSO 464 during growth in degassed and gassed beer. Bergsveinson et al. (2016) *Int J Food Microbiol* 235:28-35

^{**} Transcriptome Sequence and Plasmid Copy Number Analysis of the Brewery Isolate *Pediococcus clausenii* ATCC BAA-344T during Growth in Beer. Pittet et al. (2013)

PLOS ONE 8(9):e73627



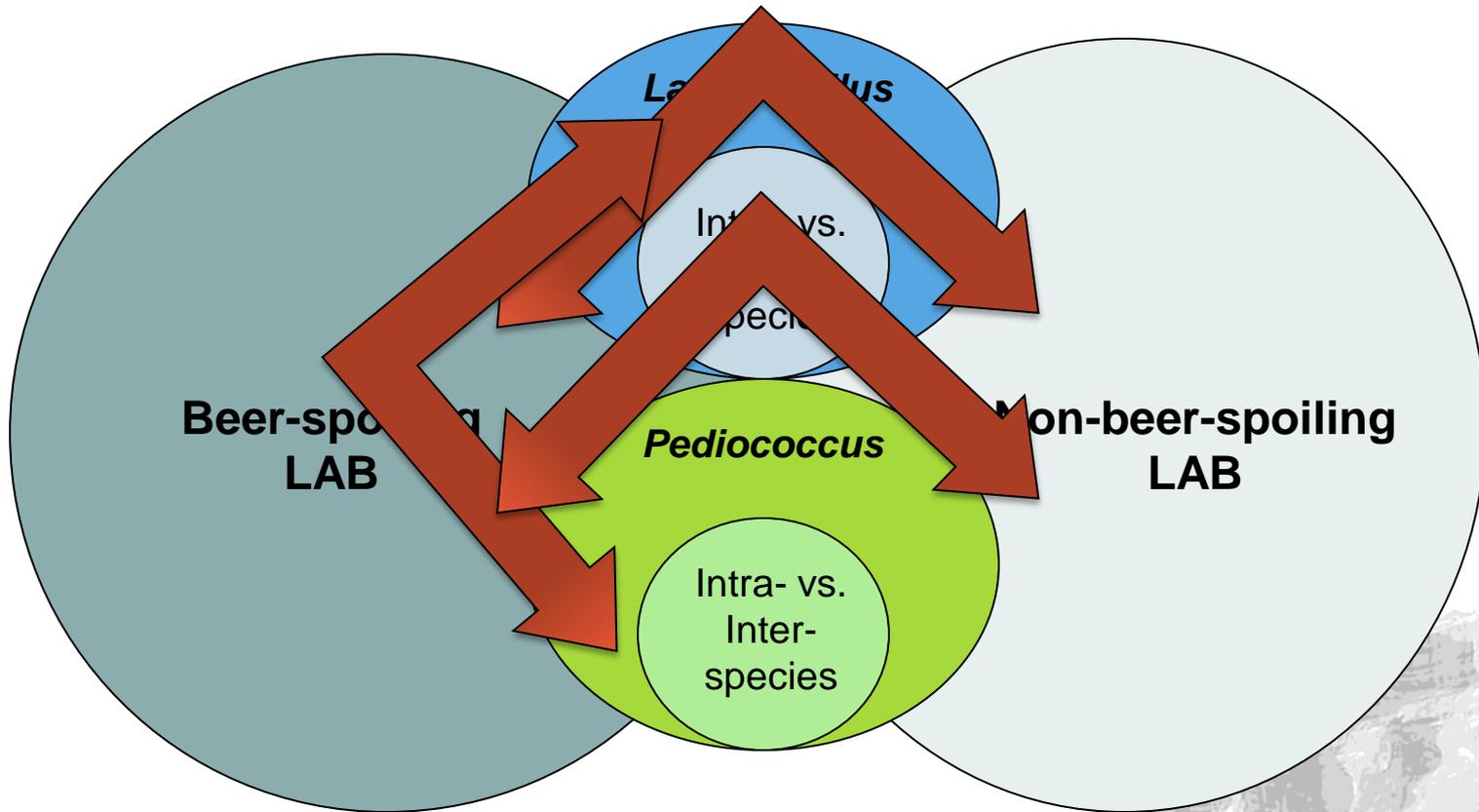
Mining *meta*-data for utility...

- But where to start!?
 - Limited # of complete genomes of beer-spoilage-related LAB
 - Quality of genetic data publically available?
 - Confounding role of plasmids/mobile genetic elements





Genomic comparisons – where to start?!





Beer-spoiling genomes

Lactobacillus
spp.
ATCC 15578

Lactobacillus
brevis
BSO 310

Lactobacillus
brevis
BSO 464

Pediococcus
claussenii
ATCC BAA-
344^T

Lactobacillus
casei
CCC B1025

Pediococcus
Damnosus
96-b

Lactobacillus
backii
101

**Increasing beer-spoilage virulence
(growth in gassed/pressurized beer).**

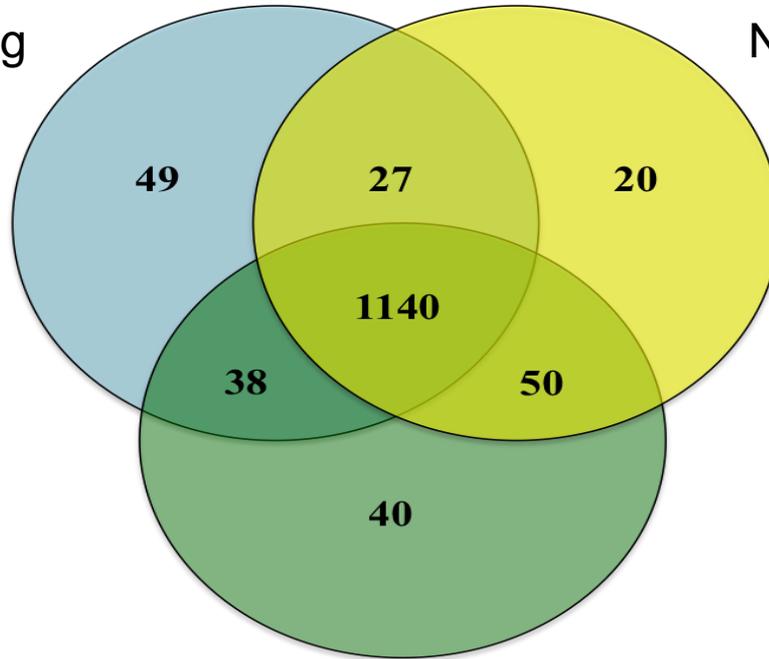
Dissolved carbon dioxide selects for lactic acid bacteria able to grow in and spoil packaged beer. Bergsveinson et al. (2015) **J Am Soc Brew Chem** 73: 331-338



To get anywhere, had to start at intra-species = *L. brevis* level

Beer-Spoiling

4 isolates



Non-Beer-Spoiling

3 isolates

Environmental

7 isolates

Common ortholog group (COG) terms assigned by OrthoMCL enriched in three different groupings of *L. brevis* isolates.

*8 environmental isolates total; however, *L. brevis* subsp. *gravensis* not included in COG term analysis



The leads...

Previous analysis of *L. brevis* beer-spoiling organisms vs. non-beer-spoilage isolates for “eco-type” specific markers (Behr et al., 2015. Food Microbiol, 51).

Beer-spoilage isolates isolated from one geographical location.

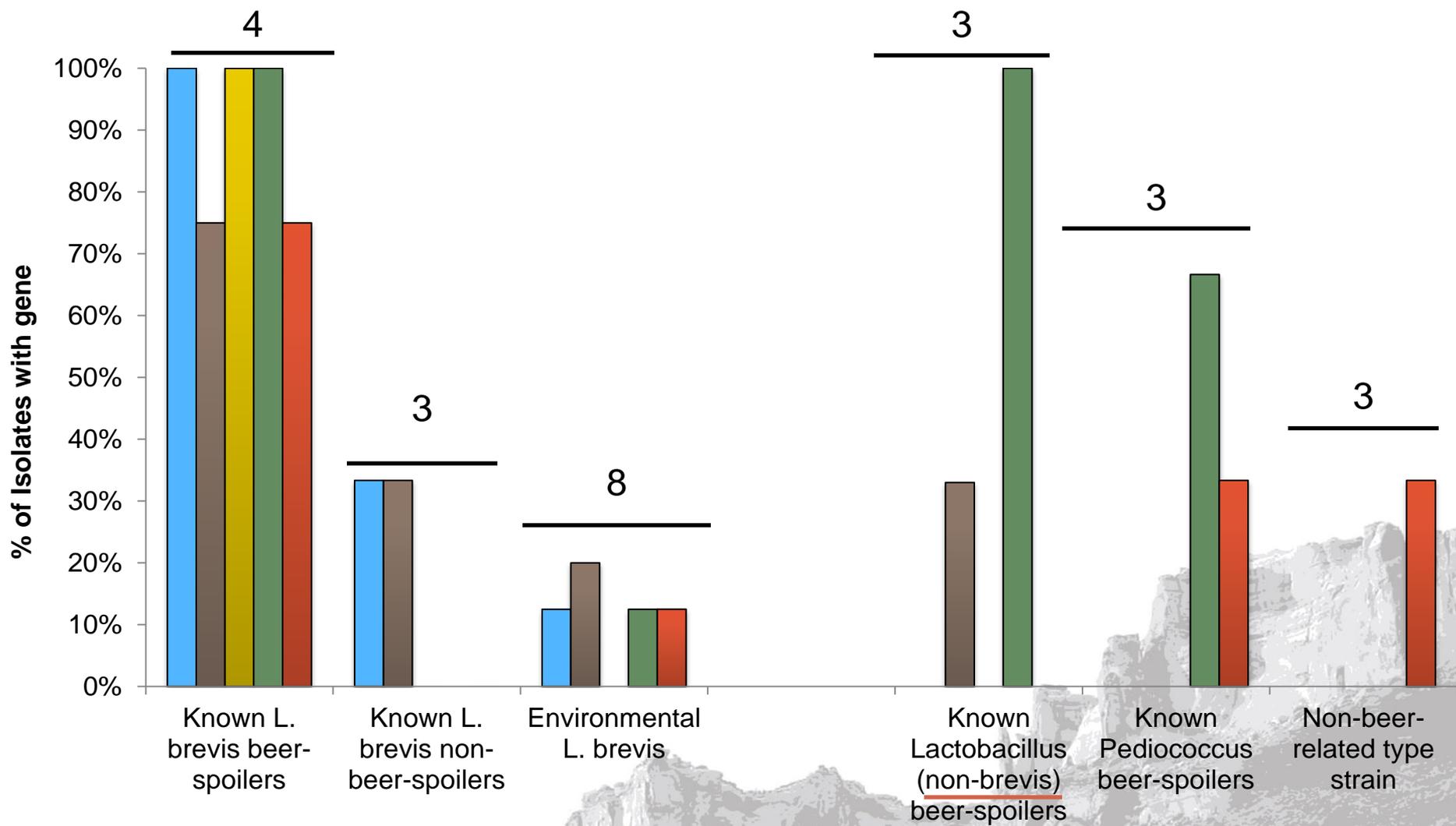
- histidine kinase
- specific transcriptional regulator *arsR* and *cinA*
- polygalacturonase (enzyme related to plant-degradation)

Transcriptional analysis during growth in beer:

- Nutrient ABC transporters
- Specific transcriptional regulators
- enzymes related to plant-degradation

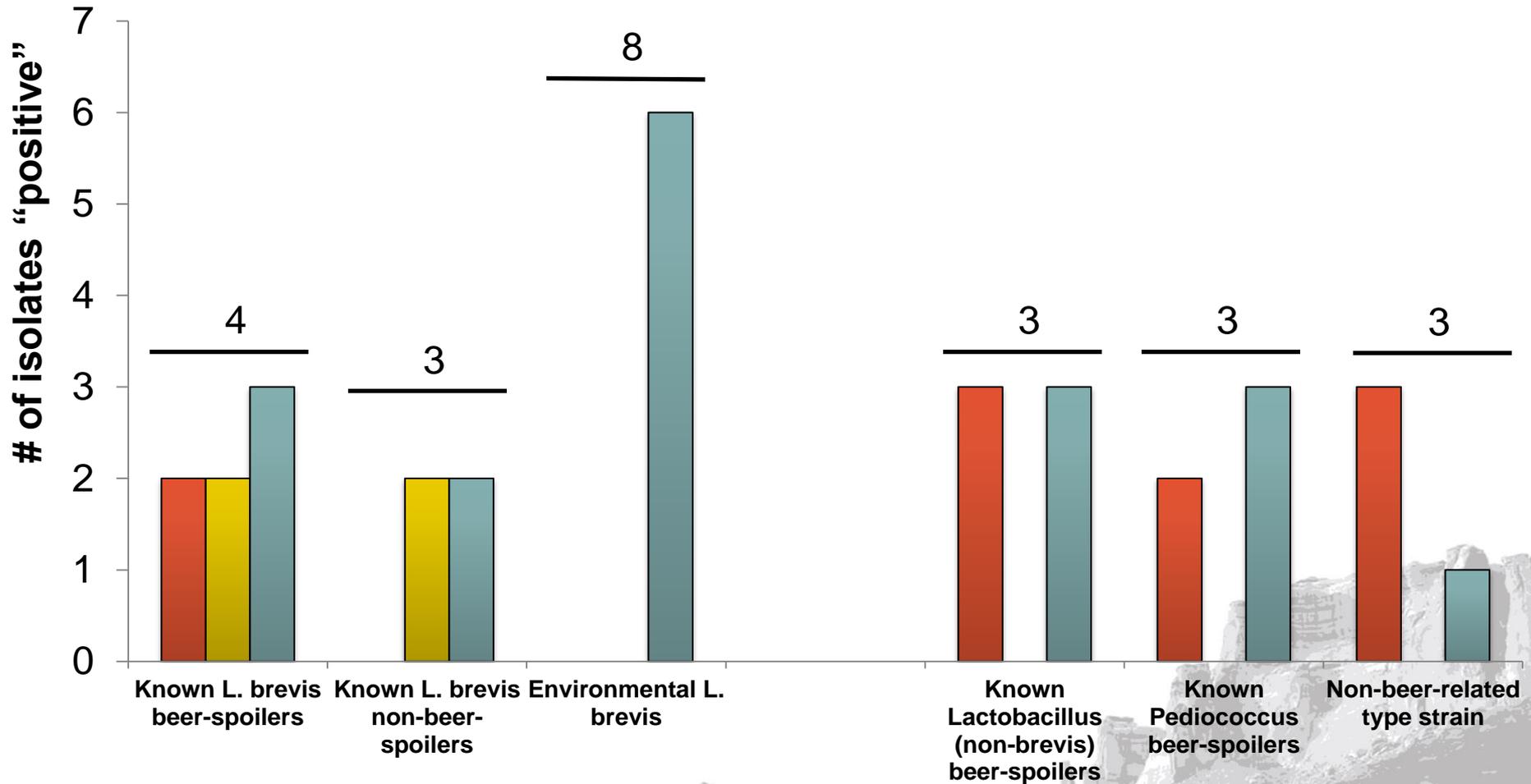
- Arabinose ABC permease
- Polygalacturonase
- Signal transduction histidine kinase

- Coniferyl Aldehyde Dehydrogenase
- PTS Sorbose-specific IIC Component

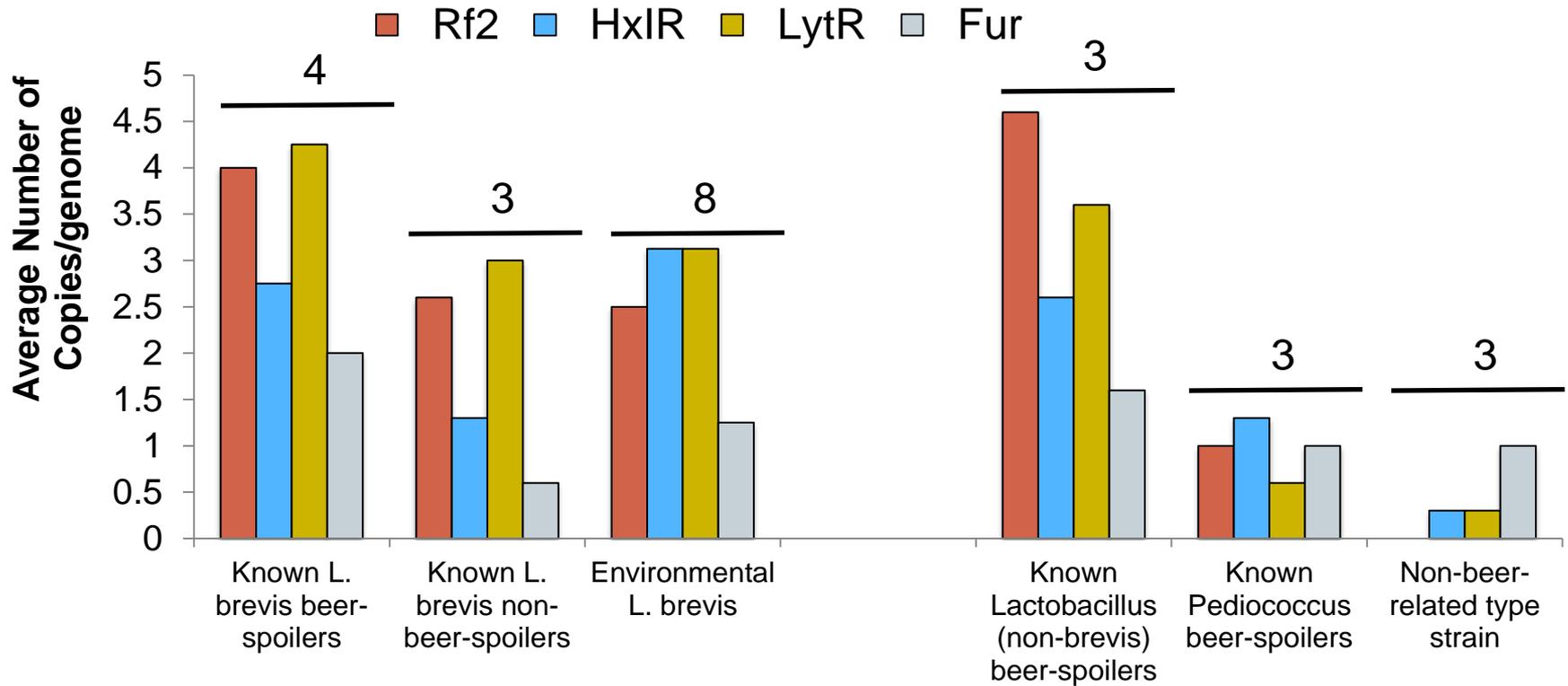


Transcriptional regulator enrichment

■ positive arsR ■ positive cinA (primers) ■ positive cinA



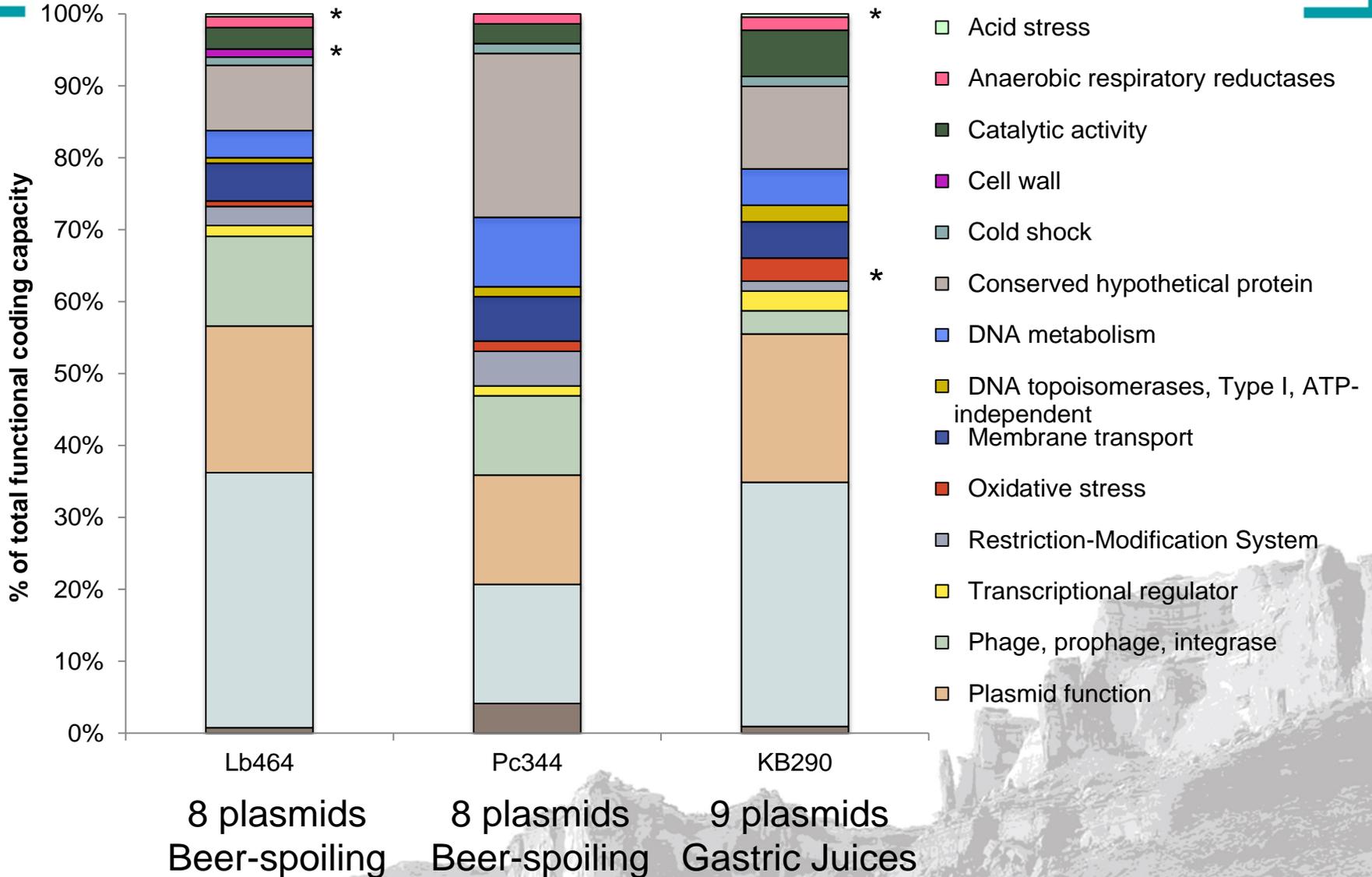
Transcriptional regulator enrichment



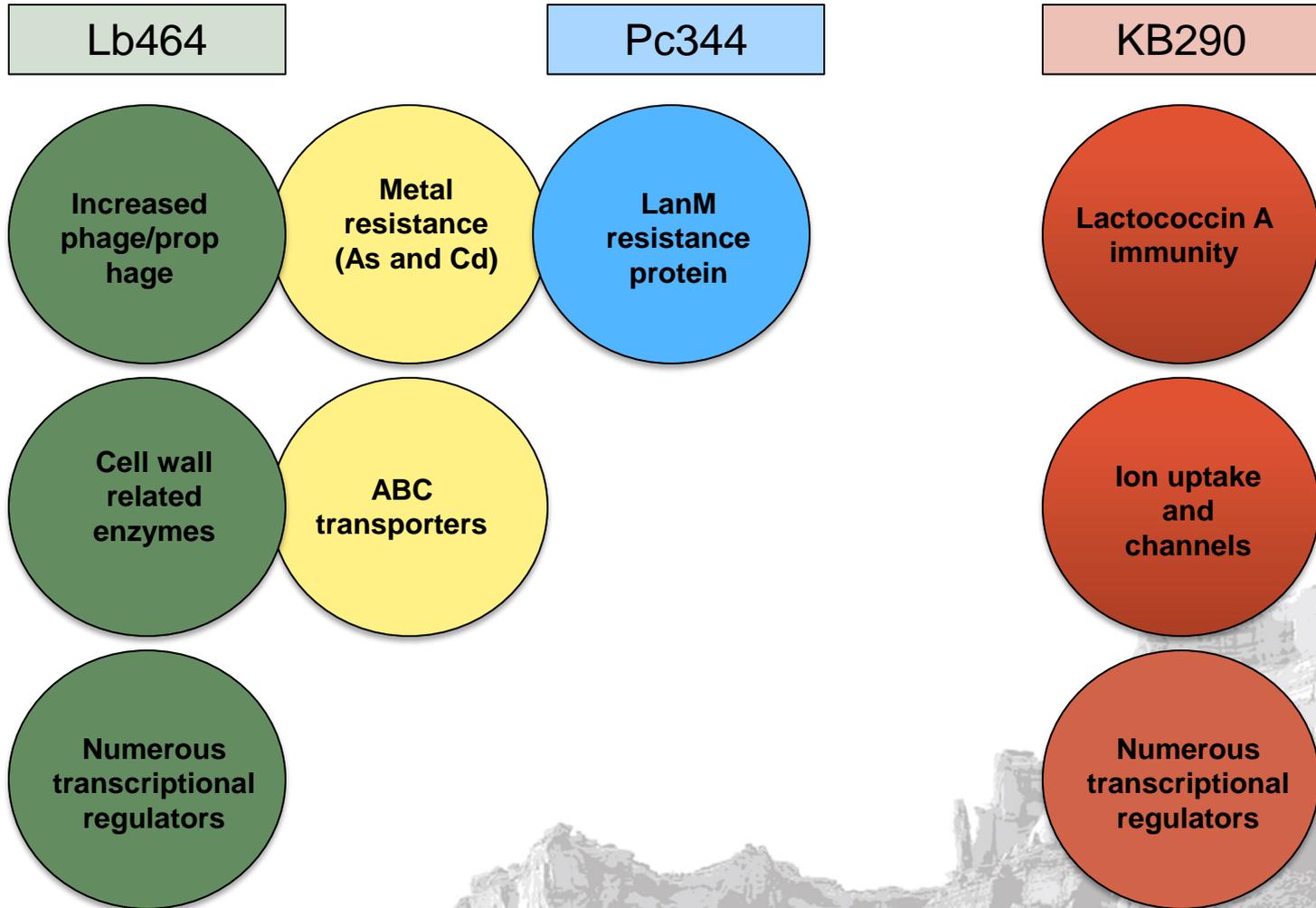
Phenotype	Isolate Label	Rrf2/ HxIR/ LytR /Fur
Non-beer-related type strain isolates	Lc334	-/-/-
	Ls23K	-/-/-2
	Pp25745	-1/1/1
Known <i>Pediococcus</i> beer-spoilers	Pc344 ^a	-1/-/-
	Pd58 ^a	1/2/-1
	Pd28219 ^b	2/1/2/2



Plasmids – High Level



Plasmids - Detailed



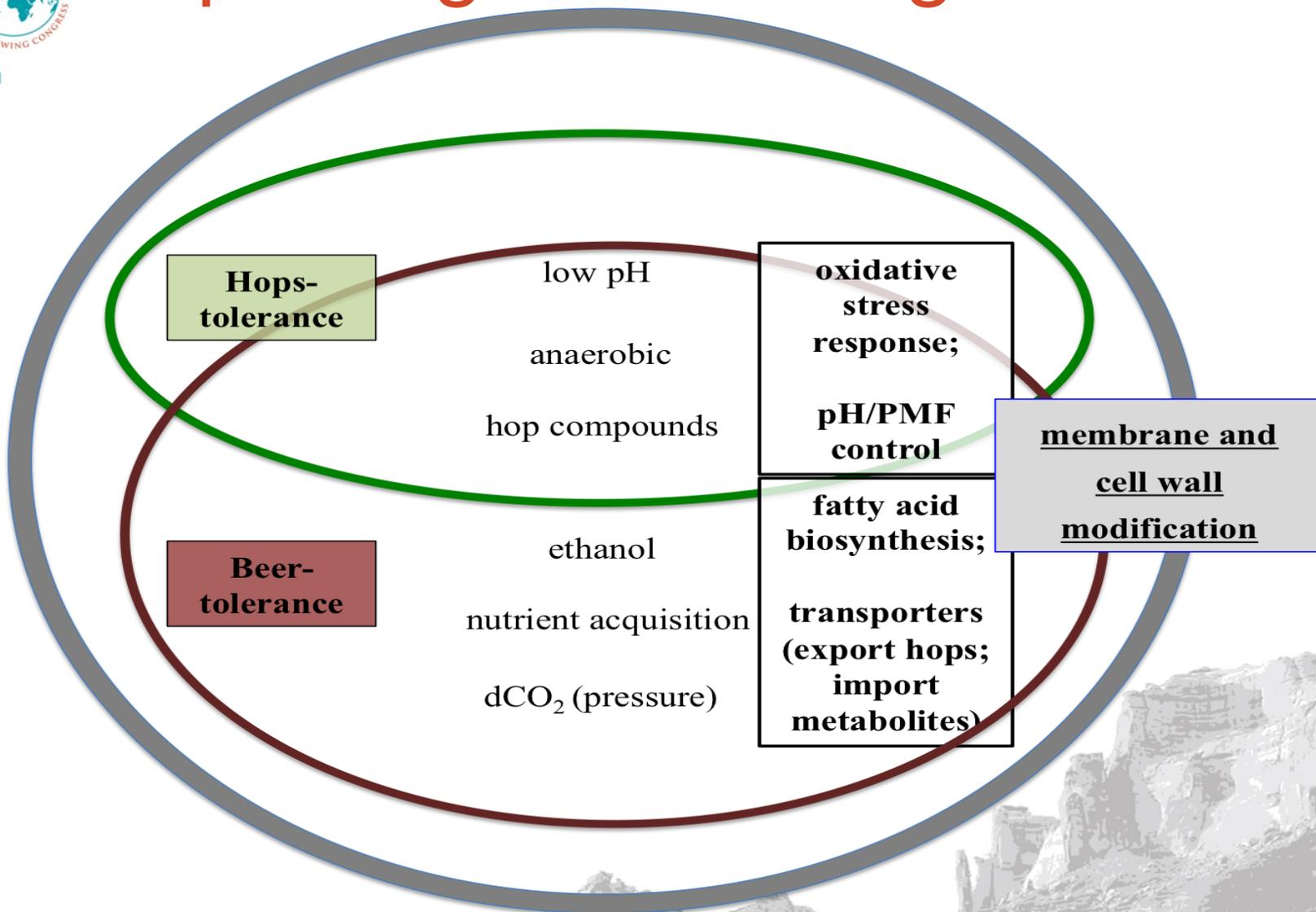


Conclusions and Future

- Enzymes related to cell wall modifications and nutrient scavenging
- Plasmids confer very specific adaptations
- Can't underestimate the influence of selection environment (location~brewery)
- Increased need for quality, publicly available beer-spoilage-related LAB genomes
- Further “omics” studies!!



Improving the working model...



Acknowledgements



The Science of Beer

- U of S College of Medicine; College of Graduate Studies and Research
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- Ilkka Kajala