



**Genetic markers for LAB beer-spoilage
and the role of dissolved CO₂/pressure
on bacterial growth in beer.**

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June 15th, 2015
ASBC Annual Meeting, La Quinta CA

nutrient
acquisition

pressure
tolerance?

ethanol
tolerance

Phenotype → Genotype → Universal Detection

acidic pH
tolerance


export hop
compounds

pH/PMF
control

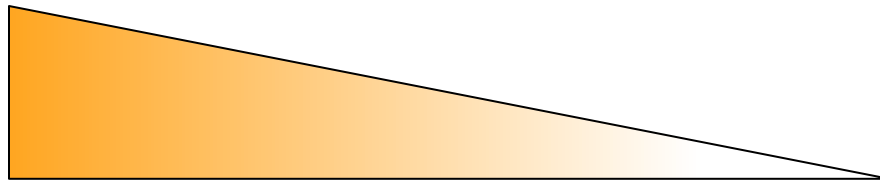
anaerobic

(1) Pittet, V. (2012). *Adaptation of lactic acid bacteria for growth in beer*. Unpublished doctoral dissertation, University of Saskatchewan, Saskatoon, Saskatchewan

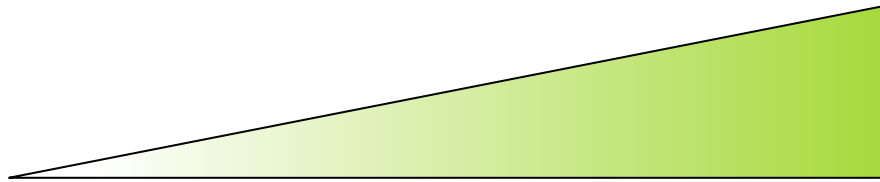
We stress “stress” – what about dCO₂?

Beer Stress	Relevance for LAB
Hops	Defining – relative scale of tolerance
Low O ₂	LAB ~ anaerobic
Low pH, ethanol (%)	LAB intrinsically tolerant
Low Nutrient Availability	General starvation
 Pressure/dCO ₂	?? – Process vs. Product contamination

Beer-Spoilage \neq Binary Phenotype



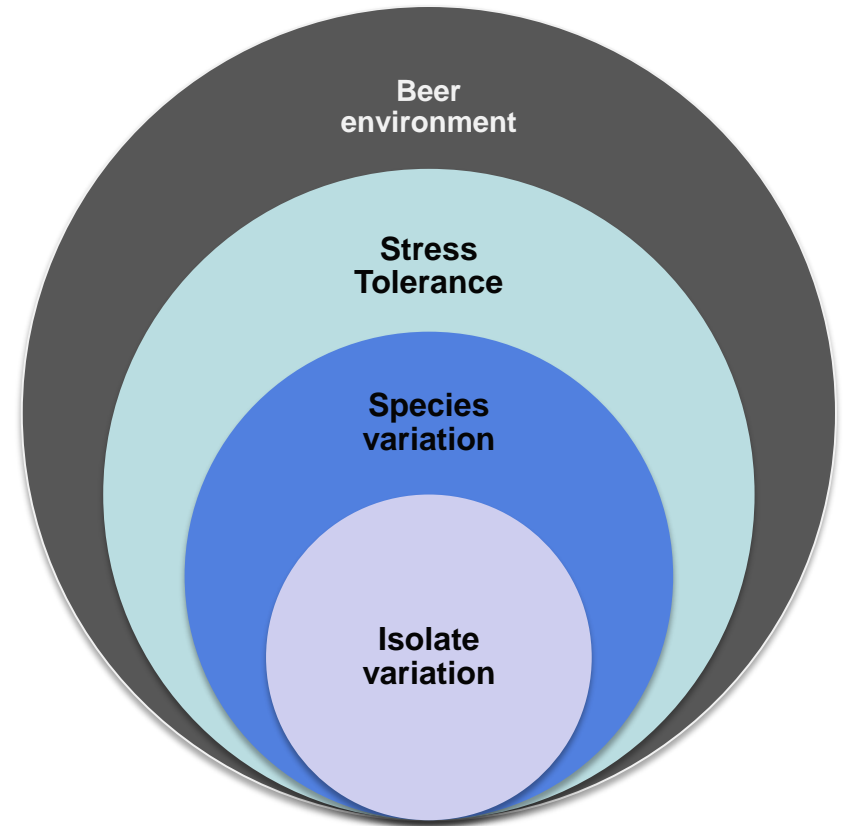
Beer A



Beer B



Beer-spoilage phenotype
of a BSO



Hop-tolerance \neq “the Holy Grail

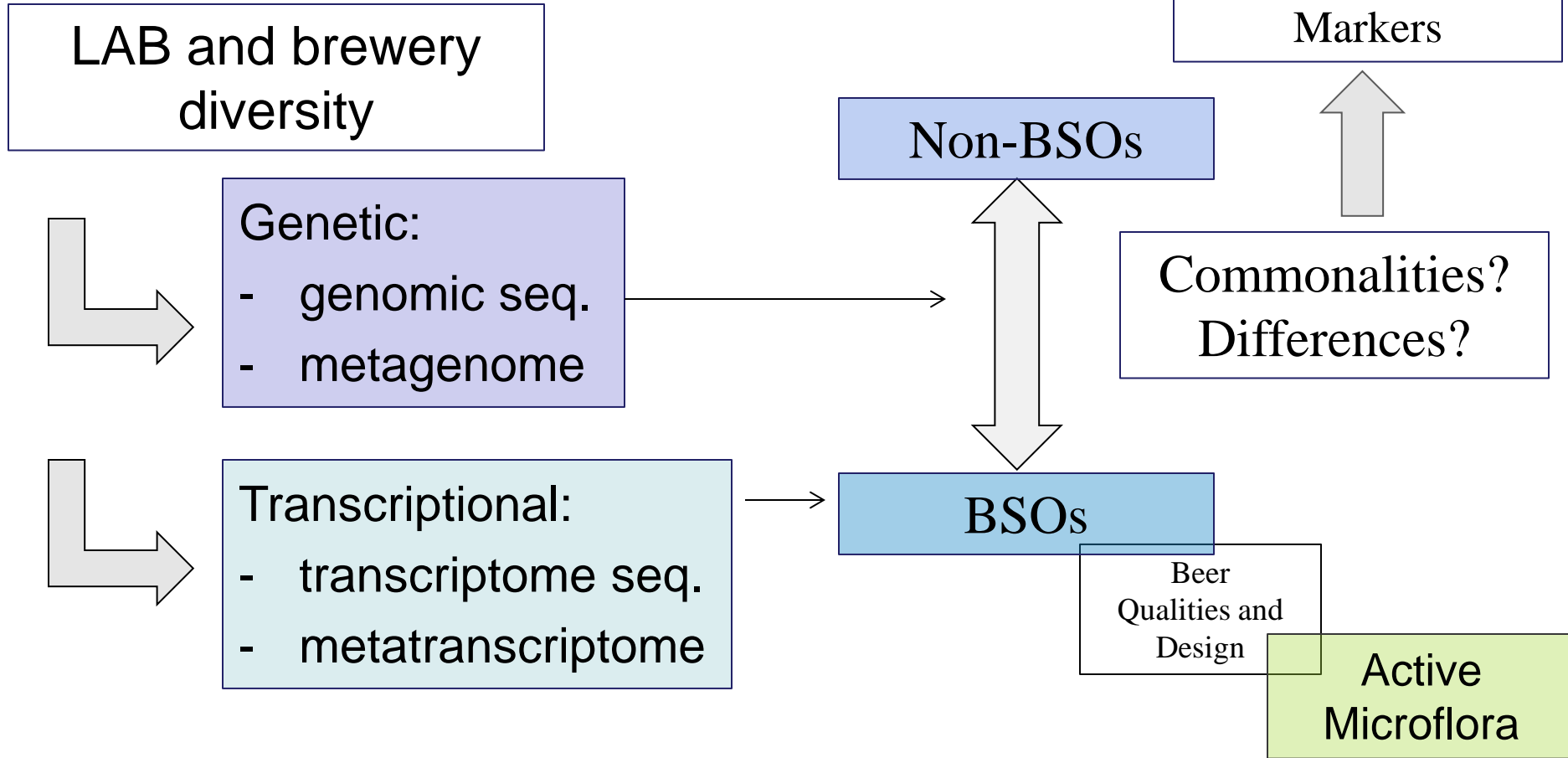
- *hitA, horA, horB, horC, bsrA*
 - not universal – false (+) and false (-)
 - detection does not guarantee function
 - plasmid-harbored...assumed conservation
- **3-4 genes to describe all of LAB BSOs!?**
 - **Need to expand search \rightarrow dCO₂!**

Effect of standard pressure/dCO₂

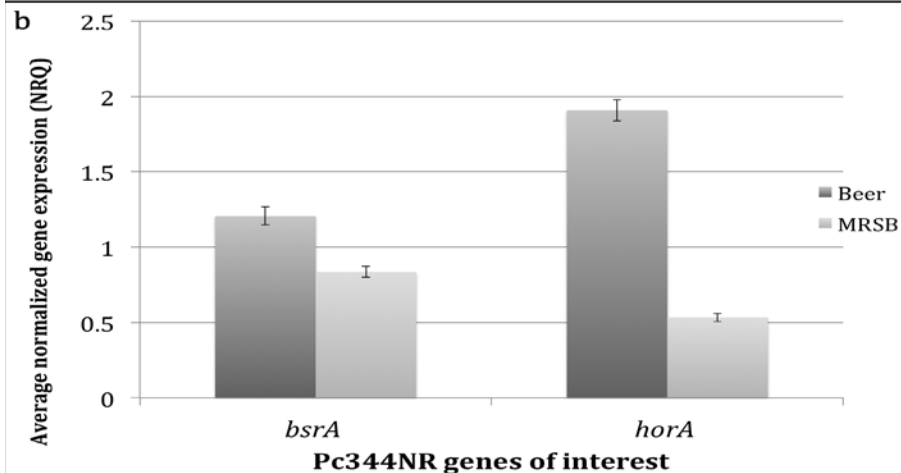
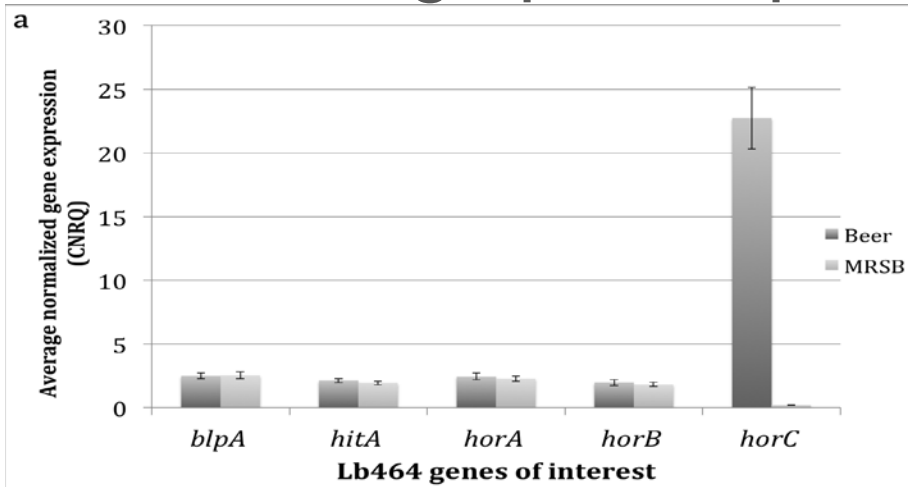
- 20 LAB isolates assayed +/- growth in +/- dCO₂ b
 - 10 isolates no growth
 - 5 isolates static
 - 5 isolates grow = True BSOs
- No genus/species correlation
- No correlation with hop and ethanol-tolerance
- **Highlights difference between process contamination vs. product contamination**



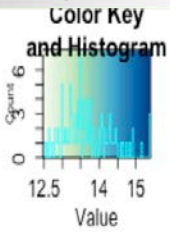
Power of “omics”:



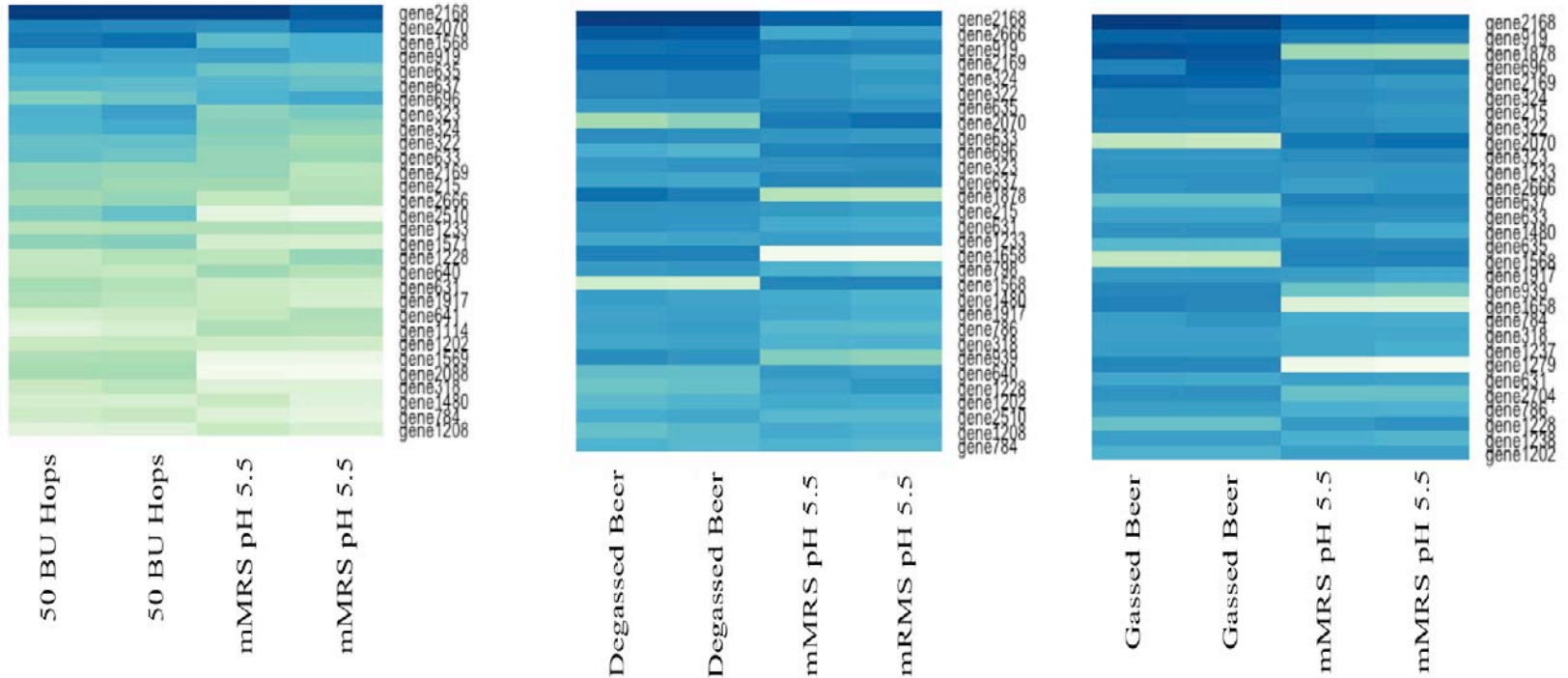
Teasing apart hops, beer, (and dCO₂)



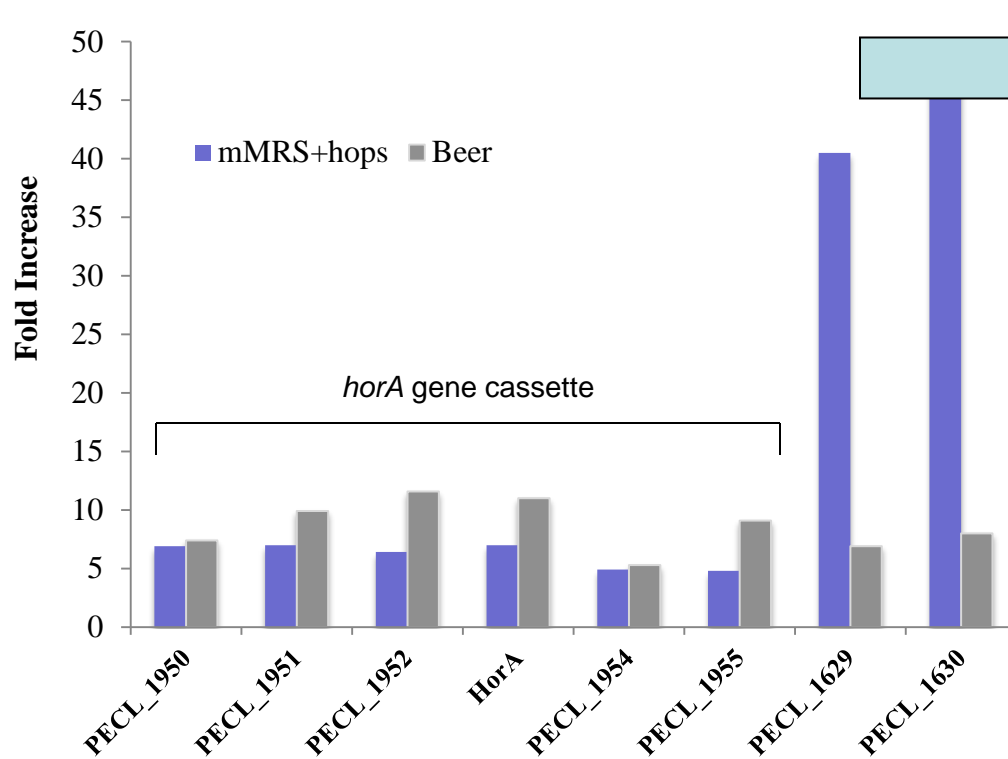
Lb464	Pc344
~135 BU hops	~54 BU hops
48 h in degassed beer	72 h in degassed beer
Growth in gassed beer	No growth in gassed beer
8 plasmids	8 plasmids
2.5 MB 2,425 CDS (184 ~plasmids)	1.8 MB 1,740 CDS (129 ~ plasmids)



Patterns of Lb464 gene expression across conditions



Hops vs. Beer Pc344:



Locus_tag	Fold Increase 30 BU hops	Gene; Description	Location	Fold Increase in Beer ^a
PECL_1630		<i>Brewery-specific</i>		7.8
PECL_2059	42.8	putative ncRNA	chromosome	32*
PECL_1629	40.5	TetR family transcriptional regulator	chromosome	2.9
PECL_2042	22.4	hypothetical protein	pPECL-8	7.8
PECL_1743	21.9	VIT family protein	chromosome	NDE ^b
PECL_1544	18.7	AAA ATPase	chromosome	15.5
PECL_1708	17.3	putrescine carbamoyltransferase	chromosome	90*
PECL_1605	16.5	prolyl oligopeptidase family protein	chromosome	25*
PECL_1744	14.8	hypothetical protein	chromosome	NDE
PECL_1591	13.9	monooxygenase	chromosome	2.1
PECL_1707	13.4	<i>aguD</i> , agmatine/putrescine transporter	chromosome	269*

Brewing-related plasmids:

- Toxin/antitoxin systems
- bacteriophage – BSO tolerance *vs.* susceptibility
- Metal ion transport and efflux pumps
- Carbohydrate fermentation patterns

TRACKING DISTRIBUTION IN BREWERY??

Omics → Specifics

- dCO_2 a selective pressure for BSOs
- Tentative species-specific responses
 - conserved responses in species
 - isolate-specific?
- Membrane modification & unique energy producing pathways appear key
- BSO plasmids harbor other non-hop tolerance genes of interest

Acknowledgments



UNIVERSITY OF
SASKATCHEWAN

College of Medicine



NSERC
CRSNG



Anna Redekop
Sheree Zoerb
Dr. Vanessa Pittet

BARTH-HAAS GROUP
FOR YOUR SUCCESS