



UNIVERSITY OF ALBERTA
FACULTY OF AGRICULTURAL,
LIFE & ENVIRONMENTAL SCIENCES

Ecological fitness of *Lactobacillus reuteri* in food and intestinal ecosystems provides insights to probiotic applications

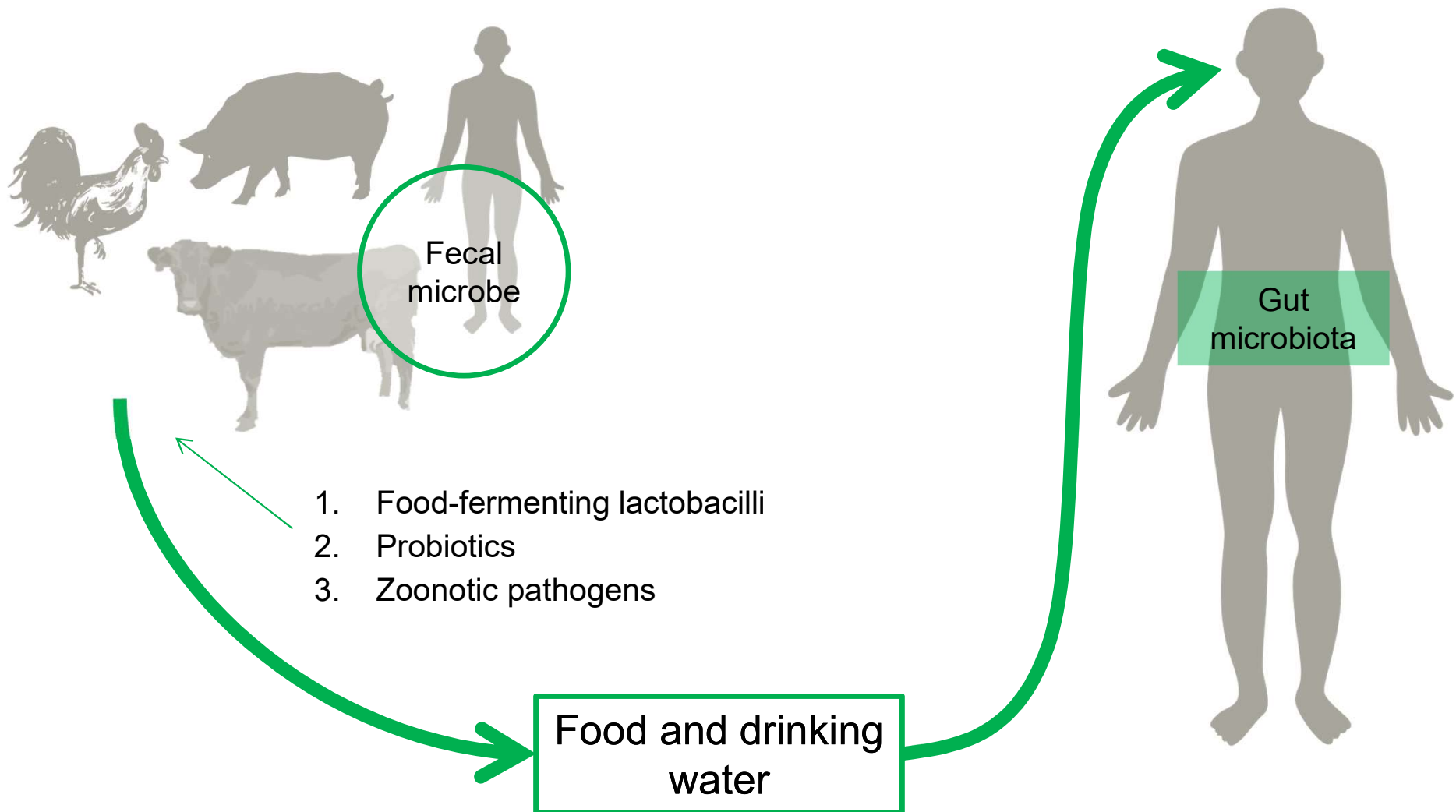
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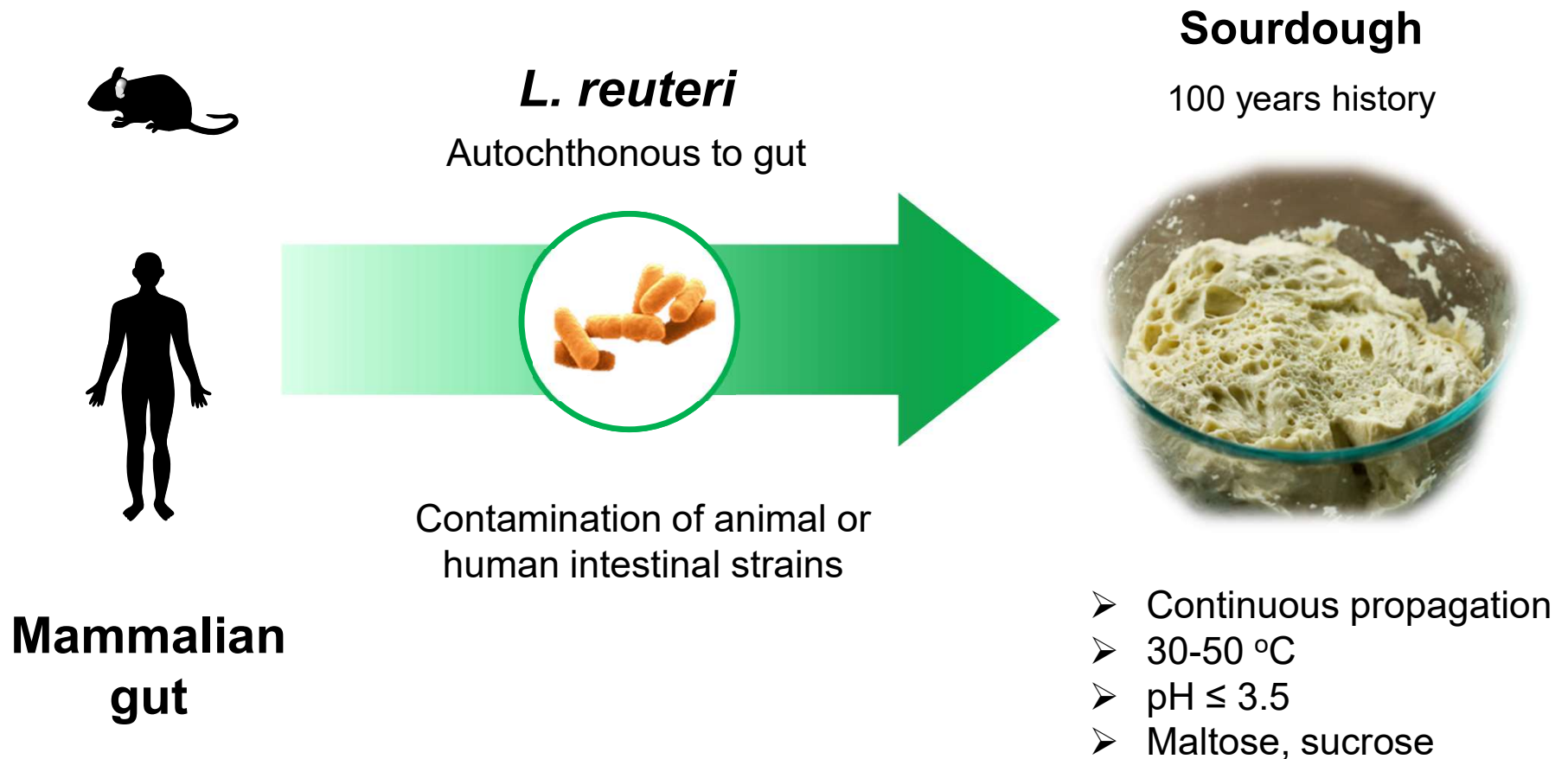
Ph.D. in Food Science and Technology
Thesis Defense

March 13, 2018

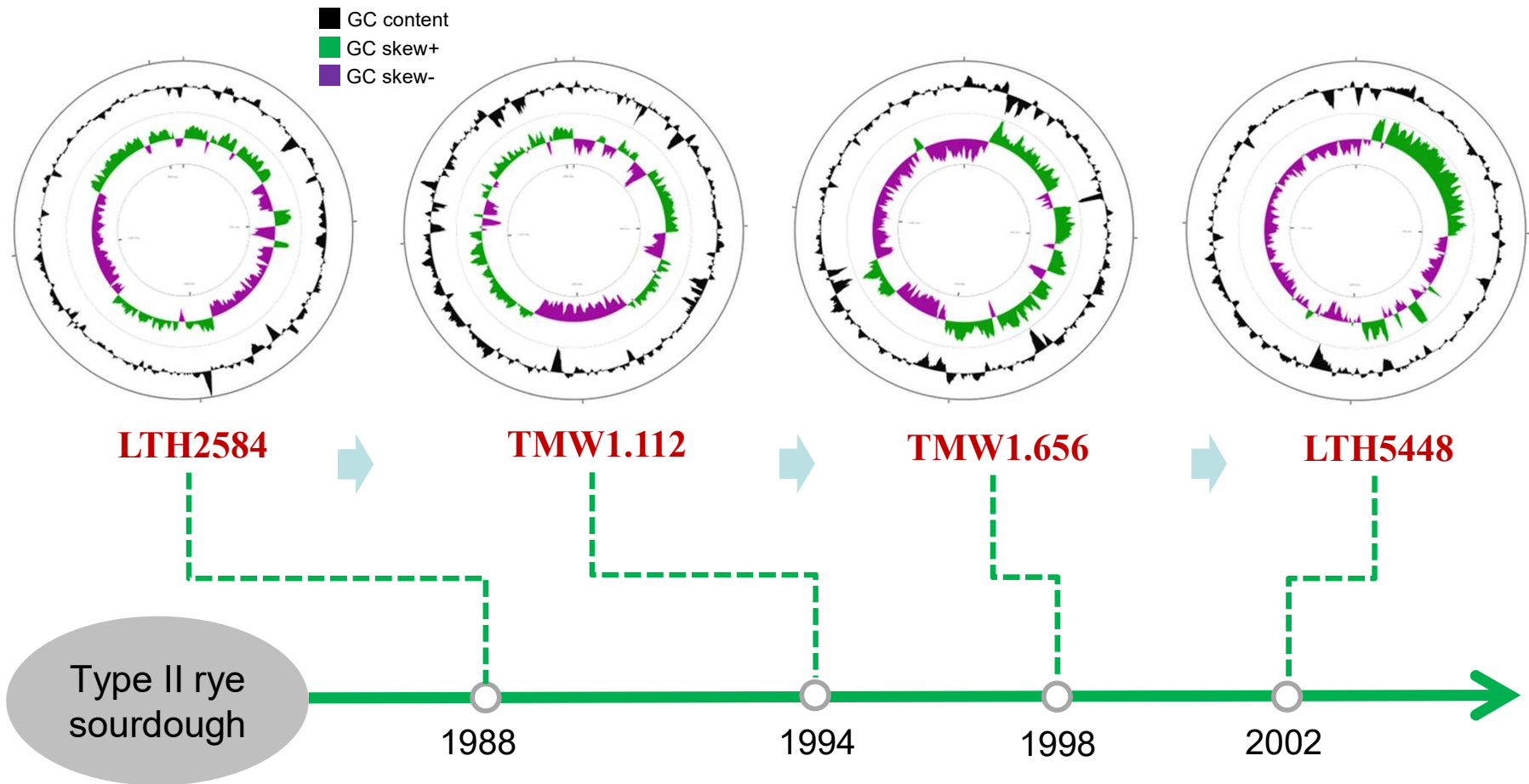
Food microbiota are partly derived from guts



Lactobacillus reuteri a model organism to study the association of fermented foods, (food) animals and human



Isolation of cereal *L. reuteri* strains



Hypothesis and objectives

Sourdough and intestinal strains of *L. reuteri* are indistinguishable with respect to genotypic and phenotypic characters.

- To access carbohydrate transport and metabolism in sourdough and intestinal strains of *L. reuteri*
- To identify drivers of species adaptation of *L. reuteri* by comparing genomes of sourdough and intestinal strains of *L. reuteri*
- To characterize the competitiveness of sourdough strains of *L. reuteri* in the pig gut
- To determine the correlation of acid resistance of probiotics with probiotic efficacy

Theme 1

**Carbohydrate utilization of sourdough strains
and intestinal strains of *L. reuteri* in relation to
niches and genotypes**

Homofermentative and heterofermentative lactobacilli co-exist in food and gut habitats

Homofermentative lactobacilli

1. PTS transporters for sugar uptake
2. Carbon catabolite repression mediated by PTS transporters
3. Sugar utilization is repressed by glucose

Heterofermentative lactobacilli

1. Much fewer PTS transporters for sugar uptake
2. Absence of carbon catabolite repression
3. Preferably use different sugars over glucose

To provide a molecular basis for resource partitioning between homo- and hetero- fermenters using *L. reuteri* as a model.

Experimental design

Genotype
In silico studies

Re-annotate carbohydrate active
enzymes at genome wide



Re-construct sugar transport
and metabolism pathways



Predict sugar transporters and
their substrates from the
inferred protein sequence

Phenotype
Lab experiments

Growth profile on 40+
carbon sources



Gene expression of
predicted transporter in the
medium and sourdough



Substrate spectrum for *L. reuteri* strains

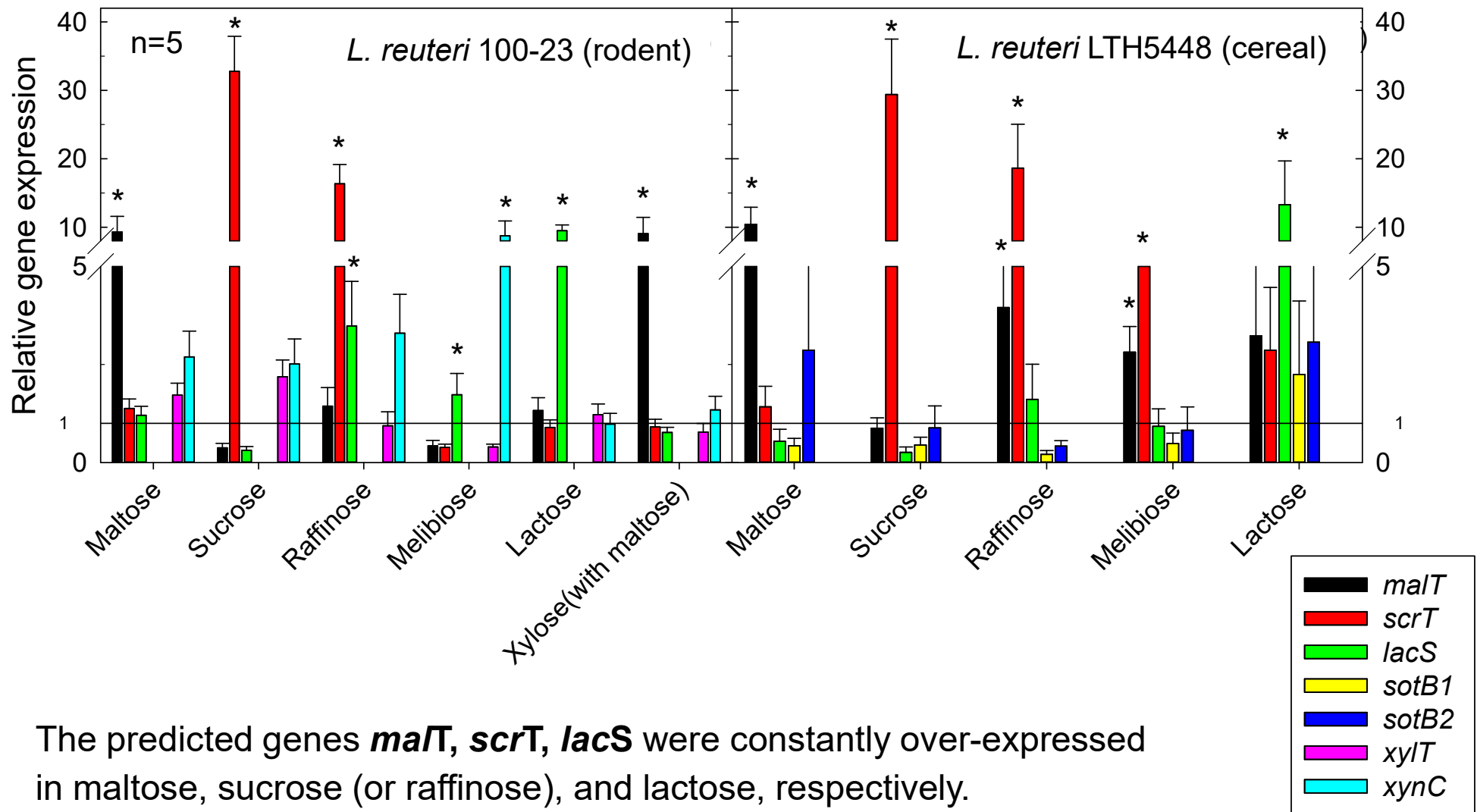
	Cereal isolates				Rodent isolates		
	LTH2584	LTH5448	TMW1.656	TMW1.112	100-23	Ipuph	mlc3
L-arabinose	-	+	-	+	+	+	+
D-ribose	+	+	+	+	+	+	+
D-xylose	+	-	-	-	+	-	+
D-galactose	+	+	+	+	+	+	+
D-glucose	+	+	+	+	+	+	+
D-maltose	+	+	+	+	+	+	+
D-lactose	+	+	+	+	+	+	+
D-melibiose	+	+	+	+	+	+	+
D-sucrose	+	+	+	+	+	+	+
D-raffinose	+	+	+	+	+	+	+

- Of 50 carbon sources tested, 10 sugars are fermentable to *L. reuteri* strains.
- Pentose utilization is strain specific.

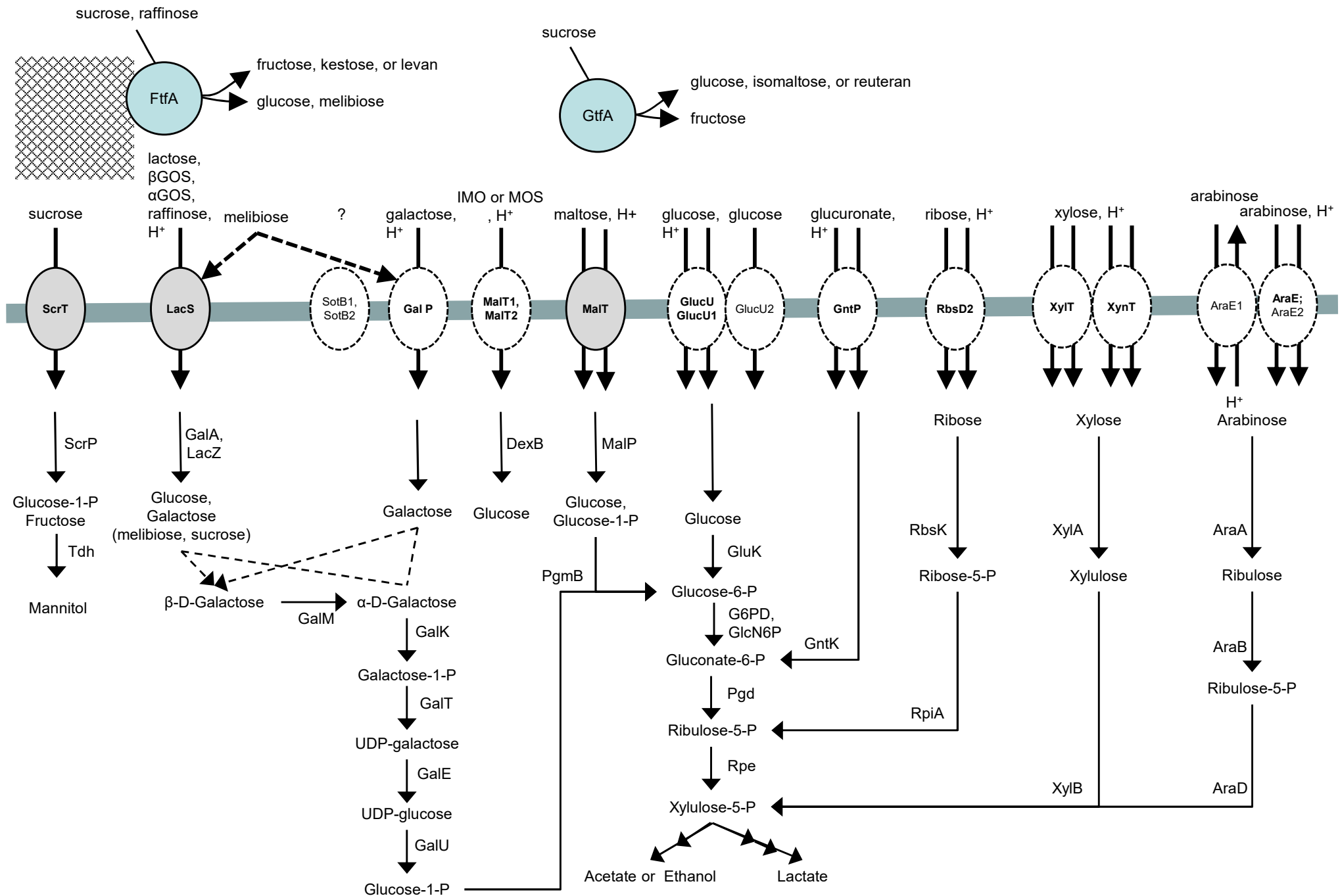
Transporter genes and substrates predicted from the primary sequence

Protein symbols	transmembrane sequence #	Transporter superfamily	Homologous proteins	Similarity (%)	Substrates
RbsD2	11	MFS	Q8XEV7	51.5	D-ribose
AraE1	12	MFS	P31122	29.8	L-arabinose
AraE	12	MFS	C4B4V9	72.7	L-arabinose
AraE2	12	MFS	P96710	30.6	L-arabinose
XylIT	12	MFS	O52733	67.2	D-xylose
XynC	11	MFS	P96792	35.3	D-xylose
GlcU	10	No PTS sugar transporter !	P40420	38.0	D-glucose
GlcU1	10		A0A0E0ZTM0	38.6	D-glucose
GlcU2	12		Q04DP6	26.8	D-glucose
GalP	13		Q9X761	41.8	D-galactose
MalT	12	MFS	Q9A612	44.9	D-maltose
MalT1	12	MFS	Q9A612	27.2	MOS
MalT2	12	MFS	Q8EEC4	27.6	MOS
ScrT	12	DMT	Q04DP6	26.8	D-sucrose
LacS	12	MFS	P23936	38.2	D-lactose/ D-raffinose
SotB1	12	* MFS: Major Facilitator Superfamily	Q9S3J9	29.6	D-melibiose
SotB2	12		Q9S3J9	26.2	D-melibiose

Gene expression of predicted sugar transporters in the chemically defined medium

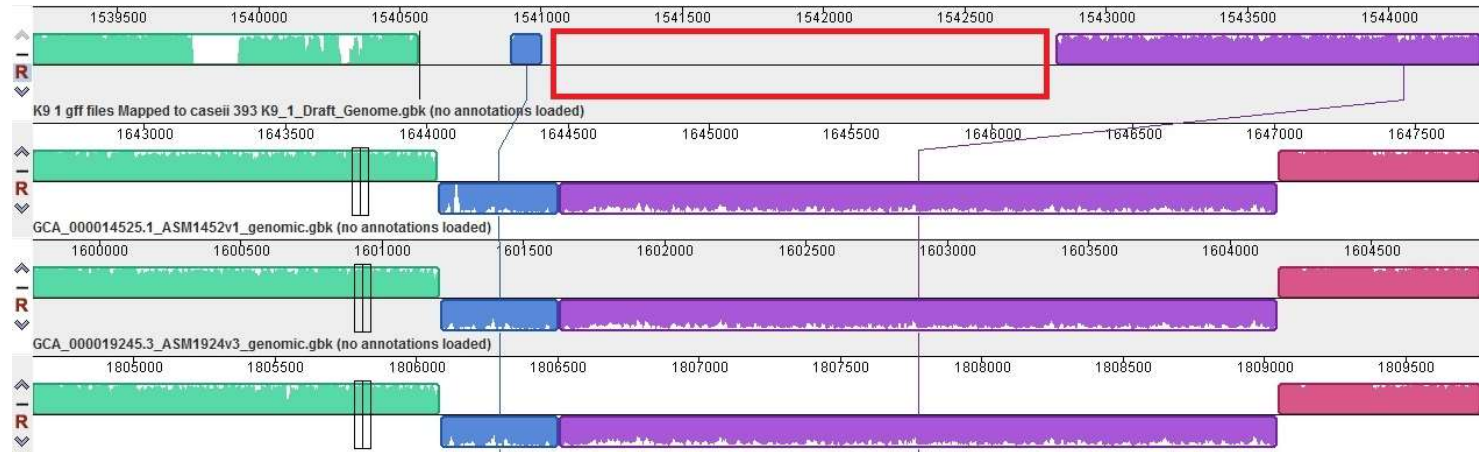


The predicted genes ***malT***, ***scrT***, ***lacS*** were constantly over-expressed in maltose, sucrose (or raffinose), and lactose, respectively.

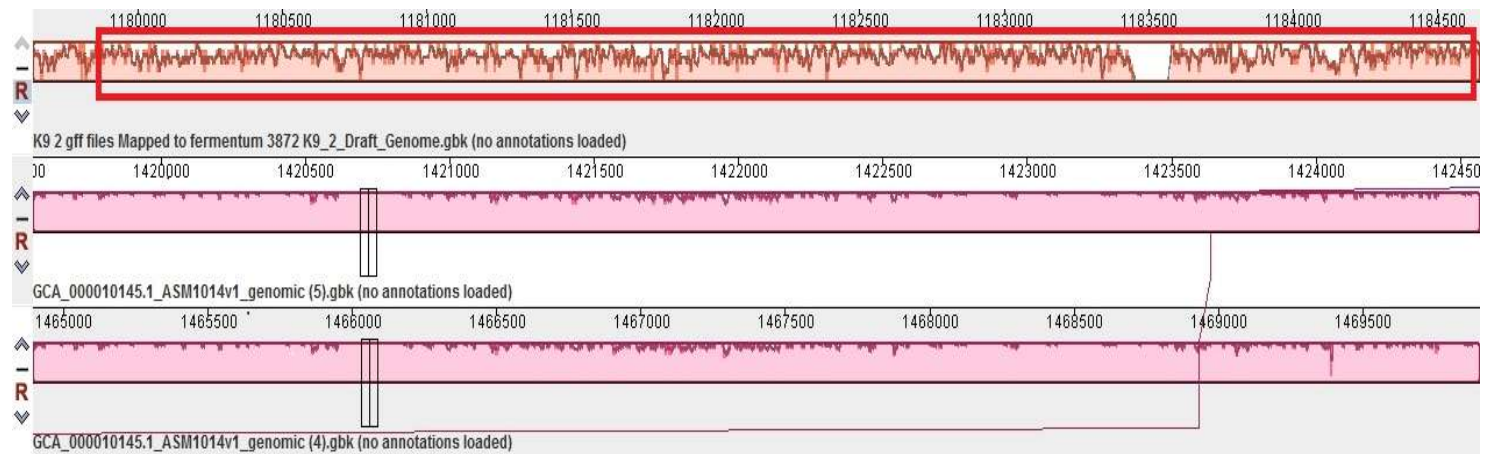


Establishment of strain-specific primers

L. casei K9-1
vs
33 genomes



L. fermentum K9-2
vs
19 genomes

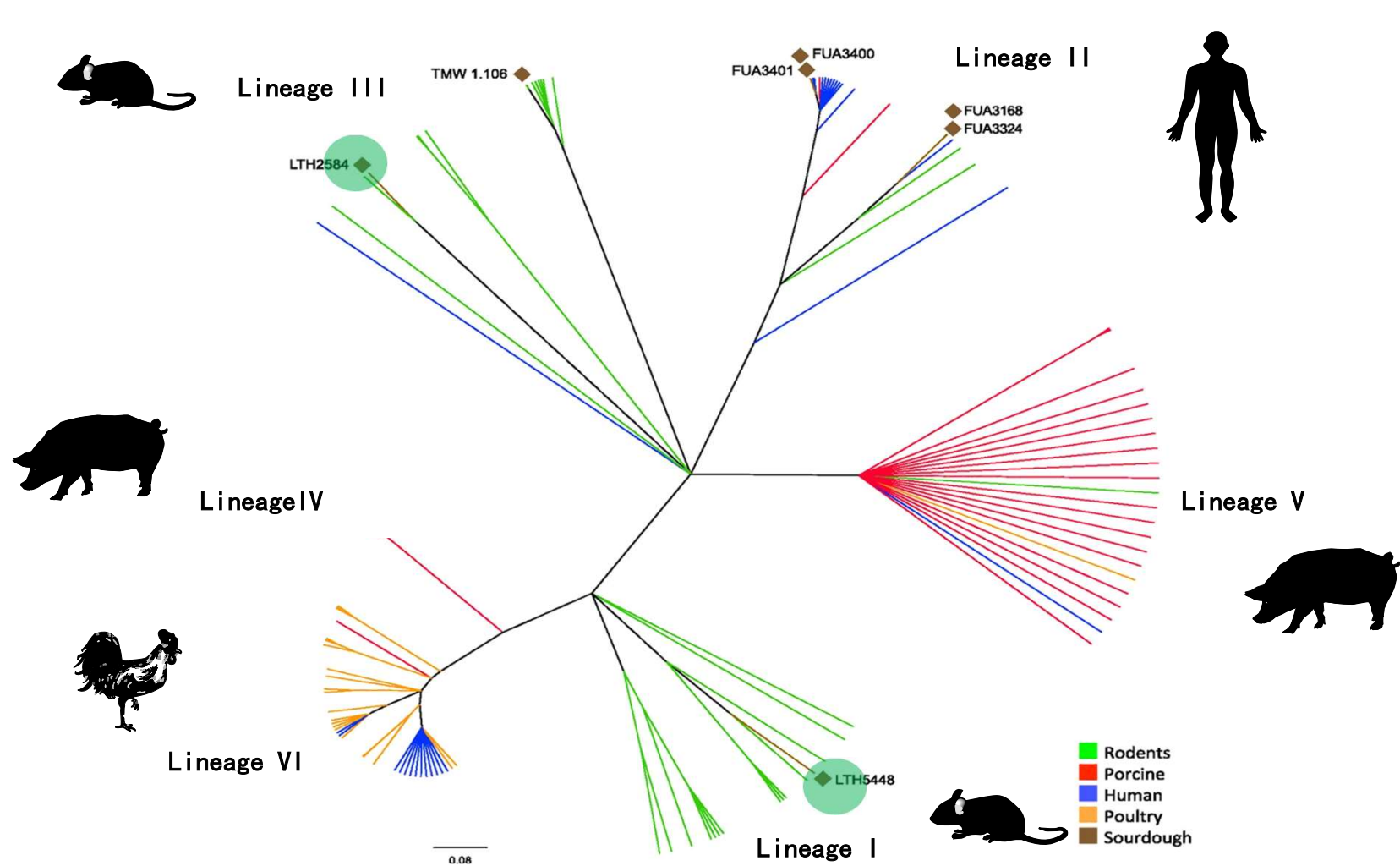


Interim Summary

- *L. reuteri* preferentially uses maltose, sucrose and raffinose. Pentose utilization in *L. reuteri* is strain-specific.
- Carbohydrate transport is mediated exclusively by major facilitator superfamily whereas the PTS sugar transporter is absent.
- *L. reuteri* as a heterofermentative model provides a molecular explanation to the resource partitioning between homo- and hetero- fermenters.

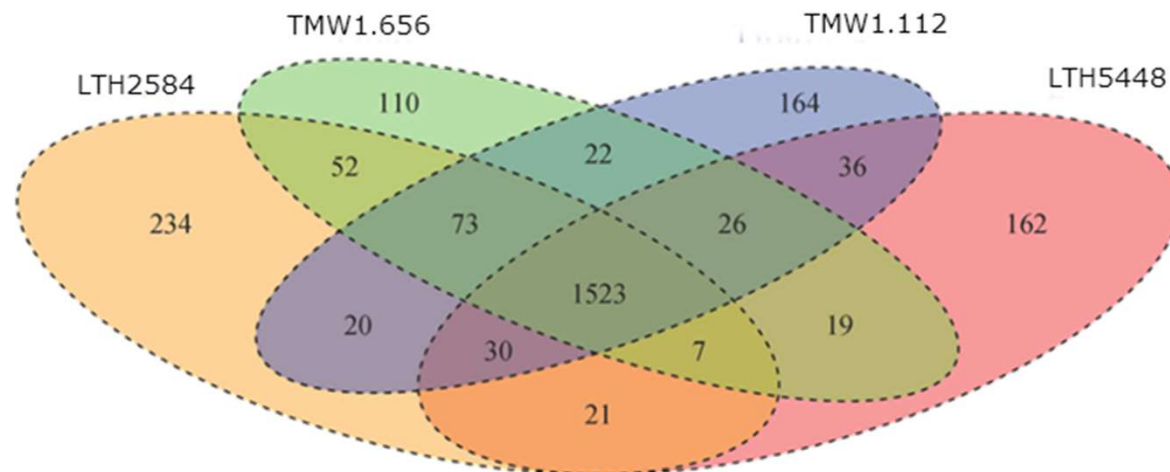
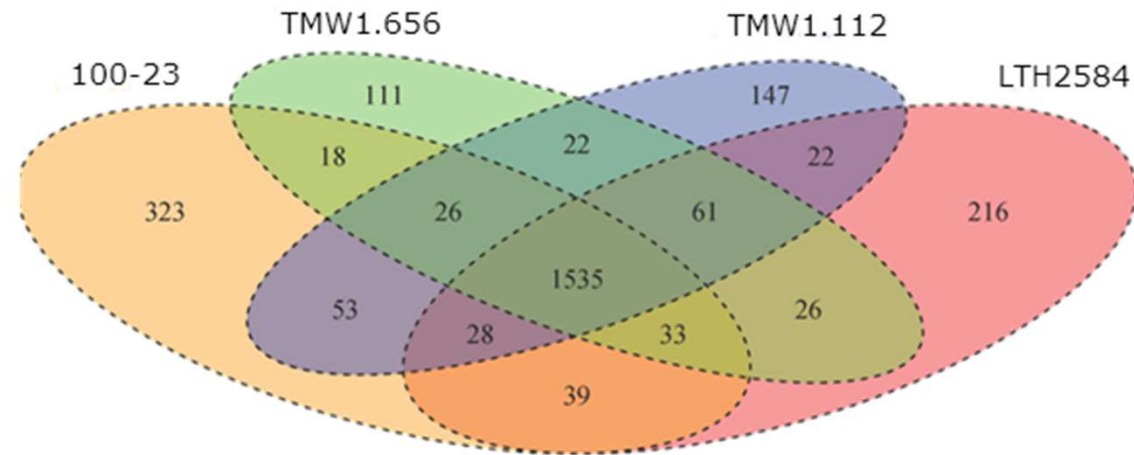
Theme 2

**Comparative genomics and competition experiments
reveal adaptation of intestinal *L. reuteri* to
sourdough fermentations**

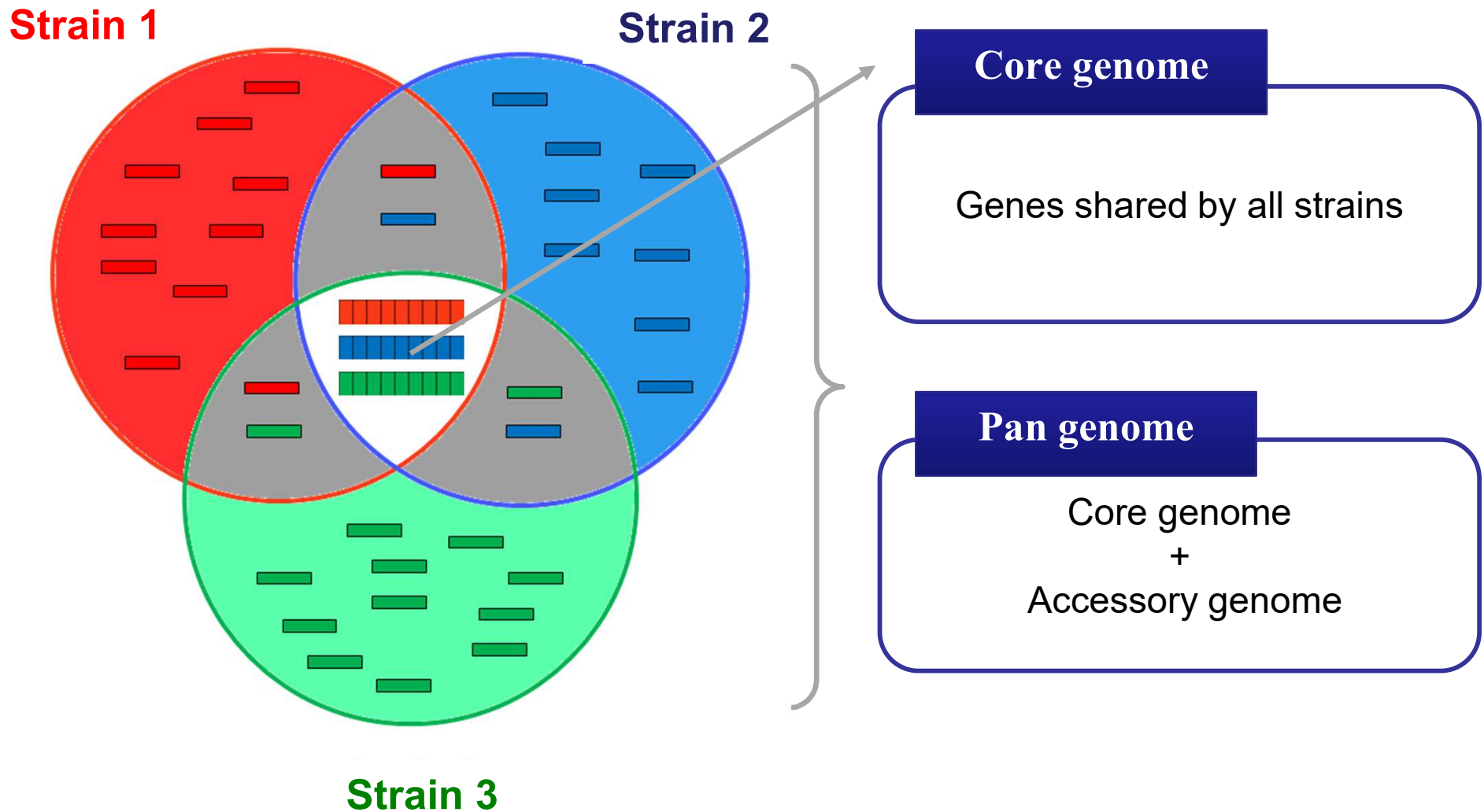


Phylogenetic tree based on MLST

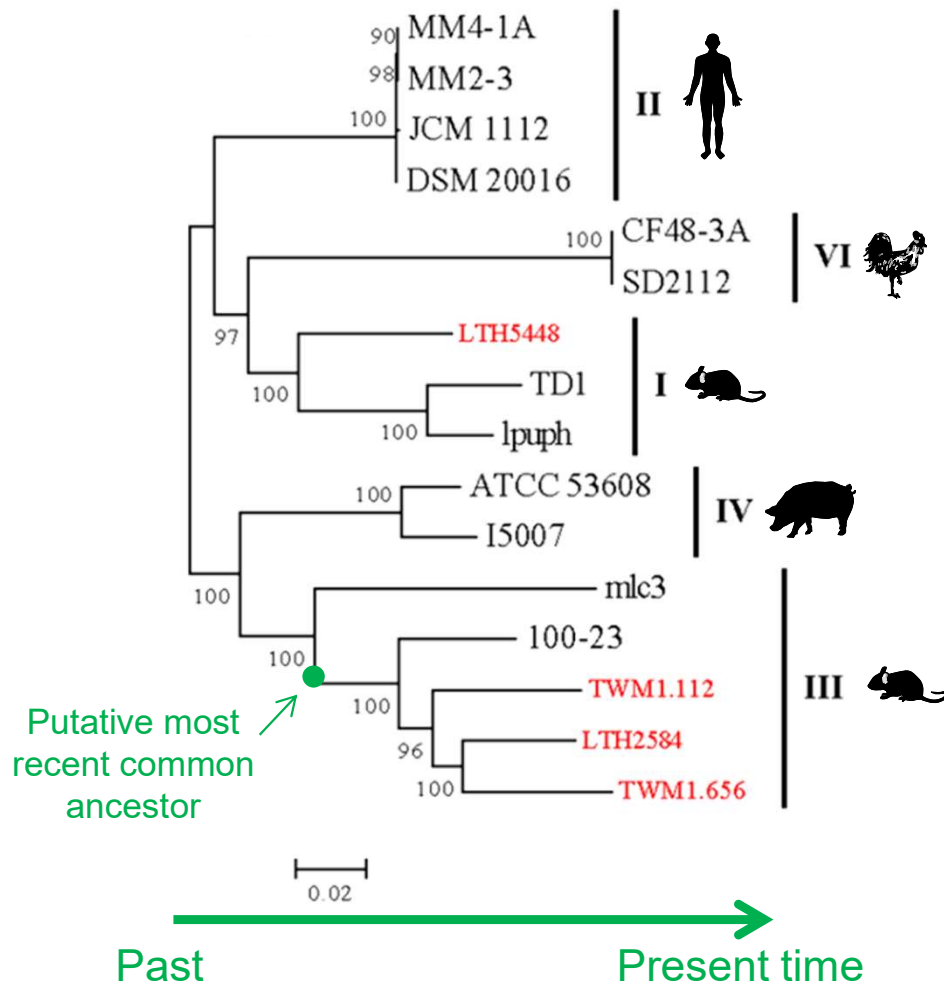
Distribution of shared and unique genes in the selected strains



Comparative genomics: core- and pan- genome



Sourdough isolates are derived from the animal gut



Core-genome tree

Mouse isolates
Human isolates
Chicken isolates
Pig isolates

Competition in mice

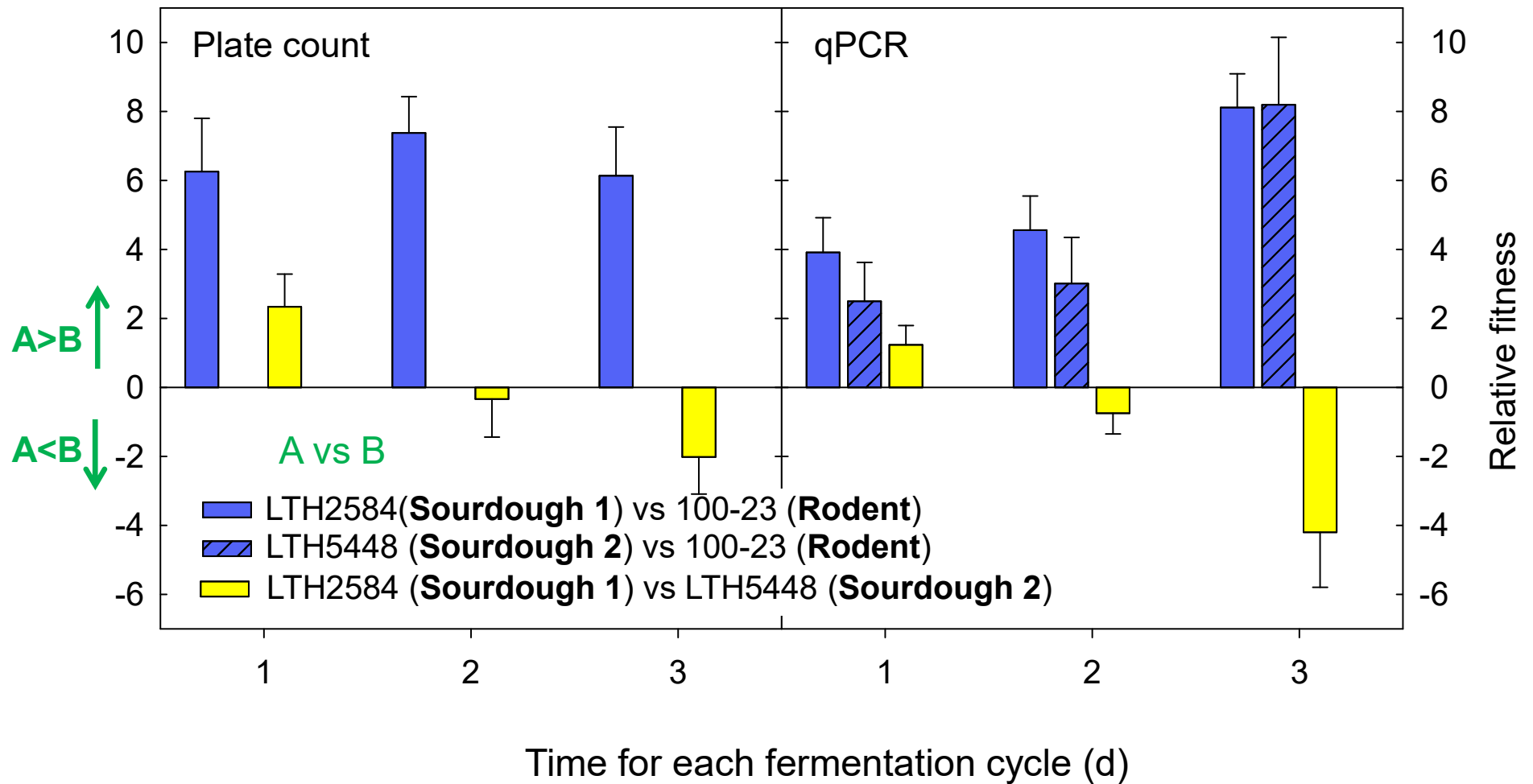
"Epithelial selection of L. reuteri strains is highly specific in mice."



Mouse isolates win.

Sourdough isolates maintain the ability to colonize mouse gut.

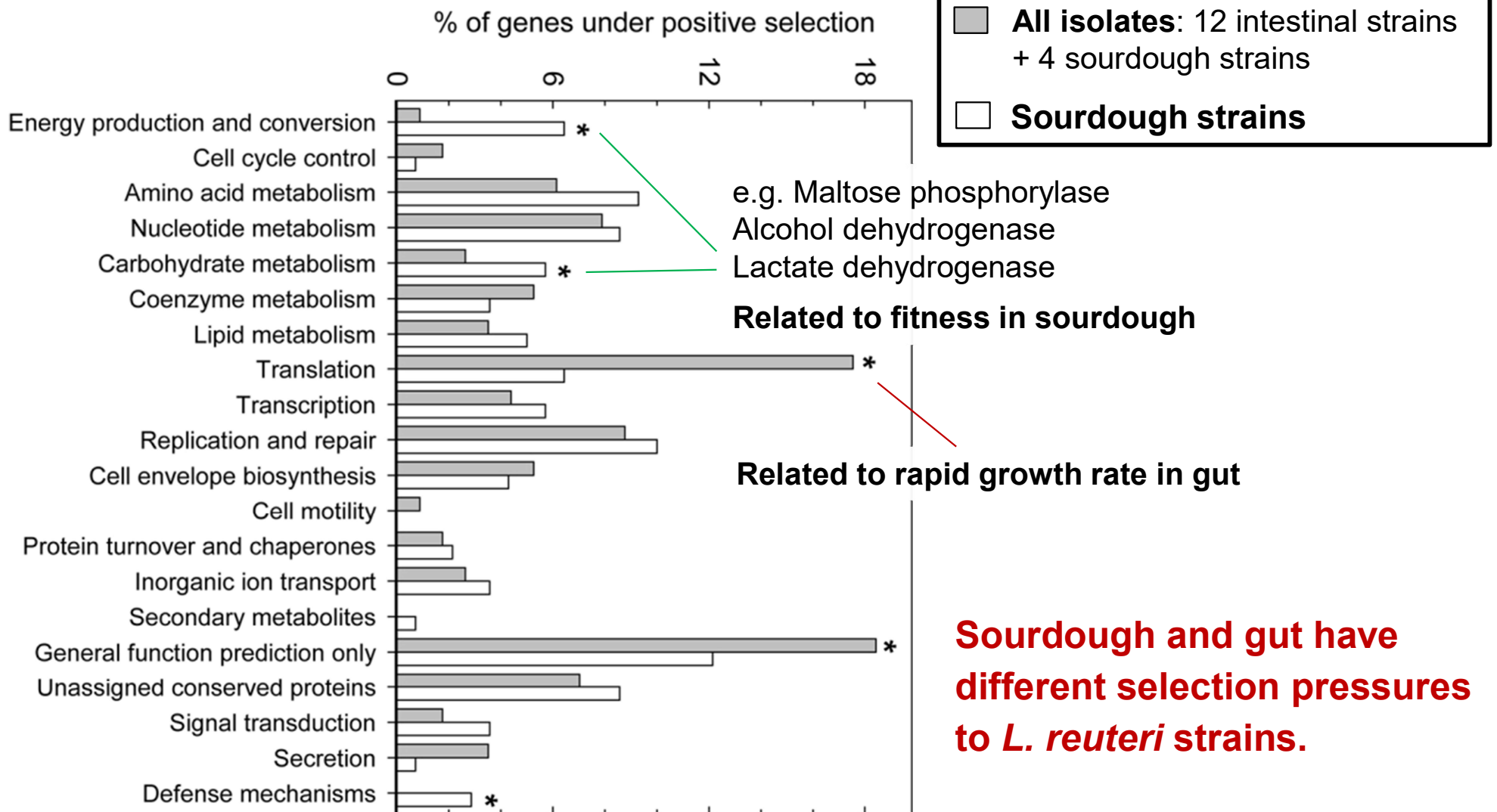
Competition in sourdough: sourdough isolates win



Accessory-genome analysis: genes exclusive to the sourdough isolates

Gene or gene cluster	Putative function	Ecological role
Reutericyclin genomic island	Reutericyclin biosynthesis and resistance ¹	Profit equals to cost
Components of an ABC transporter	Unknown	Undetermined
Aspartate racemase		Undetermined
LytTr DNA-binding domain	Unknown	Undetermined
GntR		Undetermined
Membrane transport protein	Unknown	Undetermined
Hydroxyglutarate dehydrogenase	Use of ketoglutarate as electron acceptor ²	Undetermined
GT8_A4GalT_like proteins	Protein glycosylation	Undetermined
YkuD	Peptidoglycan crosslinking	Undetermined

Core-genome analysis: Positive selection shapes the evolution of *L. reuteri*



Interim Summary

- Positive selection, in addition to horizontal gene transfer, contributes to the adaptive evolution of *L. reuteri* strains.
- Sourdough and intestine exert different selective pressure.
- This study improves our understanding of the adaptation of bacteria from intestine to food ecosystems.

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