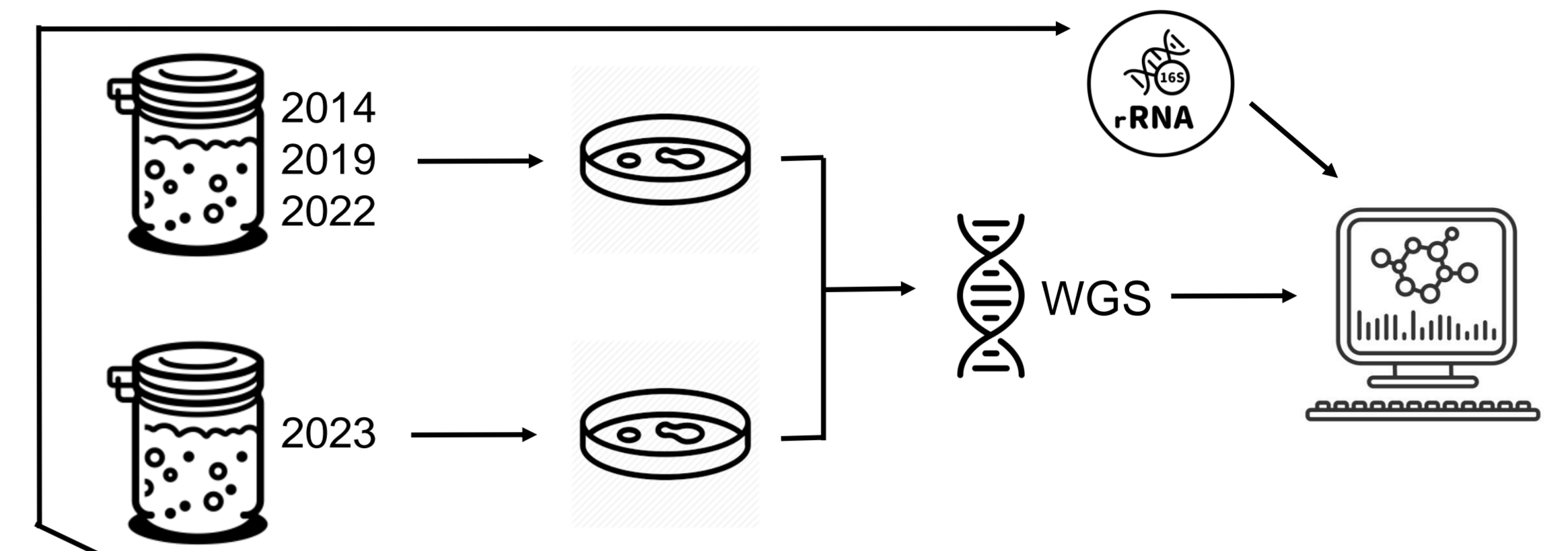


## Introduction

The performance of back-slopped sourdoughs that are continuously maintained in commercial settings relies on the stability of the microbial composition. Methods for strain-level monitoring sourdough microbial communities have included quantitative PCR with strain-specific primers, colorimetric detection using DNAzyme and RAPD typing but these methods did not reveal the genetic relatedness among the strains and impeded downstream analyses. Whole genome sequencing (WGS) enables strain identification at the strain level and further downstream analysis. This study aims to use WGS to assess the stability of microbial community of two sourdoughs collected from the same facility at different time points. A SNP cutoff of 20 was used to identify identical strains [1].

### Objectives

- Use whole genome sequencing to determine the strain-level stability of three sourdoughs that were back-slopped over a period of 3 – 17 years between sampling dates
- Apply computational tools to assess the phylogeny of identified strains



## Results

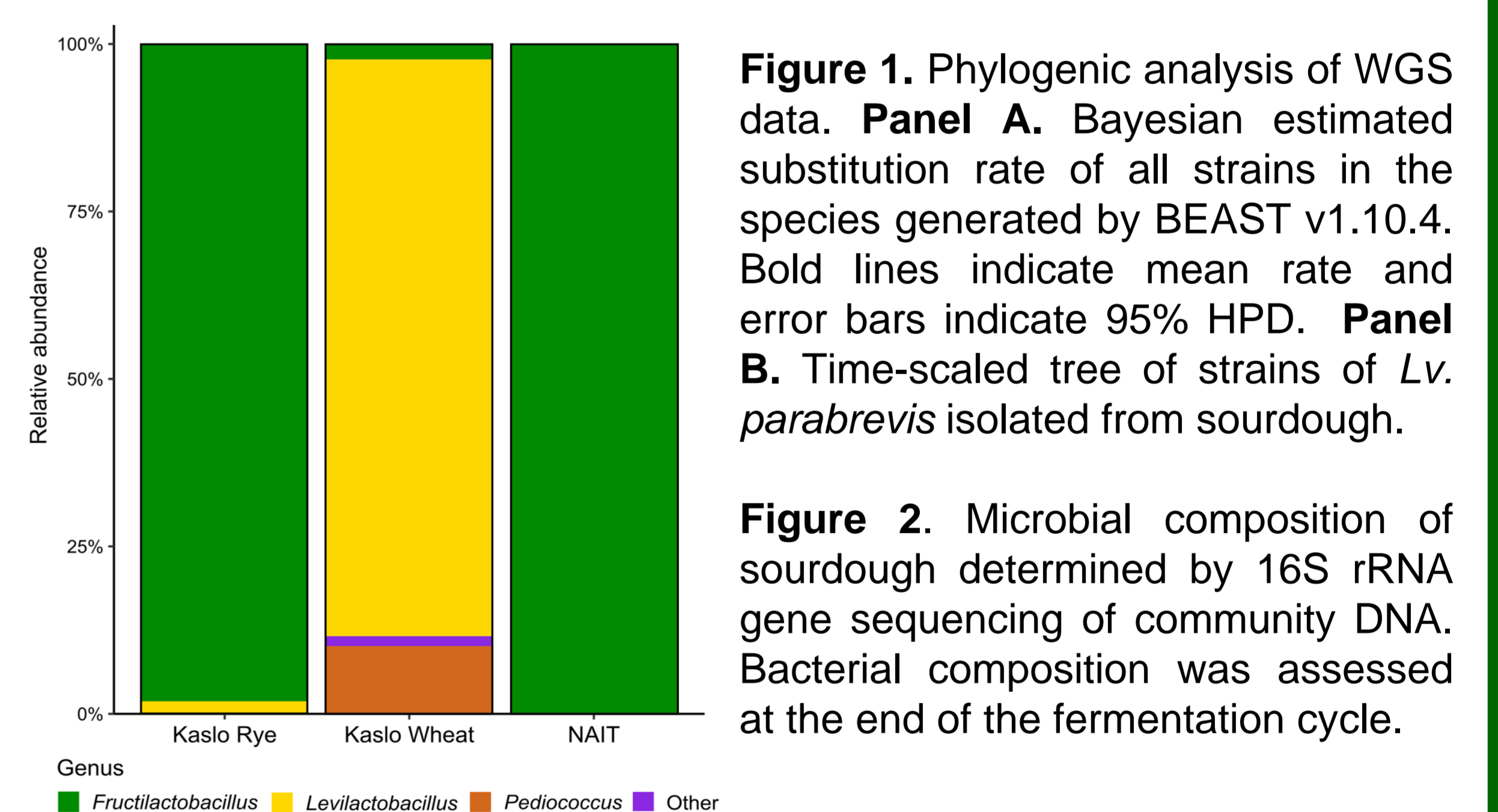
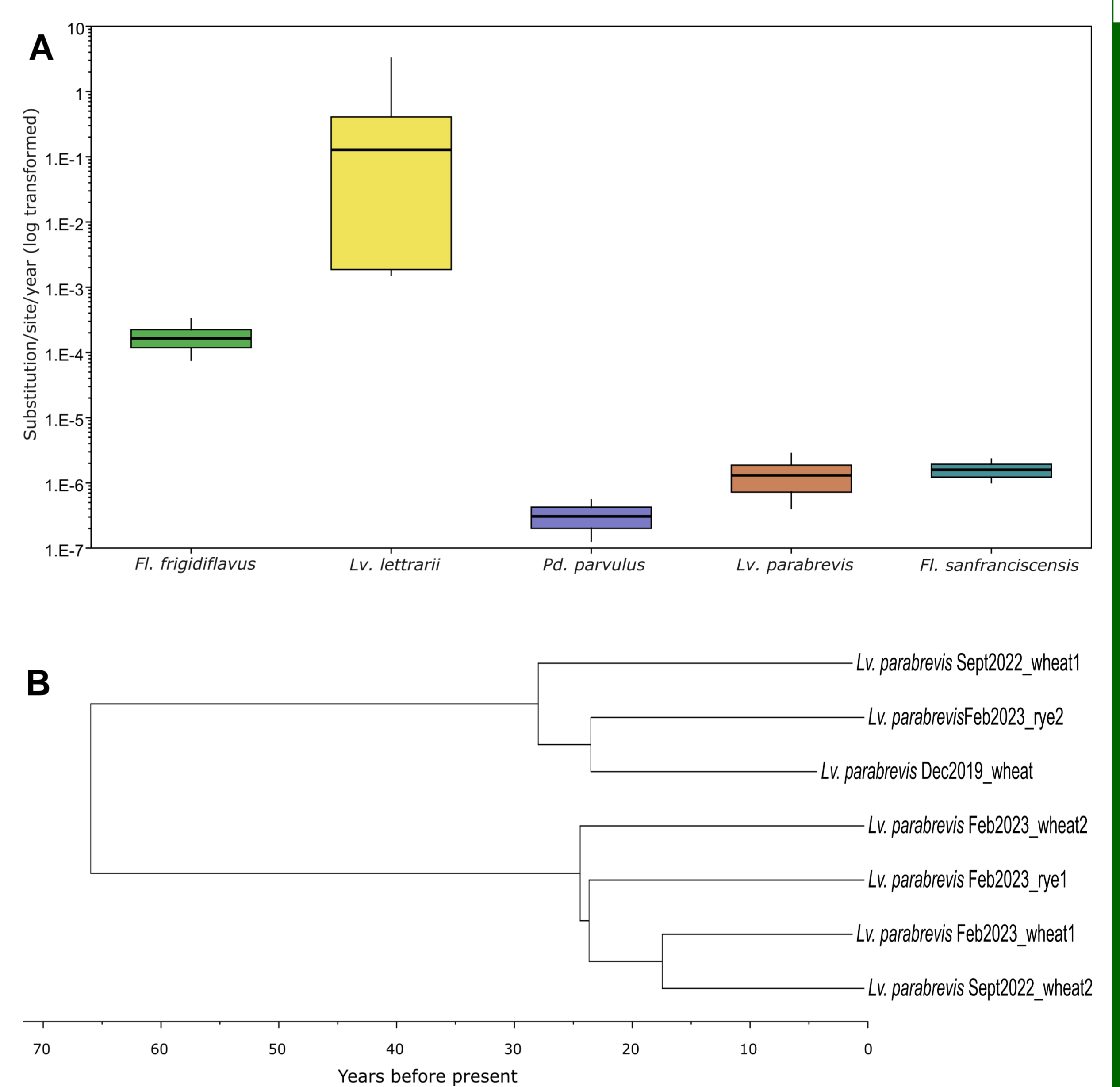
**Table 1.** Identification of microbial community in 3 sourdoughs at 4 time points in a span of 10 years. Microbial composition was assessed at the beginning of the fermentation cycle. Numbers show approximate abundance and hyphens indicate absence using culture-dependent method.

	May 2013	Dec 2019	Sept 2022	Feb 2023
<b>NAIT</b>				
<i>Fructilactobacillus sanfranciscensis</i>	99	-	-	99
<b>Kaslo; whole wheat</b>				
<i>Levilactobacillus parabrevis</i>	-	n.d.	48	35
<i>Pediococcus parvulus</i>	-	n.d.	28	55
<i>Levilactobacillus</i> spp.*	-	n.d.	23	10
<i>Fructilactobacillus</i> spp.*	-	n.e.	1	0
<i>Levilactobacillus koreeensis</i>	-	-	-	<1
<i>Acetobacter malorum</i>	-	-	<1	<1
<i>Acetobacter cerevisiae</i>	-	-	<1	<1
<i>Gluconobacter</i> spp.	-	-	<1	<1
<b>Kaslo; rye</b>				
<i>Levilactobacillus parabrevis</i>	-	-	-	88
<i>Companilactobacillus crustorum</i>	-	-	-	6
<i>Pediococcus parvulus</i>	-	-	-	3
<i>Fructilactobacillus sanfranciscensis</i>	-	-	-	3
<i>Levilactobacillus brevis</i>	-	-	-	<1
<i>Lactiplantibacillus pentosus</i>	-	-	-	<1
<i>Bacillus pumilus</i>	-	-	-	<1

\* The organisms could not be assigned to a currently described species

**Table 2.** Single nucleotide polymorphism (SNP) analysis with and without plasmids of the most abundant isolates. Strains with a SNP cutoff close to 20 are considered identical. SNP analysis was performed using Snippy. Since only one strain was isolated at 2013 and 2019 for each sourdough, it was used as a reference.

Strain	SNP range with plasmids	SNP range without plasmids	Total number of strains	Total number of plasmids
<i>Fl. sanfranciscensis</i>	226	226	2	0
<i>Lv. parabrevis</i>	671 - 1042	671 - 987	7	12
<i>Pd. parvulus</i>	24	24	4	5
<i>Levilactobacillus</i> spp.	34 - 37	33 - 34	3	6
<i>Fructilactobacillus</i> spp.	124 - 128	124 - 128	3	2



**Figure 1.** Phylogenetic analysis of WGS data. **Panel A.** Bayesian estimated substitution rate of all strains in the species generated by BEAST v1.10.4. Bold lines indicate mean rate and error bars indicate 95% HPD. **Panel B.** Time-scaled tree of strains of *Lv. parabrevis* isolated from sourdough.

**Figure 2.** Microbial composition of sourdough determined by 16S rRNA gene sequencing of community DNA. Bacterial composition was assessed at the end of the fermentation cycle.

## Conclusions

- *Lv. parabrevis* dominates both Kaslo wheat and rye sourdough in culture-dependent method followed by *Pd. parvulus* and *Fructilactobacillus* spp. while *Fl. sanfranciscensis* dominates the NAIT sourdough
- Microbial composition of back-slopped sourdough remained stable over several years
- At the strain level, only strains of *Pd. parvulus* are identical while other strains are closely related but differ by more than 20 SNPs
- *Lv. parabrevis* evolves at a much faster rate than *Pd. parvulus*. The time scaled tree indicates that two strains of *Lv. parabrevis* were populated the sourdough about 25 years ago, followed by rapid evolution during continuous propagation.

### References:

[1] Pightling AW et al. Interpreting Whole-Genome Sequence Analyses of Foodborne Bacteria for Regulatory Applications and Outbreak Investigations. Front Microbiol. 2018 Jul 10;9:1482