Bacterial and Fungal Succession in a Sourdough Fermentation Time Course

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Abstract

Sourdough starters provide a valuable model for studying microbial community dynamics over time. In this study, we investigated the succession of fungal and bacterial species during the sourdough fermentation process. Microorganisms were cultured on agar plates, and colony morphology was documented through imaging and microscopic analysis. DNA was extracted from selected colonies, specific genes were amplified via PCR, and sequencing techniques were employed to identify the microbial taxa present at various stages of fermentation. Additionally, we monitored pH levels and quantified microbial cell counts throughout the experiment. Our results revealed a temporal shift in microbial populations, with fungi such as *Pichia* and *Saccharomyces*, and bacteria including *Lactiplantibacillus plantarum* and *Levilactobacillus brevis*, emerging as dominant species. Metabarcoding analysis indicated that plant-derived DNA from flour degraded rapidly, resulting in a predominance of microbial DNA in later samples. These findings enhance our understanding of microbial community succession and ecosystem dynamics in fermented food systems.

Introduction

Sourdough starters are created through the interaction of flour and water, forming a habitat where diverse microbes, particularly fungi and bacteria, flourish. As fermentation progresses, these microbial communities undergo succession, shaped by environmental factors such as acidification, nutrient availability, and interspecies competition. This project explores these temporal changes in microbial composition using both culture-based methods and molecular techniques. Initially, microbes were isolated from a developing starter and grown on agar plates to observe colony morphology. Fungal colonies were further examined under a light microscope to capture defining cellular structures. DNA was extracted from selected colonies using a simple boiling protocol. Target regions of the 16S rRNA gene for bacteria and the internal transcribed spacer (ITS) region for fungi were amplified through polymerase chain reaction (PCR), followed by product verification using gel electrophoresis. Selected DNA samples were sent for Sanger sequencing, and BLASTn was used for species identification. Sequence alignments confirmed accuracy. In parallel, next-generation sequencing (NGS) metabarcoding was used to study community-level shifts over time. This revealed a rapid decline in plant-derived DNA from the flour and a growing dominance of microbial DNA, providing a detailed view of microbial succession and ecological function during fermentation. A special focus was given to Lactobacillus, a key genus within the lactic acid bacteria (LAB) group. These Gram-positive rods are known for fermenting carbohydrates into lactic acid, contributing to food preservation and human health. Found in environments like the human gut, oral cavity, and vaginal epithelium, Lactobacillus species help maintain low pH, produce antimicrobial compounds, and interact with the immune system. Industrially, they play essential roles in fermenting dairy, meat,

Methods and Materials

To investigate microbial succession and activity in a sourdough starter over time, we employed a combination of culture-based, microscopic, molecular, and bioinformatic techniques. pH and microbial growth were tracked over the course of fermentation (Fig. 1), and cell density measurements for both fungi and bacteria were taken at regular intervals to assess changes in population dynamics (Fig. 2). Samples were plated on selective media to isolate and distinguish colony morphologies (Fig. 3). Fungal colonies showed clear morphological differences, allowing differentiation between *Pichia* (larger, clustered growth) and *Saccharomyces* (smaller, more dispersed colonies). Bacterial colonies also exhibited distinct forms, with *Lactiplantibacillus plantarum* forming faster-growing, white colonies, and *Levilactobacillus brevis* forming slower-growing, translucent ones. Microscopic examination using wet mount preparations further confirmed fungal identity, showing *Pichia* growing in clusters, while *Saccharomyces* appeared as individual cells (Fig. 4). DNA was extracted from representative colonies using a Chelex-based boiling method, and specific genetic markers were amplified: the 16S rRNA gene for bacteria and the internal transcribed spacer (ITS) region for fungi. Amplicons were verified by gel electrophoresis (Fig. 5). Selected PCR products were subjected to Sanger sequencing, with resulting electropherograms analyzed using BLASTn to identify microbial species. Sequence alignment was performed using MUSCLE to confirm identity (Figs. 6–8). To complement culture-dependent analyses, next-generation sequencing (NGS) metabarcoding was performed to monitor microbial community composition and the degradation of plant DNA in the starter over time.

A decay curve was generated to show the rapid breakdown of mitochondrial and chloroplast rRNA from flour, indicating a shift toward a predominantly microbial DNA profile as fermentation progressed (Fig. 9a). A MiAn stacked bar plot was used to visualize shifts in microbial taxa throughout the fermentation process (Fig. 9)

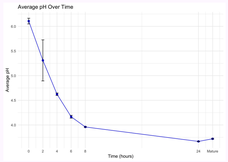

Fig 1: pH and microbial biomass (OD or CFU/mL) measured daily from sourdough starter culture. This line graph shows pH decrease and microbial growth increase over fermentation time. pH drops rapidly in early days, stabilizing as lactic acid bacteria dominate.

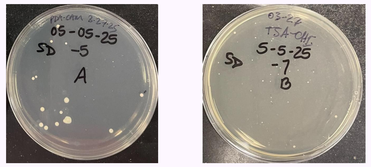

Fig 3: Visual differentiation of dominant fungal and bacterial colonies on selective media. Fungal: Distinct colony morphology—Pichia (clustered, large), Saccharomyces (smaller, dispersed). Bacterial: L. plantarum (whiter, faster), L. brevis (translucent, slower).

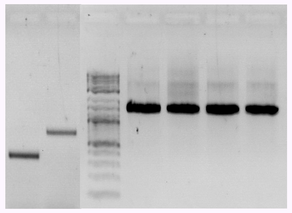

Fig 5: DNA amplicons from fungal and bacterial colonies confirmed via gel electrophoresis. Bands at ~500 bp (ITS) and ~1500 bp (16S).

Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand
2726 bits(1476)	0.0	1476/1476(100%)	0/1476(0%)	Plus/Plus

Lactiplantibacillus plantarum strain LLY-606 chromosome, complete genome
Sequence ID: CP023066.1 Length: 3194447 Number of Matches: 5
Range 1: 1110176 to 1111651 GenBankGraphics Next Match Previous Match

```
Query  1        GAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAACGAACTCTGGTATTGATTGGTGCT  60
Sbjct  1110176  GAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAACGAACTCTGGTATTGATTGGTGCT  1110235
Query  61       TGCATCATGATTTACATTTGAGTGAGTGGCGAACTGGTGAGTAACACGTGGGAAACCTGC  120
Sbjct  1110236  TGCATCATGATTTACATTTGAGTGAGTGGCGAACTGGTGAGTAACACGTGGGAAACCTGC  1110295
```

Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand
2719 bits(1472)	0.0	1472/1472(100%)	0/1472(0%)	Plus/Plus

Lactobacillus brevis strain 100D8, complete genome
Sequence ID: CP023139.1 Length: 2351988 Number of Matches: 5
Range 1: 920309 to 921780 GenBankGraphics Next Match Previous Match

```
Query  1       ACGCTGGCGGCATGCCTAATACATGCAAGTCGAACGAGCTTCCGTTGAATGACGTGCTTG  60
Sbjct  920309  ACGCTGGCGGCATGCCTAATACATGCAAGTCGAACGAGCTTCCGTTGAATGACGTGCTTG  920368
Query  61      CACTGATTTCAACAATGAAGCGAGTGGCGAACTGGTGAGTAACACGTGGGGAATCTGCCC  120
Sbjct  920369  CACTGATTTCAACAATGAAGCGAGTGGCGAACTGGTGAGTAACACGTGGGGAATCTGCCC  920428
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Fig 7: Species identification based on 16S (bacteria) and ITS (fungi) sequences.Screenshots or formatted results showing best hits from NCBI BLASTn for each sample.

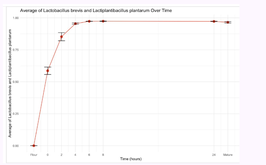
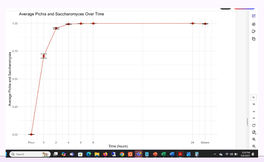


Fig 2: Quantification of viable fungal and bacterial cells at multiple timepoints. Two line plots representing CFU counts (colony-forming units) for fungi and bacteria, tracked over time. Bacteria peak earlier, fungi show slower, steady growth.



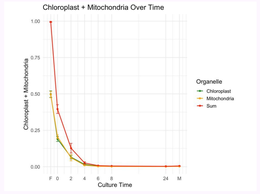


Fig 9a: Rapid degradation of plant DNA (rRNA genes) over time indicates microbial DNA dominance. Logarithmic plot showing exponential decay of plant mitochondrial and chloroplast DNA.

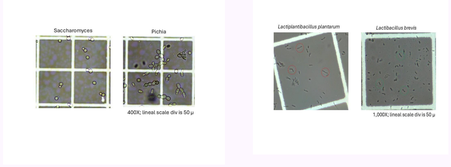


Fig 4: Light microscopy reveals morphological differences in dominant fungal species. Pichia spp. seen in dense clusters, some budding visible. Saccharomyces spp. dispersed single cells, less clustering. Lactic acid bacteria in micrographs at right

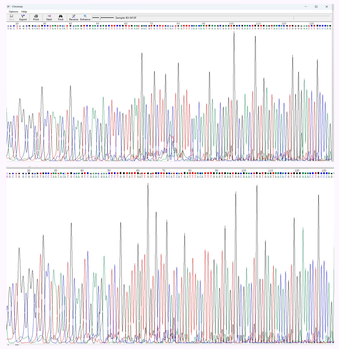


Fig 6: Representative Sanger sequencing output showing DNA base variation. Chromatograms of sequencing traces with highlighted differences in base pairs between samples.

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B4-M13F  --ACGCTGGCGGCATGCCTAATACATGCAAGTCGAACGAGCTTCCG--TTGAATGACGTG
B3-M13F  --ACGCTGGCGGCATGCCTAATACATGCAAGTCGAACGAGCTTCCG--TTGAATGACGTG
B2-M13F  --ACGCTGGCGGCGTGCCTAATACATGCAAGTCGAACGAACTCTGGTATTGATTG--GTG
B1-M13F  --ACGCTGGCGGCGTGCCTAATACATGCAAGTCGAACGAACTCTGGTATTGATTG--GTG
          ********** ************************** **   *  **** **  ***

B4-M13F  CTTGCAC--TGATTTCAACAATGAAGCGAGTGGCGAACTGGTGAGTAACACGTGGGGAAT
B3-M13F  CTTGCAC--TGATTTCAACAATGAAGCGAGTGGCGAACTGGTGAGTAACACGTGGGGAAT
B2-M13F  CTTGCATCATGATTT--ACATTTGAGTGAGTGGCGAACTGGTGAGTAACACGTGGGAAAC
B1-M13F  CTTGCATCATGATTT--ACATTTGAGTGAGTGGCGAACTGGTGAGTAACACGTGGGAAAC
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Fig 8: MSA highlights nucleotide differences confirming that LP and LB belong to separate genera. MUSCLE alignment of Lactiplantibacillus plantarum and Levilactobacillus brevis sequences shows genus-level differences.

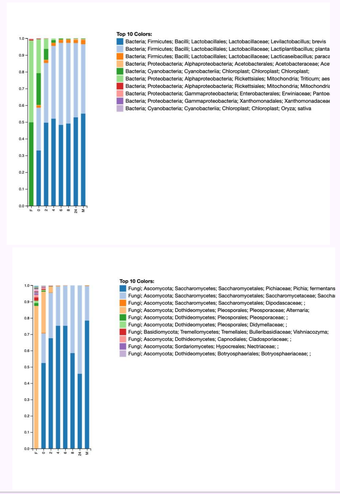

Fig 9 b-d: Metabarcoding reveals succession of fungal and bacterial communities during sourdough fermentation.

- b: Overall taxa shifts
- c: Fungal genera (Pichia, Saccharomyces)
- d: Bacterial genera (Lactiplantibacillus, Levilactobacillus)

Results

The results confirmed that microbial populations in sourdough undergo distinct and predictable succession. Pichia and Saccharomyces became the dominant fungi, while Lactiplantibacillus plantarum and Levilactobacillus brevis emerged as the leading bacterial species. These shifts appear driven by environmental pressures such as acid buildup and interspecies interactions. Morphological traits and sequencing results supported these identifications. Interestingly, our data also revealed genetic and functional diversity within the lactic acid bacteria group. Recent reclassifications splitting Lactobacillus into genera like Lactiplantibacillus and Levilactobacillus are supported by our results and underscore the ecological complexity within even closely related microbes. Furthermore, trends observed in culture-based analysis mirrored those seen in metabarcoding, suggesting consistent succession patterns across methods. The rapid decline in flour DNA and rise in microbial signal provide a strong indication of active fermentation. This microbial dominance allowed us to study the succession patterns more clearly and draw insights into ecosystem dynamics within the starter culture.

Discussion

Our investigation highlights the utility of sourdough as a model for understanding microbial succession in food systems. The combined use of microscopy, culturing, sequencing, and metabarcoding enabled us to track how microbial communities assemble, compete, and stabilize under dynamic conditions. The consistent emergence of dominant species demonstrates that even in open systems like sourdough, ecological principles like niche adaptation and competitive exclusion are at play. These microbial relationships aren't merely academic; they're responsible for the biochemical transformations that make fermented foods nutritious, safe, and flavorful. Microbes produce lactic acid and bioactive compounds that shape taste and texture, while also influencing gut health. As such, understanding these relationships opens the door to better quality control in food production, targeted use of probiotics, and microbiome-informed dietary recommendations. Finally, this study contributes to broader efforts in food transparency and microbiome literacy. As consumers demand to know more about the origins and composition of their food, molecular-level insights into fermentation offer a scientific basis for labeling, safety assurance, and personalized nutrition. By charting microbial succession in a staple like sourdough, we not only explore a fascinating biological system but also pave the way for a more sustainable and informed food future.

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