



Research Paper

Identification of dominant lactic acid bacteria and yeast in rice sourdough produced in New Zealand

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ABSTRACT

This study characterised a commercial New Zealand gluten free (GF) rice sourdough and its starter culture composition. Acidity of the mother sourdough, dough before proofing and dough after proofing was determined during the production of rice sourdough bread, and colour was measured for the baked bread. Yeast and lactic acid bacteria (LAB) were enumerated in the rice sourdough samples and representative colonies characterised using API kits and sequenced by the Internal Transcribed Spacer and 16 S rRNA region. Sourdough LAB isolates were identified as *Lactobacillus* (*L.*) *papaplantarum* DSM 10667 and *L. fermentum* CIP 102980 and the yeast isolates as *Saccharomyces* (*S.*) *cerevisiae* CBS 1171. Dough acidity increased significantly ($p < 0.05$) during fermentation due to the metabolic activities of the sourdough cultures. After baking, the colour of the rice sourdough bread crust was similar to that of unleavened wheat bread (golden brown). The improved colour of the rice sourdough bread crust may be a result of combined use of sourdough technique and optimal baking conditions. The results of this study may allow bakers to improve the overall quality of GF rice sourdough baked bread by selecting suitable fermentation and baking parameters.

1. Introduction

The need for gluten free products is increasing due to the special dietary requirements of celiac patients and non-celiac consumers (Witczak et al., 2016). Celiac patients are recommended to consume a gluten-free (GF) diet throughout their lifetime to avoid the immune responses triggered by gluten (Pérez-Quirce et al., 2014).

Most GF breads, including rice bread are perceived to have a poor mouth feel, dry crumb, and bland flavor, thus research in this field has aimed to improve the characteristics of gluten free breads (Gobbetti and Gänzle, 2012; Moroni et al., 2009). Using hydrocolloids and non-gluten proteins as additives is not ideal, as they have been associated with challenges such as induction of allergies and relatively high cost (Gobbetti et al., 2008; Moroni et al., 2009). An alternative approach would be to use the sourdough technique, which has been recently employed for improving the sensory qualities of GF bread due to its low cost and efficiency at improving colour and texture (Moroni et al., 2009; Mantzourani et al., 2019).

Sourdough is made by mixing flour and water followed by fermentation using inherent LAB and yeast (Lhomme et al., 2015; Todorov and

Holzappel, 2014). Previous research on GF products indicated that fermentation of sourdough increased volume and improved texture, flavour and the nutrient content of bakery products (Gobbetti et al., 2008; Gobbetti and Gänzle, 2012). Furthermore, compared to unleavened cereal flour, higher levels of free amino acids, vitamins and bioactive minerals are released during sourdough fermentation, resulting in the improved nutrient availability for the consumer (Moroni et al., 2009).

Since the quality and other characteristics of sourdough are related to the activities of the starter cultures used, knowledge of the composition of the cultures and their metabolic activities are important (Galli et al., 2019; Mantzourani et al., 2019). The LAB and yeast in the starter culture produce metabolites such as organic acids, carbon dioxide and exopolysaccharides (EPS) which contribute to sourdough flavour, texture and volume (De Vuyst and Neysens, 2005). Mature sourdoughs, which have a stable performance, have been reported to contain more than 8 log CFU/g LAB (Ehrmann and Vogel, 2005; Ercolini et al., 2013). The number of co-existing yeast are usually 1–2 logarithmic magnitudes lower than the LAB (Ehrmann and Vogel, 2005; Ercolini et al., 2013; Hounbédji et al., 2019; Minervini et al., 2014). Currently, over 90

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species of LAB and more than 40 species of yeast have been isolated from wheat and rye sourdoughs (Comasio et al., 2020).

Some GF sourdoughs have been reported to contain novel starter culture strains with potential to produce high quality GF bread (Gobbetti and Gänzle, 2012; Foschia et al., 2016). However, published data show that there is only rudimentary information on the composition of GF rice sourdough starter cultures (De Vuyst and Neysens, 2005; Meroth et al., 2004; Park et al., 2017). Therefore, research on the composition of rice sourdough starter cultures of an existing well-accepted commercial sourdough product will provide information on their growth and metabolic characteristics, which may help to optimise fermentation conditions resulting in improved quality of GF sourdough baked products.

2. Materials and methods

2.1. Sample collection

Samples of sourdough and sourdough bread produced by a local commercial manufacturer were collected once per week for 10 consecutive weeks at four stages of production: mother sourdough (MSD) ($n = 10$), dough before proofing (DBP) ($n = 10$), dough-after-proofing (DAP) ($n = 10$) and sourdough bread (SDB) ($n = 10$) as shown in Fig. 1. All samples were transported under chilled conditions (4°C) to Massey University, Auckland Campus, New Zealand, for analysis. Independent experiments were repeated ten times ($n = 10$) and analyses/measurements of samples were conducted in duplicate. Chemicals and reagents used in the experiments were of reagent grade or higher.

The initial MSD was prepared by mixing water, brown rice flour and sourdough starter culture. This mixture designated as the MSD was stored at 4°C for two days. A portion of the stored MSD starter culture was used in sourdough bread-making, while the remainder (MSD) was refreshed every two days by mixing with water and brown rice flour (Ceres Organics, NZ). Refreshed MSD was stored at 4°C for two days and

then used for bread-making.

To obtain the DBP, a portion of the two-day old MSD was mixed with potable water, brown rice flour, white rice flour, tapioca starch, corn starch, guar gum, brown rice malt syrup and salt (Ceres Organics, NZ). The DBP was mechanically divided into equal portions and placed into individual aluminium bread baking tins ($38\text{ cm} \times 14\text{ cm}$) and then allowed to ferment at 38°C for 3–3.5 h in a humidity-controlled incubator (95–102% relative humidity) to produce bread dough after proofing (DAP). Following fermentation (proofing), the fermented and leavened bread dough loaves were baked in a steam oven (703 Multi-rack, Revent Oven, Sweden) at 265°C for 40 min to obtain sourdough baked bread (SDB).

2.2. pH and total titratable acidity (TTA)

Ten (10) grams of each sourdough sample (MSD, DBP, DAP) containing starter cultures was measured in duplicate into a sterile stomacher bag (Global Science, NZ) and homogenised with 90 mL of distilled water using a stomacher laboratory paddle (Masticator 400 ml, IUL, Spain) (Lhomme et al., 2015). The pH of the homogenate was measured using a standardised glass electrode pH meter (HI 2221, Hanna Instruments, UK). Titratable acidity of the homogenate was determined by titration using 0.1 M NaOH to a faint persistent light pink and the results were expressed as grams of lactic acid/gram sample.

2.3. Colour measurement of bread crust

Bread crust colour was measured using a Minolta CR-300 model Chroma Meter (Japan) with CIE colour space for a^* coordinates (redness), b^* coordinates (yellowness) and L^* coordinates (lightness) (Pérez-Quirce et al., 2014). Twelve (12) crust pieces from six SDB loaves were measured for each sampling time. The colour of the top crust and bottom crust of each bread was measured.

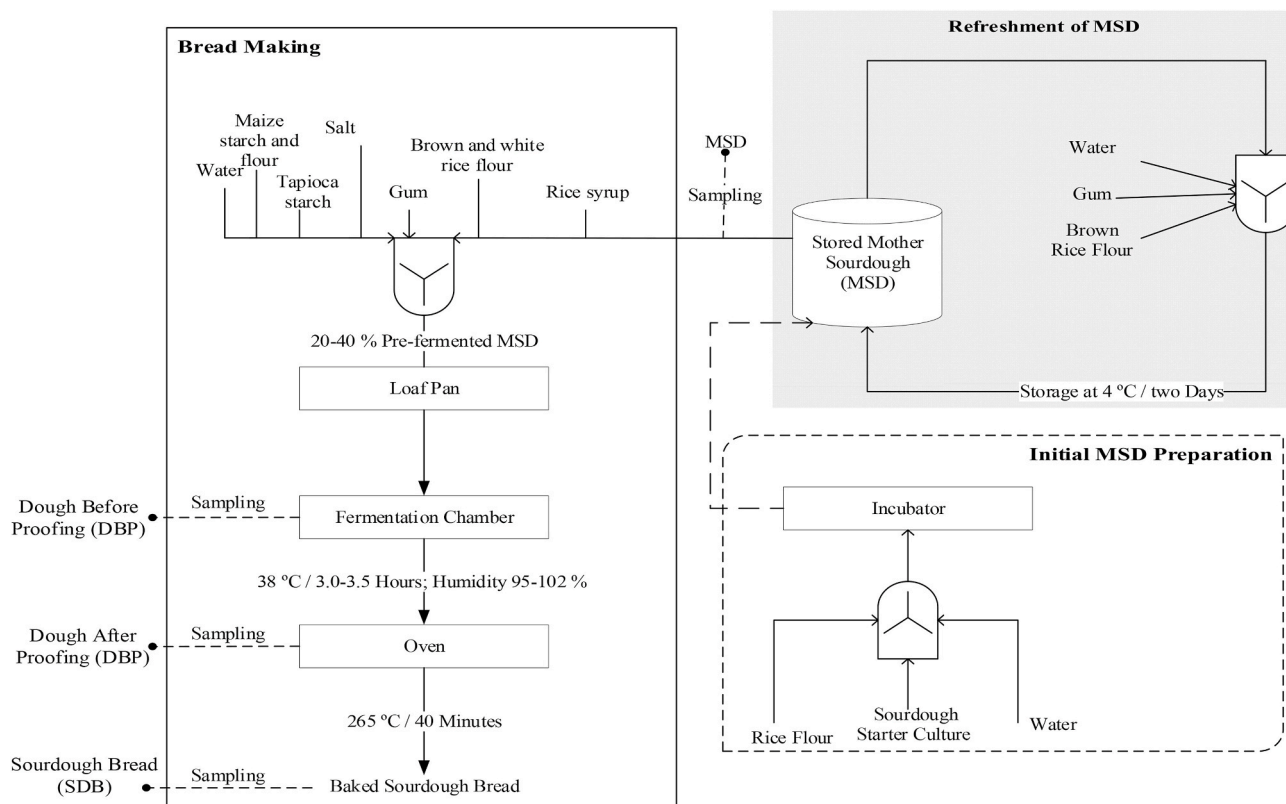


Fig. 1. Generalised production of whole meal rice sourdough bread.

2.4. Enumeration and isolation of LAB and yeast

Ten (10) grams of each sourdough sample (MSD, DBP, DAP) was mixed with 90 mL of sterile peptone water using a stomacher laboratory paddle blender. Suitable serial decimal dilutions were prepared using the mixed suspensions of samples and plated in duplicate on suitable media. LAB were enumerated on MRS agar (Oxoid, UK) under anaerobic incubation for 2 d at 35 °C, while yeast were enumerated on YGC agar (Merck, Germany) under aerobic conditions for 5 d at 25 °C (Lee and Lee, 2008; Gobetti and Gänzle, 2012; Lhomme et al., 2016). Grown colonies were enumerated, and morphologically distinct colonies were selected for purification by streaking before conducting API tests.

2.5. Carbohydrate fermentation profiles and identification of isolates

Purified LAB and yeast isolates were characterised using the API 50 CHL and API 32 C identification test kits respectively, according to the manufacturer's instructions (<https://apiweb.biomerieux.com>). Analysis of the results was aided by the apiweb™ identification software database V 5.1.

2.6. Molecular analysis

Following the presumptive identification of the isolates using the API test system, partial genome sequencing of LAB and yeast was carried out. Due to the high microbial diversity of LAB, high-throughput sequencing analysis (Illumina sequencing) was first applied to total LAB DNA isolated from MSD, DBP and DAP sourdough samples. For identification of the selected distinct LAB colonies, 16 S rRNA gene segments were subjected to PCR amplification and a portion of the amplicon was sequenced. For identification of yeast isolates, ITS 1 DNA analysis was conducted on total yeast DNA isolated from samples of MSD, DBP, DAP as well as the distinct yeast colonies from the same samples (Chen et al., 2001; Liu et al., 2016).

2.6.1. Bacteria and yeast DNA extraction

Total DNA extractions from purified yeast isolates, LAB isolates and sourdough samples were performed using a DNeasy® Blood & Tissue Kit (Qiagen, USA), according to the manufacturer's instructions (Qiagen, USA), with some modifications for DNA extracted from yeast isolates as described later.

Following the suspension of yeast cells in lyticase at 30 °C for 30 min, the sample (suspension) was placed at −80 °C for 1 min and then immediately heated at 90 °C for 1 min. This step was repeated once more, and then the suspension was centrifuged at 300 g for 10 min (Heraeus Multifuge × 1 R, Thermo Fisher, Germany) to obtain the spheroblasts. The remainder of the DNA extraction procedure for the yeast lysate and determination of DNA yield and purity procedures were the same as for the DNA extraction of LAB lysate (Qiagen, USA).

2.6.2. High-throughput sequencing of LAB

After analysis of the quality of the DNA and quantification, the V3 and V4 regions of the 16 S rRNA genes were amplified according to 16 S Metagenomic Sequencing Library Preparation instructions of Illumina (Illumina, n.d.) using the forward primer (5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGACAGCCTACGGGNGGCWGCAG-3') and reverse primer (5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3') supplied by Integrated DNA Technologies (IDT®, New Zealand). After PCR amplification, the products were purified, then 25 µl of each purified LAB PCR product was transferred to sterile PCR tubes and transported to Illumina Inc. For sequencing (Palmerston North, New Zealand).

2.6.3. DNA sequencing of LAB and yeast isolates

DNA extracted from four purified LAB and four yeast colonies were sent by courier to MacroGen Inc. (Seoul, Korea) for further PCR

amplification, purification and sequencing. Universal primers 27 F (5'-AGAGTTTGTATCMTGGCTCAG-3') and 1492 R (5'-TACGGY-TACCTTGTACGACTT-3') were used to amplify LAB 16 S rRNA genes. PCR reactions for LAB were conducted as follows: (1) denaturation at 94 °C for 5 min, (2) 30 cycles: 30 s at 94 °C; followed by 30 s at 56 °C then 1 min at 72 °C; (3) final extension for 10 min at 72 °C and the sample was held at 4 °C (Liu et al., 2016).

For yeast, universal primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS 4 (5'-TCCTCCGCTTATTGATATGC-3') were selected (Liu et al., 2016) and PCR reaction conditions were: 95 °C for 6 min, followed by 25 cycles at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, then one final extension at 72 °C for 10 min. After sequencing, the results were retrieved from the MacroGen Inc. Website: (<https://dna.macrogen.com/eng/index.jsp>).

2.6.4. Analysis of sequenced isolates

Data for total DNA LAB pyrosequencing were analysed by the Massey Genome Centre (Massey University, Manawatu Campus, Palmerston North, New Zealand) to obtain taxa count information. The taxonomic information for each operational taxonomic unit (OUT) was obtained by comparing with the 97 OUT database (Hildebrand et al., 2014). 16 S rRNA sequences of single LAB colonies and ITS region sequences were compared with Targeted Loci Nucleotide Blast Database of National Centre for Biotechnology Information (NCBI, <http://www.ncbi.nlm.nih.gov/blast>). For LAB 16 S rRNA gene BLAST, the LAB species was positively identified when a sequence similarity of more than 97.6% was found. For yeast, the species was aligned with existing database (NCBI database and the ISHAM Barcoding database (<http://its.mycologylab.org/>)) species when at least 80% of the sequence length was covered and 99% of sequence similarity was achieved.

2.7. Statistical analysis

Results for acidity (pH and total titratable acidity), colour of bread crust and microbiological colony counts (LAB and yeast) were analysed by univariate analysis of variance, descriptive and Tukey's multi-comparison tests ($p < 0.05$) (SPSS Version 25, IBM™ Company, USA).

3. Results and discussion

3.1. pH and titratable acidity

The total titratable acidity and pH of MSD, DBP, DAP and SDB samples collected weekly over a 10-week period are shown in Figs. 2 and 3, respectively. The MSD samples had the highest TTA (1.05–1.34%) and the lowest pH (3.66–4.12) compared to the DBP, DAP and SDB samples collected in the same week. The acidity of MSD was similar to that reported for German rice MSD, which had a TTA of 1.3–1.8% and pH of 3.8–3.9 (Meroth et al., 2003). The acidity of MSD obtained in this study was higher than levels obtained for DBP and DAP which may be due to the organic acids produced by the metabolic activities of LAB and yeast during storage of MSD as previously reported (Todorov and Holzapfel, 2014).

The acidity of rice sourdough increased significantly for the DAP sample compared to the DBP ($p < 0.05$). According to previous studies (De Vuyst and Vancanneyt, 2007), the increased acidity indicates the level of carbohydrate metabolism of the LAB, which produce lactic acid and acetic acid through the phosphoketolase pathway (heterofermentative LAB) or the Embden–Meyerhof pathway (homof fermentative LAB).

When comparing the TTA of the DAP and DBP samples from each week, samples obtained in weeks 4 and 9 had the highest increases in acidity during proofing (0.49%), whereas the TTA in week 5 showed the lowest increase of only 0.22%. Generally, an increase in acidity is related to the activity of LAB in the dough (De Vuyst and Vancanneyt, 2007). However, the LAB counts in week 5 (8.41 log CFU/g) were markedly

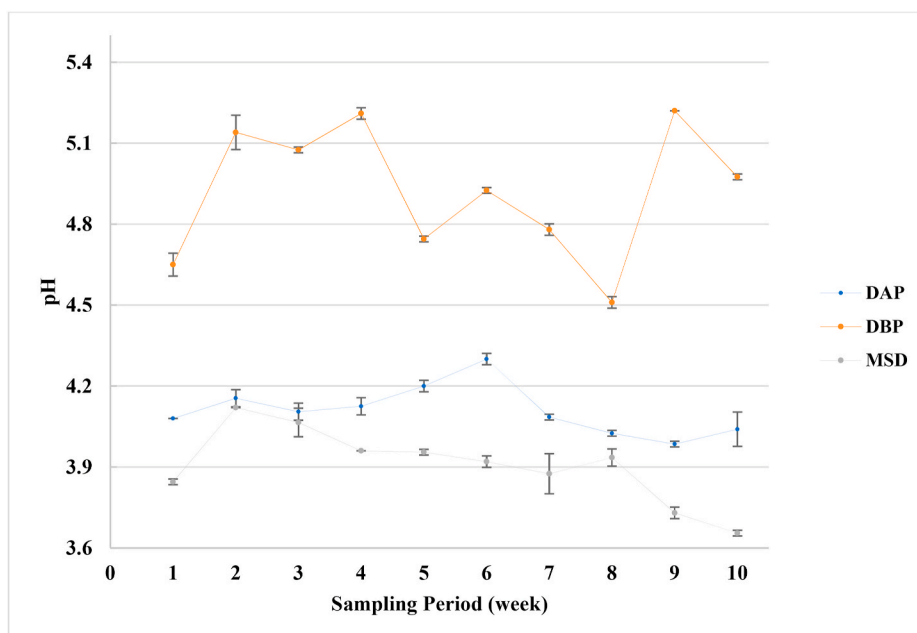


Fig. 2. pH of mother sourdough (MSD), dough before proofing (DBP) and dough after proofing (DAP) collected over a 10-week period. Mean \pm SEM; error bars = SEM; n = 10 replications with duplicate analysis/measurements of samples.

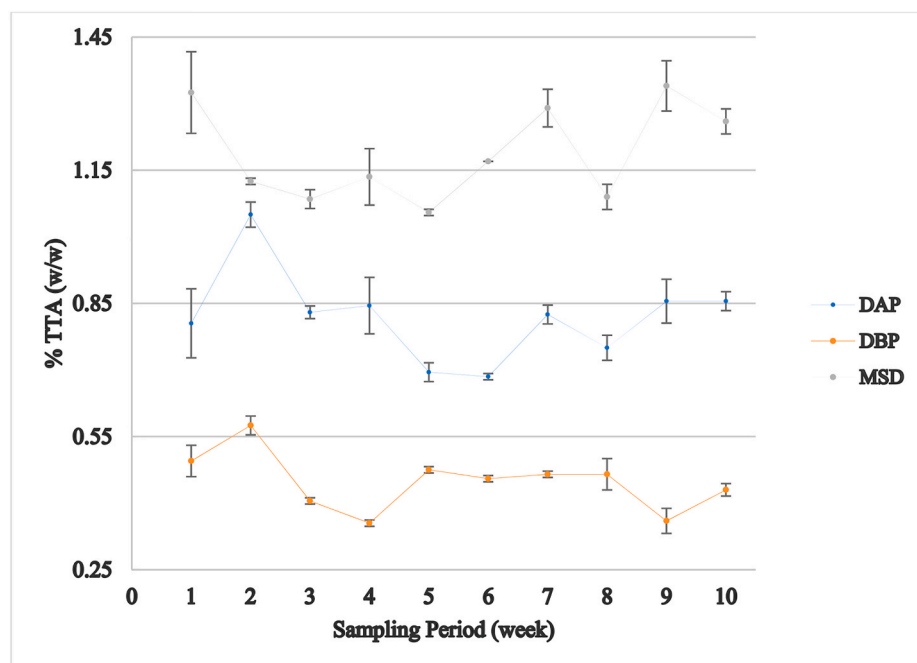


Fig. 3. Total titratable acidity (TTA) of mother sourdough (MSD), dough before proofing (DBP) and dough after proofing (DAP) collected over a 10-week period. Mean \pm SEM; error bars = SEM; n = 10 replications with duplicate analysis/measurements of samples.

higher than week 4 (7.85 log CFU/g) and slightly higher than week 9 (8.33 log CFU/g), yet the levels of acidity produced during proofing were the lowest, suggesting that the LAB activity in week 5 was weaker than that in weeks 4 and 9.

The fermentation and growth of sourdough LAB are affected by several factors including pH, temperature and salt concentration (Gobbetti and Gänzle, 2012). The optimum pH for sourdough LAB growth and metabolism generally lies between pH 5.0 and 6.0 (Gobbetti and Gänzle, 2012). The pH of DBP in weeks 4 and 9 was within the optimum pH range for LAB at the beginning of proofing, whereas in week 5 the pH was below the optimum range. Therefore, the acid-producing ability of

the LAB in week 5 may have been affected by the unfavourable acidity.

3.2. Bread crust colour

Bread crust colour is an important indicator of bread quality and a desirable bread crust should be golden brown (Phattanakulkaewmorie et al., 2011). Bread crust colour is affected by its raw materials, baking temperature, baking time and other factors such as acidity (Moghaddam et al., 2020; Najafi et al., 2016; Shittu et al., 2007). According to Moghaddam et al. (2020), LAB fermentation in the presence of functional ingredients such as olive leaf extract could be applied to GF breads to

improve the colour because the sourdough technique may generate more colour variation to simply changing the ratio of raw materials.

The lightness (L^*) of SDB ranged from 41.27 to 59.29. Breads baked in different zones in industrial ovens may have variable lightness as shown by the differences in the L^* -values (Therdthai et al., 2002). The lightness of NZ sourdough bread was closer to the reported L^* for wheat bread (51.27) than unleavened Jasmine Rice Bread (80.14) baked at 200 °C for 20 min (Pongjaruvat et al., 2014). While LAB fermentation can increase the lightness of sourdough bread (Moghaddam et al., 2020; Najafi et al., 2016), the lower lightness of NZ sourdough was probably caused by the higher baking temperature (265 °C) and longer baking time (40 min), which was used because more brown pigments are generated using an increased time-temperature combination (Shittu et al., 2007).

The a^* (redness) of SDB ranged from 3.93 to 8.36 and b^* yellowness from 12.85 to 16.45). A^* and b^* values of LAB-fermented rice bread decreased compared with its unleavened controlled group (Moghaddam et al., 2020). However, a^* and b^* values increased for LAB-fermented corn bread and wheat bread with additional date seed flour (Moghaddam et al., 2020; Najafi et al., 2016). The change of a^* and b^* values during fermentation could relate to the interaction between acids and coloured plant materials (Najafi et al., 2016). Baking temperature and time have also been reported to affect bread a^* and b^* values, however, the effect was not linear (Shittu et al., 2007).

GF bread is usually characterised by poor colour (Phimolsiripol et al., 2012). However, the colour of NZ rice SDB was close to that of yeast leavened wheat bread (a^* value 6.68; b^* value 15.18) baked at 240 °C for 32.5 min (Shittu et al., 2007). Having additional coloured plant raw materials such as brown rice and maize flour in NZ sourdough fermentation may improve its bread crust colour (Najafi et al., 2016). In addition, the improved colour is likely related to increased levels of reducing sugars and free amino acids which have been reported during fermentation, that can undergo Maillard reactions during baking to produce coloured compounds (Gänzle, 2014; Mantzourani et al., 2019). Therefore, to improve GF bread colour, experiments with various combinations of coloured raw materials, sourdough technology, baking temperature and baking time should be conducted.

3.3. LAB and yeast counts

The viable cell counts of LAB and yeast from MSD, DBP and DAP over the 10-week sampling period are shown in Fig. 4. MSD contained the high amounts of viable LAB (8.06 ± 0.01 log CFU/g to 9.08 ± 0.02 log CFU/g) required to initiate a new batch of fermentation. LAB counts in MSD were similar to the cell counts reported in German rice mother sourdough (8.1–9.2 log CFU/g), which was also produced using a commercial MSD but its raw materials were not specified (Meroth et al., 2004). However, the cell counts found in this study were higher than the Korean rice sourdough starter culture counts (6.53–7.87 log CFU/g) prepared using a commercial makgeolli (a type of rice wine) (Park et al., 2017). The differences in cell counts between the Korean MSD and the New Zealand MSD may be due to differences in the raw materials used (Korean study used: rice powder, commercial makgeolli, water, sucrose, salt) (Park et al., 2017). If the makgeolli was the source of the fermenting microorganisms in the Korean MSD, this may also help explain the differences in cell counts. During proofing, LAB counts of the DAP increased significantly compared to the DBP counts ($p < 0.05$), indicating that the proofing conditions used were favourable for the growth of LAB in the NZ rice sourdough.

Yeast counts from the MSD (4.39 ± 0.09 – 6.88 ± 0.02 log CFU/g) were lower than those reported in German rice sourdough (Meroth et al., 2004), which was dominated by *S. cerevisiae*, which is similar to the NZ rice sourdough in this study. Yeast cell counts from the DBP and DAP were similar ($p > 0.05$), suggesting that there was no marked growth of yeast during proofing. The growth of yeast is dependent on proofing temperature and acidity. The proofing temperature used for the NZ rice sourdough was 38 °C, which was higher than the optimum growth temperature of *S. cerevisiae* (30–35 °C) (Walsh and Martin, 1997). This probably explains the lack of significant yeast growth during proofing observed in this study.

The stress tolerance of yeast to acidity, is both species- and strain-dependent (Houngbédji et al., 2019). The viability of yeast is affected by non-dissociated forms of acids, especially acetic acid (Houngbédji et al., 2019). In this study, yeast in the MSD were exposed to organic acids during the two-day storage period and this, along with a high acidity and nutrient-limiting environment may have affected their viability (Casal et al., 2008). German MSD had a higher TTA than the MSD in this study, yet the yeast cell counts (7.7 log CFU/g) were higher

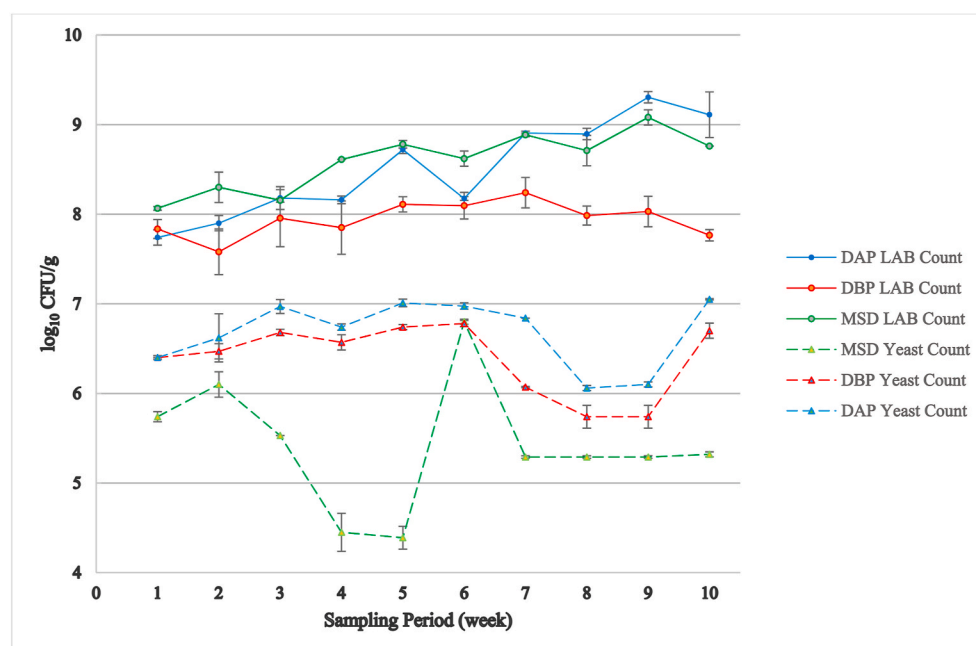


Fig. 4. Lactic acid bacteria (LAB) and yeast counts in rice sourdough over a 10-week period. Mother sourdough was used as the starter culture to initiate the fermentation. MSD = mother sourdough; DBP = dough before proofing; DAP = dough after proofing; Mean \pm SEM; error bars = SEM; $n = 10$ replications with duplicate analysis/measurements of samples.

in the former. It is possible that the strains of *S. cerevisiae* in the German MSD could tolerate higher levels of acidity than the yeast found in the NZ MSD (Houngbédji et al., 2019). As yeast are responsible for bread leavening and production of flavour compounds, it is desirable for sourdough yeast to be able to tolerate stress conditions such as high acidity, osmotic pressure and low carbon source concentration to function well during bread making (Gobbetti and Gänzle, 2012).

3.4. API identification and carbohydrate fermentation profiles

Four morphologically distinct LAB colonies (A, B, C, and D) and three yeast colonies (I, II, and III) isolated from MSD, DBP and DAP were tested for their carbohydrate fermentation profiles. All selected LAB colonies fermented galactose, glucose, fructose, ribose, maltose, lactose, melibiose, sucrose and raffinose. LAB isolate C also fermented D-mannitol and sorbitol, but not L-arabinose, which was fermented by LAB isolates A, B, and D. Based on the fermentation profiles of the isolates, LAB isolate C was presumptively identified as *L. plantarum* 1 with a 99.9% identity, while isolates A, B and D were identified as *L. fermentum* 1 with 98.8% identity for isolate A, 98.8% for isolate B and 89.7% for isolate D.

The carbohydrate fermentation profiles of the three selected yeast isolates from MSD, DBP and DAP were the same. All the yeast fermented galactose, sucrose, raffinose, maltose, trehalose and glucose. The three yeast isolates were presumptively identified as *S. cerevisiae* with high percentages of identity ranging from 98.8 to 99.9% using the API 32 C test kits.

3.5. High throughput sequencing of LAB

For pyrosequencing of LAB, the number of reads of each isolated sample (A, B, C, and D) was around 1000 and the length of the amplicons for the V3 and V4 regions were about 500 bp (Fig. 5).

Results showed that the genus *Lactobacillus* (>99.9% taxonomy count in MSD, DBP and DAP samples) was predominant during rice sourdough fermentation, which is similar to other studies (Speranza et al. (2016)). The genus *Pediococcus* was present in low amounts

(<0.01% taxonomy count in MSD, DBP and DAP samples) similar to other studies, where this genus was present as a subdominant LAB group (Corsetti et al., 2007). In the DAP, other LAB of the genera *Leuconostoc* and *Fructobacillus* were also detected. These genera are inherent in flour and cereal kernels and potentially grow during proofing (Corsetti et al., 2007).

3.6. 16 S rRNA sequencing analysis of LAB isolate A, B, C and D

According to 16 S rRNA sequencing results, isolate C was identified as *L. paraplantarum* DSM 10667 which has previously been isolated from human faeces and beer, while the other isolates A, B and D were identified as *L. fermentum* CIP 102980, which is commonly found in fermented cereals and plant materials (Gänzle and Zheng, 2019). The rRNA sequence identification for isolate C was different to that identified using the API 50 CHL test (*L. plantarum*). As molecular methods are considered superior for identification than phenotypic methods such as API tests, LAB isolate C should be aligned to *L. paraplantarum* (De Vuyst and Vancanneyt, 2007; Gobbetti and Gänzle, 2012).

The biodiversity of sourdough starter culture is highly associated with the ingredient content. GF sourdough can have different starter cultures to those from wheat- or rye-based sourdough. *L. fermentum* and *L. plantarum* have been frequently reported in sourdoughs prepared from flours other than wheat and rye such as tef, maize, sorghum or millet (Gänzle and Zheng, 2019; Ogodo et al., 2016). In contrast, a recent study, reported that *L. fermentum* was not isolated from over 500 wheat- or rye-based sourdough starter cultures (Landis et al., 2021). Therefore, the presence of *L. fermentum* in GF sourdough from NZ is likely to have originated from the GF maize flour.

3.7. ITS sequencing analysis of yeast

All recovered yeast isolates were identified as *S. cerevisiae*, which agrees with the results from the API 32 C tests. Distinct yeast isolates obtained from MSD, DBP and DAP were all identified as *S. cerevisiae* CBS 1171. During fermentation, the yeast isolate was able to grow at a high temperature (38 °C) and low pH (pH 3.66). *S. cerevisiae* is the most

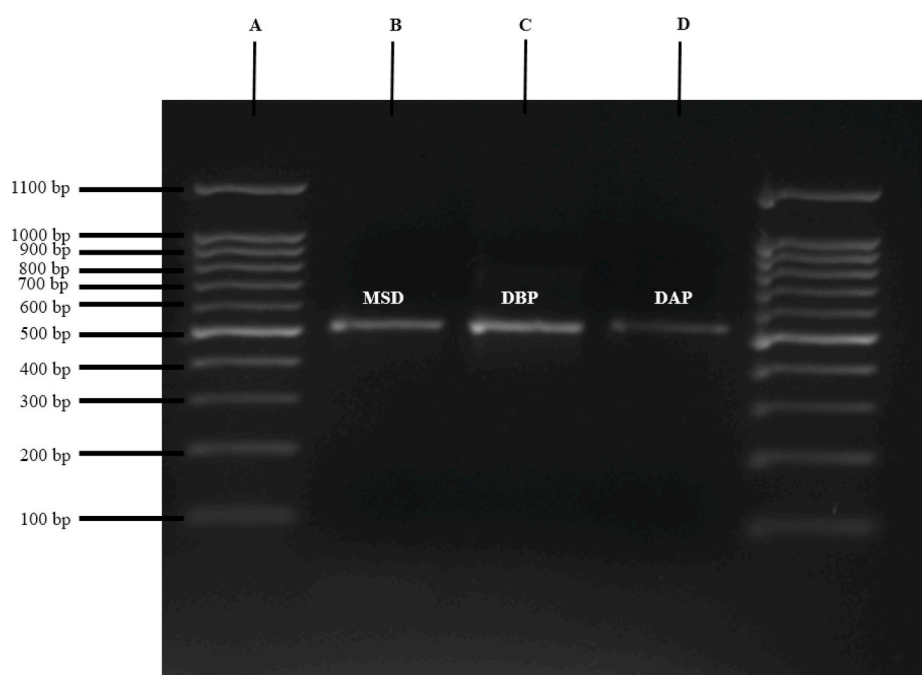


Fig. 5. Agarose gel electrophoresis of polymerase chain reaction (PCR) products of total DNA extracted from 4 LAB isolates from mother sourdough (MSD), dough before proofing (DBP), dough after proofing (DAP). **Note:** Lane A: 100 bp DNA ladder; Lane B: MSD amplicon; Lane C: DBP amplicon; Lane D: DAP amplicon. Image captured by Gel DocTM EZ Imager (BIO-RAD, USA).

resistant yeast to stress conditions such as low pH (pH 3.4) and can grow in the presence of acetic acid, lactic acid and ethanol (Houngbédji et al., 2019). *S. cerevisiae* has also been reported to dominate in similar GF products such as maize Kenkey and rice sourdough (Moroni et al., 2009).

4. Conclusions

The identified LAB in NZ rice sourdough belonged to the genus *Lactobacillus*, with typical LAB isolates identified as *Lactobacillus paraplantarum* DSM 10667 and *Lactobacillus fermentum* CIP 102980. *S. cerevisiae* predominated in the NZ rice sourdough and typical yeast isolates were identified as *S. cerevisiae* CBS 1171. NZ rice sourdough starter cultures were able to tolerate high acidity (TTA 1.34%; pH 3.66) and high temperature (38 °C). Further studies are recommended to determine whether use of a lower proofing temperature of 35 °C, which falls within the optimum growth temperature of *S. cerevisiae* may improve the overall quality of NZ rice sourdough bread. In addition, another strain of *S. cerevisiae* that has better acid tolerance than *S. cerevisiae* CBS 1171 could be considered for NZ rice sourdough production to improve the leavening of the bread.

CRedit authorship contribution statement

Qiwei Yang: Conceptualization, planning, Investigation, Data curation, Writing – original draft, editing, Writing – review & editing. **Kay Rutherford-Markwick:** Conceptualization, planning, Writing – review & editing. **Anthony N. Mutukumira:** Project Leader and overall management of the study, Conceptualization, planning, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

- Casal, M., Paiva, S., Queirós, O., Soares-Silva, I., 2008. Transport of carboxylic acids in yeasts. *FEMS Microbiol. Rev.* 32 (6), 974–994. <https://doi.org/10.1111/j.1574-6976.2008.00128.x>.
- Chen, Y.C., Eisner, J.D., Kattar, M.M., Rassoulou-Barrett, S.L., Lefe, K., Bui, U., et al., 2001. Polymorphic internal transcribed spacer region 1 DNA sequences identify medically important yeasts. *J. Clin. Microbiol.* 39 (11), 4042–4051.
- Comasio, A., Verce, M., Van Kerrebroeck, S., De Vuyst, L., 2020. Diverse microbial composition of sourdoughs from different origins. *Front. Microbiol.* 11, 1212.
- Corsetti, A., Settanni, L., Valmorri, S., Mastrangelo, M., Suzzi, G., 2007. Identification of subdominant sourdough lactic acid bacteria and their evolution during laboratory-scale fermentations. *Food Microbiol.* 24 (6), 592–600.
- De Vuyst, L., Neysens, P., 2005. The sourdough microflora: biodiversity and metabolic interactions. *Trends Food Sci. Technol.* 16 (1–3), 43–56.
- De Vuyst, L., Vancanney, M., 2007. Biodiversity and identification of sourdough lactic acid bacteria. *Food Microbiol.* 24 (2), 120–127.
- Ehrmann, M.A., Vogel, R.F., 2005. Molecular taxonomy and genetics of sourdough lactic acid bacteria. *Trends Food Sci. Technol.* 16 (1), 31–42.
- Ercolini, D., Pontonio, E., De Filippis, F., Minervini, F., La Stora, A., Gobbetti, M., Di Cagno, R., 2013. Microbial ecology dynamics during rye and wheat sourdough preparation. *Appl. Environ. Microbiol.* 79 (24), 7827–7836.
- Foschia, M., Horstmann, S., Arendt, E.K., Zannini, E., 2016. Nutritional therapy—facing the gap between coeliac disease and gluten-free food. *Int. J. Food Microbiol.* 239, 113–124.
- Galli, V., Venturi, M., Pini, N., Guerrini, S., Granchi, L., Vincenzini, 2019. MLiquid and firm sourdough fermentation: microbial robustness and interactions during consecutive backslippings. *LWT - Food Science and Technology* 105, 9–15.
- Gänzle, M.G., 2014. Enzymatic and bacterial conversions during sourdough fermentation. *Food Microbiol.* 37, 2–10.
- Gänzle, M.G., Zheng, 2019. J. Lifestyles of sourdough lactobacilli—Do they matter for microbial ecology and bread quality? *Int. J. Food Microbiol.* 302, 15–23.
- Gobbetti, M., De Angelis, M., Di Cagno, R., Rizzello, C.G., 2008. Sourdough/lactic acid bacteria. *Gluten-free Cereals Products and Beverages* 267–288.
- Gobbetti, Marco, Gänzle, Michael (Eds.), 2012. *Handbook on Sourdough Biotechnology*. Springer Science and Business Media.
- Hildebrand, F., Tadeo, R., Voigt, A.Y., Bork, P., Raes, J., LotuS., 2014. An efficient and user-friendly OTU processing pipeline. *Microbiome* 2 (1), 30.
- Houngbédji, M., Johansen, P., Padonou, S.W., Hounhouigan, D.J., Siegmundfeldt, H., Jespersen, L., 2019. Effects of intrinsic microbial stress factors on viability and physiological condition of yeasts isolated from spontaneously fermented cereal doughs. *Int. J. Food Microbiol.* 304, 75–88.
- Landis, E.A., Oliverio, A.M., McKenney, E.A., Nichols, L.M., Kfoury, N., Biango-Daniels, M., et al., 2021. The diversity and function of sourdough starter microbiomes. *Elife* 10, e61644.
- Lee, H.M., Lee, Y., 2008. A differential medium for lactic acid-producing bacteria in a mixed culture. *Lett. Appl. Microbiol.* 46 (6), 676–681.
- Lhomme, E., Lattanzi, A., Dousset, X., Minervini, F., De Angelis, M., Lacaze, G., Gobbetti, M., 2015. Lactic acid bacterium and yeast microbiotas of sixteen French traditional sourdoughs. *Int. J. Food Microbiol.* 215, 161–170.
- Lhomme, E., Urien, C., Legrand, J., Dousset, X., Onno, B., Sicard, D., 2016. Sourdough microbial community dynamics: an analysis during French organic bread-making processes. *Food Microbiol.* 53, 41–50.
- Liu, T., Li, Y., Chen, J., Sadiq, F.A., Zhang, G., Li, Y., He, G., 2016. Prevalence and diversity of lactic acid bacteria in Chinese traditional sourdough revealed by culture dependent and pyrosequencing approaches. *LWT - Food Sci. Technol. (Lebensmittel-Wissenschaft -Technol.)* 68, 91–97.
- Mantzourani, I., Plessas, S., Odatzidou, M., Alexopoulos, A., Galanis, A., Bezirtzoglou, E., Bekatorou, A., 2019. Effect of a novel *Lactobacillus paracasei* starter on sourdough bread quality. *Food Chem.* 271, 259–265.
- Meroth, C.B., Hammes, W.P., Hertel, C., 2004. Characterisation of the microbiota of rice sourdoughs and description of *Lactobacillus spicheri* sp. nov. *Syst. Appl. Microbiol.* 27 (2), 151–159.
- Meroth, C.B., Walter, J., Hertel, C., Brandt, M.J., Hammes, W.P., 2003. Monitoring the bacterial population dynamics in sourdough fermentation processes by using PCR-denaturing gradient gel electrophoresis. *Appl. Environ. Microbiol.* 69 (1), 475–482.
- Minervini, F., De Angelis, M., Di Cagno, R., Gobbetti, M., 2014. Ecological parameters influencing microbial diversity and stability of traditional sourdough. *Int. J. Food Microbiol.* 171, 136–146.
- Moghaddam, M.F.T., Jalali, H., Nafchi, A.M., Nouri, L., 2020. Evaluating the effects of lactic acid bacteria and olive leaf extract on the quality of gluten-free bread. *Gene Reports* 21, 100771.
- Moroni, A.V., Dal Bello, F., Arendt, E.K., 2009. Sourdough in gluten-free bread-making: an ancient technology to solve a novel issue? *Food Microbiol.* 26 (7), 676–684.
- Najafi, M.B.H., Pourfarzad, A., Zahedi, H., Ahmadian-Kouchaksaraie, Z., Khodaparast, M. H.H., 2016. Development of sourdough fermented date seed for improving the quality and shelf life of flat bread: study with univariate and multivariate analyses. *J. Food Sci. Technol.* 53 (1), 209–220.
- Ogodo, A.C., Ugbo, O.C., Onyeagba, R.A., Okereke, H.C., Agwaranze, D.I., 2016. Dynamics of functional properties of maize flours fermented with lactic acid bacteria (LAB)-consortium isolated from cereals. *FUW Trends in Science Technology* 1 (1), 134–138.
- Park, Jaehyung, Seo, Ji Sun, Seul-Ah Kim, So-Yeon Shin, Jong-Hyun Park, Han, Nam Soo, 2017. Microbial diversity of commercial makgeolli and its influence on the organoleptic characteristics of Korean rice sourdough, jeung-pyun. *J. Microbiol. Biotechnol.* 27 (10), 1736–1743.
- Pérez-Quirce, S., Collar, C., Ronda, F., 2014. Significance of healthy viscous dietary fibres on the performance of gluten-free rice-based formulated breads. *Int. J. Food Sci. Technol.* 49 (5), 1375–1382.
- Phattanakulkaewmorie, N., Paseephol, T., Moongnarm, A., 2011. Chemical compositions and physico-chemical properties of malted sorghum flour and characteristics of gluten free bread. *World Academy of Science, Engineering and Technology* 5 (7), 532–538.
- Phimolsiripol, Y., Mukprasirt, A., Schoenlechner, R., 2012. Quality improvement of rice-based gluten-free bread using different dietary fibre fractions of rice bran. *J. Cereal. Sci.* 56 (2), 389–395.
- Pongjaruvut, W., Methacanon, P., Seetapan, N., Fuongfuchat, A., Gamonpilas, C., 2014. Influence of pregelatinised tapioca starch and transglutaminase on dough rheology and quality of gluten-free jasmine rice breads. *Food Hydrocolloids* 36, 143–150.
- Shittu, T.A., Raji, A.O., Sanni, L.O., 2007. Bread from composite cassava-wheat flour: I. Effect of baking time and temperature on some physical properties of bread loaf. *Food Res. Int.* 40 (2), 280–290.
- Speranza, B., Bevilacqua, A., Corbo, M.R., Sinigaglia, M., 2016. *Starter Cultures in Food Production*. John Wiley & Sons.
- Therdthai, N., Zhou, W., Adamczak, T., 2002. Optimisation of the temperature profile in bread baking. *J. Food Eng.* 55 (1), 41–48.
- Todorov, S., Holzapfel, W., 2014. Traditional cereal fermented foods as sources of functional microorganisms. *Advances in Fermented Foods and Beverages: Improving Quality, Technologies and Health Benefits* 123.
- Walsh, R., Martin, P.A., 1997. Growth of *Saccharomyces cerevisiae* and *Saccharomyces Uvarum* in a Temperature Gradient. *Journal of the Institute of Brewing*.
- Witczak, M., Ziobro, R., Juszcak, L., Korus, J., 2016. Starch and starch derivatives in gluten-free systems—A review. *J. Cereal. Sci.* 67, 46–57.

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