



Bacterial composition in sourdoughs from different regions in China and the microbial potential to reduce wheat allergens

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ABSTRACT

Previous researches have shown microbial diversity in sourdoughs in different regions. However, the specific reasons of those differences need further study. This study has identified the bacterial composition using Illumina high-throughput sequencing technology in eighteen geographically representative sourdough in China and determined the effects of environmental factors on bacterial community. In order to identify potential strains to produce hypoallergenic wheat products, preliminary screening of bacteria based on its capabilities in decreasing allergenicity of wheat protein was used. The results showed that the predominant bacteria in 15/18 samples were *Lactobacillus*, while *Weissella* in Guangdong samples. Environmental factors (altitude, annual average temperature, annual rainfall and sunshine duration) have effects on the diversity and richness of bacteria in sourdough, while no influence on type of dominant bacteria. *Pediococcus acidilactici*XZ31, *Pediococcus pentosaceus*GD4 and *Lactobacillus sakei*GS6, isolated from XZ, GD, GS samples, respectively, showed superiority over other selected strains in reducing wheat proteins allergenicity.

1. Introduction

Sourdough is a mixture of flour, water, and fermentation strains, which can ferment and acidify a dough. The batter used to ferment the dough for the first time is called starter (L. Vuyst, De, Vrancken, Ravyts, Rimaux, & Weckx, 2009; L. D. Vuyst & Vancanneyt, 2007). In modern biotechnology of fermentation, the starter is often added to the steamed rice mixed with the Daqu, Xiaoqu and fermented with the flour to enrich the microbes in the dough. Previous studies have shown that sourdough fermentation has a great advantage in improving the texture, nutrition and shelf life of pasta and gluten-free products, such as the decrease of glycemic responses, degradation of phytate and production of antifungal factors and exopolysaccharide, compared to commercial yeast (Raffaella et al., 2006; Marco Gobbetti et al., 2017; Marco Gobbetti, Rizzello, Cagno, & Angelis, 2014; Hassani, Procopio, & Becker, 2016; Rizzello, Nionelli, Coda, De Angelis, & Gobbetti, 2010; Gobbetti et al., 2018).

“Wheat-related disorders” including wheat allergy, celiac disease, non-celiac wheat sensitivity (NCGS), and some chronic inflammation of intestinal tract, such as inflammatory bowel diseases (IBD) have an increasing prevalence around the world (Casella et al., 2010). Among them, wheat allergy can be broadly divided into IgE-mediated and non-

IgE-mediated. Avoiding the uptake of allergenic proteins in wheat is essential for people with those diseases. Thirteen kinds of ingestive allergens in wheat including Tri a 14, Tri a 18, Tri a 19, Tri a 20, Tri a 25, Tri a 26, Tri a 36, Tri a 37, Tri a 41-Tri a 45 were given from WHO/IUIS Allergen Nomenclature Sub-Committee (<http://allergen.org>). Previous studies have been revealed the immune mechanisms of IgE-dependent pathway. However, IgE-independent pathway has still not been figured out. Nonetheless, some studies have shown that IgG antibodies may compete with IgE antibodies for food allergens (Yu, Dmh, & Nadeau, 2016). The sourdough fermentation has shown the most promising result for reducing allergenic proteins (Scherf, Koehler, & Wieser, 2016; Zannini, Waters, & Arendt, 2012). Furthermore, some studies also confirmed that fermentation by bacteria related to sourdough may reduce the immunoreactivity of dairy and soy protein (Rui et al., 2019). Sourdough fermentation as a promising traditional biotechnologies for high-quality GF-foods and hypoallergenic products may deserve deeper researches. The diversity of microorganisms in sourdoughs in different regions depends on the differences in starter, grain, environment and fermentation conditions (Guohua & Guoqing, 2013; Minervini, Angelis, Cagno, & Gobbetti, 2014). Several studies have shown that the microbial community in Chinese western sourdough was different from that of northern and southern (Liu et al.,

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2016, 2018; Zhang et al., 2015). However, the specific reasons of those differences need further study. In this study, to find the relationship between environmental factors and microbiota, high throughput sequencing was used to investigate bacterial composition in eighteen representative samples. The culture-dependent bacterial from samples were screened according to the ability of wheat protein allergenicity degradation. The potential bacteria in sourdough were identified to provide a new prospect for the preparation of gluten-free or hypoallergenic wheat products.

2. Materials and methods

2.1. Materials

Sourdough were collected from local shops or private household. Human sera were obtained from Beijing Luoqi Clinical Laboratory, Beijing, China. The protocol was approved by the local ethical committee of Luoqi Clinical Laboratory and written informed consent was obtained. Goat anti-human IgG horseradish peroxidase conjugate (HRP), Goat anti-human IgG alkaline phosphatase conjugate (AP), Bovine serum albumin (BSA), Bradford Protein Assay Kit, Bacterial Genomic DNA Extraction Kit and SDS-PAGE loading buffer were purchased from Solarbio Science & Technology Co., Ltd., Beijing, China. BCIP/NBT and BeyoECL-Star kits were purchased from Beyotime Biotechnology (Shanghai, China).

2.2. Sample collection and determination of sourdough physicochemical properties

All of those samples were traditional backslopped sourdoughs and made from wheat flours. The sourdough has been used repeatedly for a long time and microbial composition has been stability adapted to the environment. Environmental information obtained from National Geomatic Center of China (NGCC, www.ngcc.cn/) and National Meteorological Information Center (www.nmic.cn/) (Table 1). All of those samples were transported to the laboratory at low temperatures and stored at -20°C before analysis.

For pH and total titratable acidity (TTA) determination, samples were 10-fold diluted with sterile distilled water and mixed in a homogenized bag for 2 min. The pH was determined by a pH meter and TTA was expressed as the volume (mL) of 0.1 M NaOH to get pH of 8.3. Moisture and ash content were measured under the guidance of AACCC (AACCC, 2002). Total protein was extracted according to Mitsugu Akagawa and determined by Bradford method (Mitsugu et al., 2007).

2.3. Determination of composition and diversity of bacteria in sourdough

After lyophilization, 0.25 g of each sample was used for the extraction of total DNA of sourdough samples by a DNA extractor kit. Bacterial universal primers were chosen to amplify 16S rRNA gene. The

amplified products were purified and recovered using 1.0% agarose gel electrophoresis method and then were paired-end sequenced by Beijing Biomarker Technologies Co., Ltd.

For bacterial composition determination, the amplicons were sequenced with Illumina HiSeq2500. The original data was spliced and filtered out to get high quality tags sequence. The optimized sequences with a limit of 3% distance were gathered into operational taxonomic units (OTU), and the representative sequences were aligned against Silva database (<http://www.arb-silva.de>), subsequently, performing the taxonomic classification use Ribosomal Database Project (<http://sourceforge.net/projects/rdpclassifier/>) at 80% threshold. Community richness and diversity of each sample were performed by alpha diversity using the index of Chao, Ace, Shannon and Simpson, which were calculated by Mothur1.30. Beta diversity analysis, such as Unweighted Pair-group Method with Arithmetic Mean Analysis (UPGMA), were calculated by using QIME. LEfSe (Line Discriminant Analysis (LDA) Effect Size) was calculated by R language3.1.1 with a LDA threshold score of 4 to find statistically different biomarker between different samples. Redundancy Analysis (RDA) were calculated to show the relationship between environmental factors and sample composition by R language3.1.1. The correlation analysis chart between bacterial community and environmental factors were created by SPSS and GraphPad Prism6.

2.4. Isolation and identification of lactic acid bacteria

Sourdough samples were ten-fold diluted with sterile peptone water and mixed in a stomacher. The mixture was serially diluted in peptone water and appropriate 10^3 fold dilutions were pour-plated on MRS agar, at 37°C for 48 h. Colonies with different morphology were isolated randomly from plates and incubated in MRS at 37°C for 24 h. Gram-positive, catalase-negative presumptive lactic acid bacterial were streaked into MRS agar for purification. Genomic DNA were extracted using Bacterial Genomic DNA Extraction Kit and amplified using the general bacterial primers 27F (5'-AGCGGATCACTTCACACAGGACTACGGCTACCTTGTACGA-3') and 1492R (5'-GCAGAGTTCTCGGAGTCACG AAGAGTTTGATCCTGGCTCAG-3') by polymerase chain reaction (94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 2 min, and 72°C for 10 min). The products were assessed by 1% electrophoresis agarose gel and sequenced by Sangon, Beijing, China.

2.5. Lactic acid bacterial (LAB) treatment of wheat protein extracts

All strains were incubated to the late exponential phase of growth, and then harvested by centrifugation at 6,000 g for 10 min at 4°C , and resuspended in 0.1 M Na-phosphate buffer (pH 7.2) before use (optical density at 600 nm, ca. 2.5). According to a modified method of previous study (Raffaella et al., 2002), wheat protein hydrolysate (WPH) was produced as follows: 30 g wheat flour were suspended in 100 mL phosphate buffer (20 mM, pH 7.2–7.4) and ultrasonicated for 30 min on

Table 1
Sourdough samples source information and physicochemical properties.

sources	AD (m)	AT ($^{\circ}\text{C}$)	AR (mm)	SD (h)	Moisture (g/100 g)	Protein (g/100 g)	Ash (g/100 g)	pH	TTA (mL)
XZ	3650	2.1	477.9	3000.00	37.2 $\pm$ 0.00 ^a	2.416 $\pm$ 0.00 ^a	0.7 $\pm$ 0.00 ^a	4.15 $\pm$ 0.00 ^c	6.35 $\pm$ 0.07 ^b
GS	1500	5.4	211.8	2982.00	38.4 $\pm$ 0.00 ^f	2.418 $\pm$ 0.14 ^a	0.9 $\pm$ 0.00 ^b	4.35 $\pm$ 0.00 ^b	6.10 $\pm$ 0.14 ^b
SX	1250	8.8	383.6	2753.95	37.0 $\pm$ 0.00 ^e	3.331 $\pm$ 0.10 ^c	0.6 $\pm$ 0.00 ^a	3.85 $\pm$ 0.00 ^a	11.15 $\pm$ 0.21 ^c
HN	260	13.7	587.7	2176.95	23.9 $\pm$ 0.00 ^c	3.042 $\pm$ 0.02 ^b	1.0 $\pm$ 0.00 ^c	3.87 $\pm$ 0.01 ^c	12.30 $\pm$ 0.28 ^d
LN	210	9.8	577.4	2612.00	36.7 $\pm$ 0.00 ^d	3.239 $\pm$ 0.16 ^{bc}	0.6 $\pm$ 0.00 ^d	4.75 $\pm$ 0.00 ^f	4.95 $\pm$ 0.07 ^a
GD	95	23.0	1944.0	2120.50	26.6 $\pm$ 0.00 ^b	3.072 $\pm$ 0.03 ^b	1.1 $\pm$ 0.00 ^d	4.84 $\pm$ 0.00 ^d	6.20 $\pm$ 0.00 ^b

XZ, Lasa City, Xizang Province. GS, Jinchang City, Gansu Province. SX, Xian City, Shanxi Province. HN, Hebi City, Henan province. LN, Jinzhou City, Liaoning Province. GD, Shenzhen City, Guangdong Province. AD, altitude annual. AT, average temperature. AR, annual rainfall. SD, sunshine duration. The data of AD and SD were received from NGCC. AR and SD information were obtained from National Meteorological Information Center. Moisture, protein, ash content were measured on dry basis. Results were expressed as means $\pm$ standard deviation ($n \geq 2$). Superscript lowercase letters in the same column were used to indicate significant differences ($p < 0.05$).

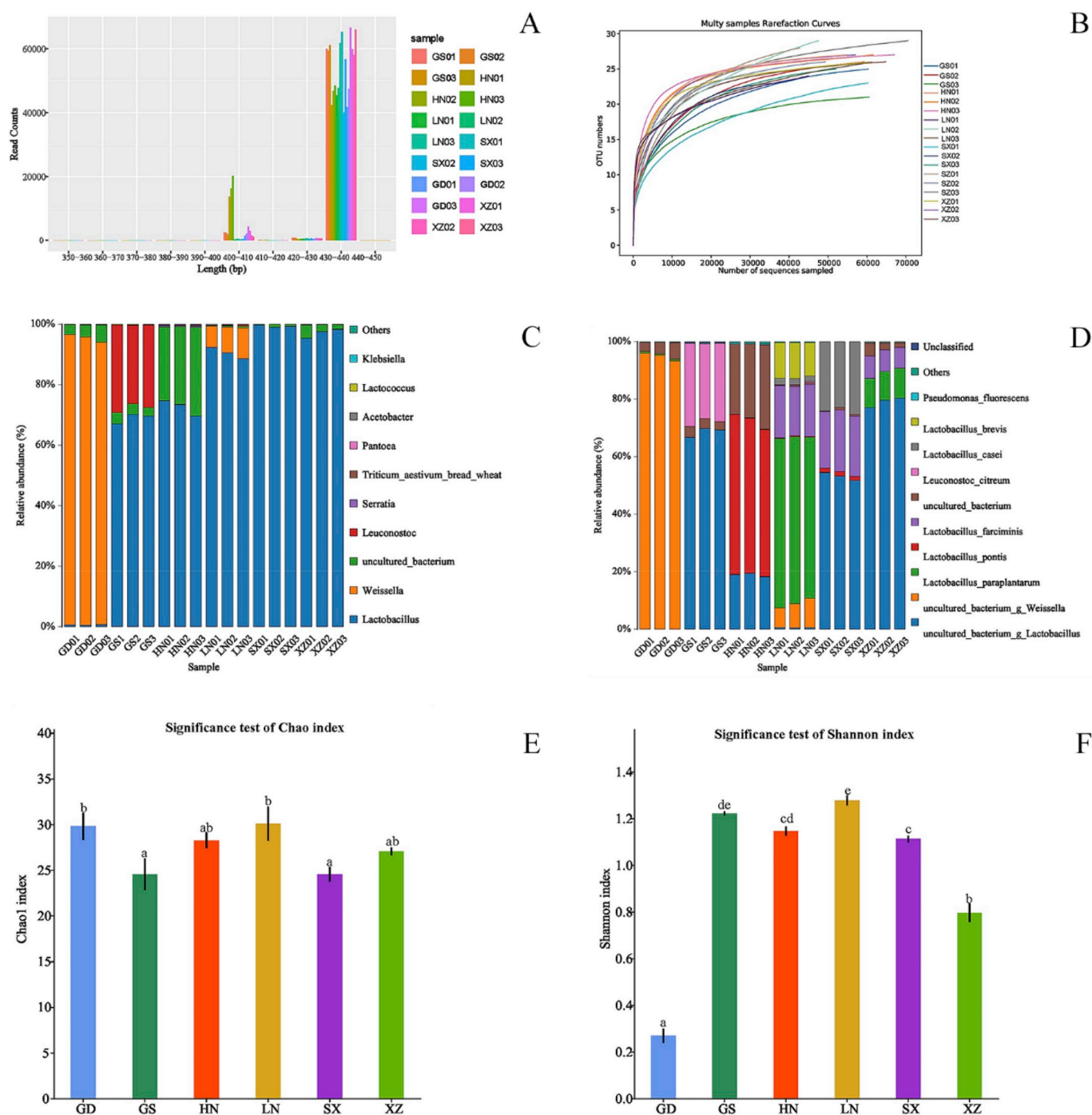


Fig. 1. (A) Effective tags distribution of eighteen samples. (B) Rarefaction curve of eighteen samples. (C) Bacterial composition at genus level. (D) Bacterial composition at species level. (E) Significance test of Chao index. (F) Significance test of Shannon index.

ice. And then, the suspension was centrifuged ($10000 \times g$ for 15 min at 4°C) and sterilized by filtration ($0.22\text{-}\mu\text{m}$ -pore-size Millex-GV; Millipore, Ireland). WPH was lyophilized and stored at -20°C for further analysis. The assay mixture, containing one volume of the blend of WPH with a concentration of ca. 1.25 mg/mL and an equivalent volume of the cellular suspension ($\text{OD}_{600} = 2.5$, ca. 10^9 CFU/mL), was incubated at 37°C for 0 h, 4 h, 8 h, 12 h, then the supernatant was recovered by centrifugation. The degree of protein hydrolysis was estimated by SDS-PAGE. Immunological analysis was carried out by using Western blots and enzyme linked immunosorbent assay (ELISA). The control was mixture without cellular suspension and incubated at the same conditions.

2.6. SDS-PAGE and Western blots

The methods from previous study (Rao et al., 2018) were slightly modified as follows: samples were mixed with loading buffer and heated at 100°C for 5 min. After that, $15\text{ }\mu\text{g}$ protein were loaded onto 4/15% polyacrylamide gel. The gel was resolved under 80 v for 40 min, 110 v for 80 min and stained with coomassie brilliant blue (R-250) for 40 min and then destained in washing buffer (50% v/v Methanol, 10% v/v acetic acid) every 2 h for three times. The gray values were calculated using Image J software.

Unstained gels were transferred to nitrocellulose membranes, which were blocked with TBST (0.02 M TBS, 0.05% Tween-20, pH 7.6)

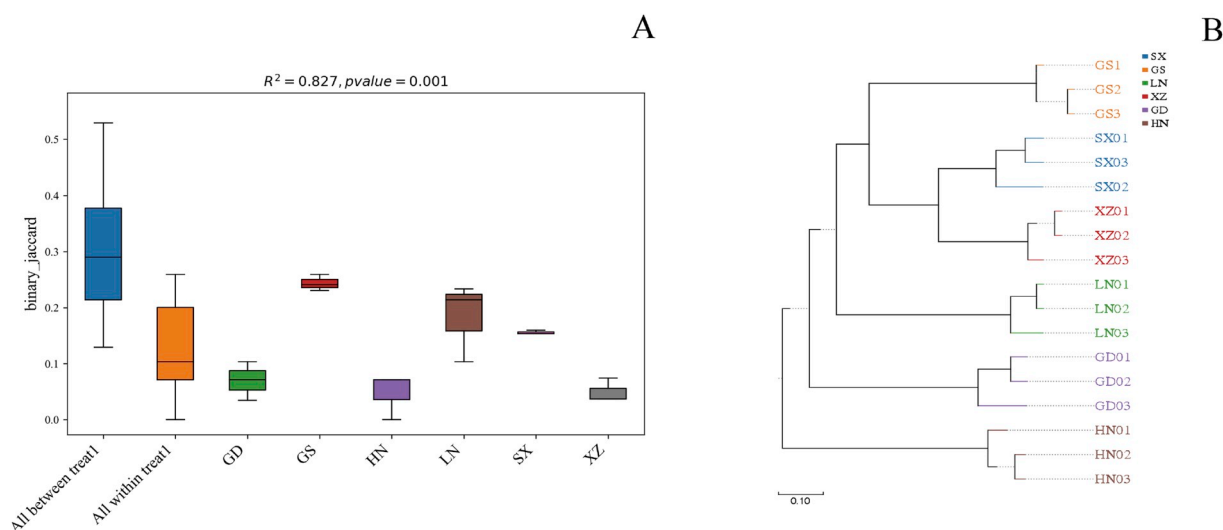


Fig. 2. (A) PERMANOVA box-plot. The R value which is closer to 1 indicating significant difference ($P < 0.05$) between groups. (B) UPGMA tree of different sourdough samples.

containing 5% (w/v) skim milk for 2 h, using a MiniProtein III system (BioRad) at 300 mA for 90 min. After 4 times washing (TBST), membranes were incubated with human serum (1:10 diluted in blocking buffer) overnight at 4 °C. Then those membranes were washed four times again and further incubated with Goat anti-human IgG labeled with HRP (1:10000 diluted in blocking buffer) at room temperature for 2 h. Membranes were washed for 4 × 15 min using TBST and added with a chemiluminescent BeyoECL-Star. The protein bands were visualized using SageCapture™ Mini610C.

2.7. Assay of IgG binding by ELISA

According to a slightly modified existing procedure ((Rao et al., 2018), 100 µL of diluted samples (5 µg/mL) were coated onto microplate and incubated overnight at 4 °C. After washing wells 4 times with wash buffer, wells were blocked with 0.02 M Tris-buffered saline containing 1% (w/v) bovine serum albumin for 2 h at 37 °C. Following 4 times re-washing, the wells were added with 100 µL human serum (1:10 diluted in blocking buffer) and incubated for 2 h at 37 °C. HRP-conjugated goat anti-human IgG (1:10,000) was added to the plate (100 µL/well) and incubated for 1 h at 37 °C, followed by 4 times washing. The assay development was performed by using a tetramethylbenzidine substrate kit. The results were read at 450 nm using a microplate reader (Thermo Fisher Multiskan FC). The absorbance of samples at 0 h, 12 h were expressed by X0 and X. The IgG-binding inhibition of a sample was expressed as $(X0 - X) \times 100/X0$.

2.8. Statistical analysis

Except as noted, the statistical analyses of appropriate data were performed by ANOVA (analysis of variance) using IBM SPSS Statistics 22.0 and all figures were processed using GraphPad Prism 6.02. Statistically significant was determined at a $P < 0.05$ level.

3. Results and discussion

3.1. Quality assessment of sequencing data and microbial community composition

The number of effective tags was more than 77% of PE Reads and the length distribution of it was also concentrated around 430 bp, which was consistent with the base length of the v 3 + v 4 region (Fig. 1A). Sequences that matched with more than 97% similarity were classified as

an OTU. OTU coverage (≥ 0.999) indicated that lower abundance of OTU has also been covered, and this sequencing result can show bacterial condition truly in these samples. The amount of total bacterial OTUs of 18 samples were 34. Rarefaction Curve tend to be flat, which suggest that the microbial in all samples was identified almost completely (Fig. 1B). The taxonomic classification of the sequences shows that, at phylum level, consistent with previous studies, *Firmicutes* was the predominant one in all samples followed by *Proteobacteria* (Liu et al., 2016; Liu et al., 2018). Fig. 1C shows the bacterial community composition at genus level. The dominant genus was *Weissella* in GD samples, however, *Lactobacillus* in other samples. In species level (Fig. 1D), there were numerous unclassified ones in XZ, indicating that new species will be more likely separated from that. Alpha diversity indices were used to perform community richness and diversity of each sample. The Chao and Ace indices were calculated to estimate community richness. The larger Shannon index value and smaller Simpson index value indicate the higher diversity of the sample (Grice et al., 2009; Lucilla et al., 2009). As shown in Fig. 1E and F, the Chao indices difference in samples were not significant ($P < 0.05$), implying that there was no significant difference in community richness between different samples. Bacterial diversity was highest (Shannon = 1.28 ± 0.04 , 1.22 ± 0.01) in LN, GS samples and Shannon values of 0.27 ± 0.05 for GD samples indicates that these samples had less bacterial diversity (Table S1).

3.2. Analysis of similarities in different sourdough samples

TTA, pH and proximate composition of sourdough samples were shown in Table 1. UPGMA by the R language tool was used to determine the similarity of microbial composition between samples. PERMANOVA tests for significant differences in beta diversity between groups of samples (Fig. 2A). UPGMA tree was shown in Fig. 2B: the shorter the branch length, the higher the similarity of the sample composition. XZ, SX and GS samples have similar bacterial composition. Interestingly, for most samples, the closer the environmental factor values were, the more similar the composition of the bacteria in the sample was. Line Discriminant Analysis (LDA) Effect Size analysis was used to find the classified bacterial taxa with significant abundance differences between different samples. As shown in Fig. 3A and B, 26 bacterial clades were determined to be significantly discriminant with a LDA threshold of 4. The abundance of *Leuconostocaceae* (family) and *Weissella* (genus) in the GD samples were much higher. *Acetobacter* (genus), *Leuconostoc* (genus) showed abundance advantage in the LN, GS samples. This finding was in agreement with previous study (X. Liu

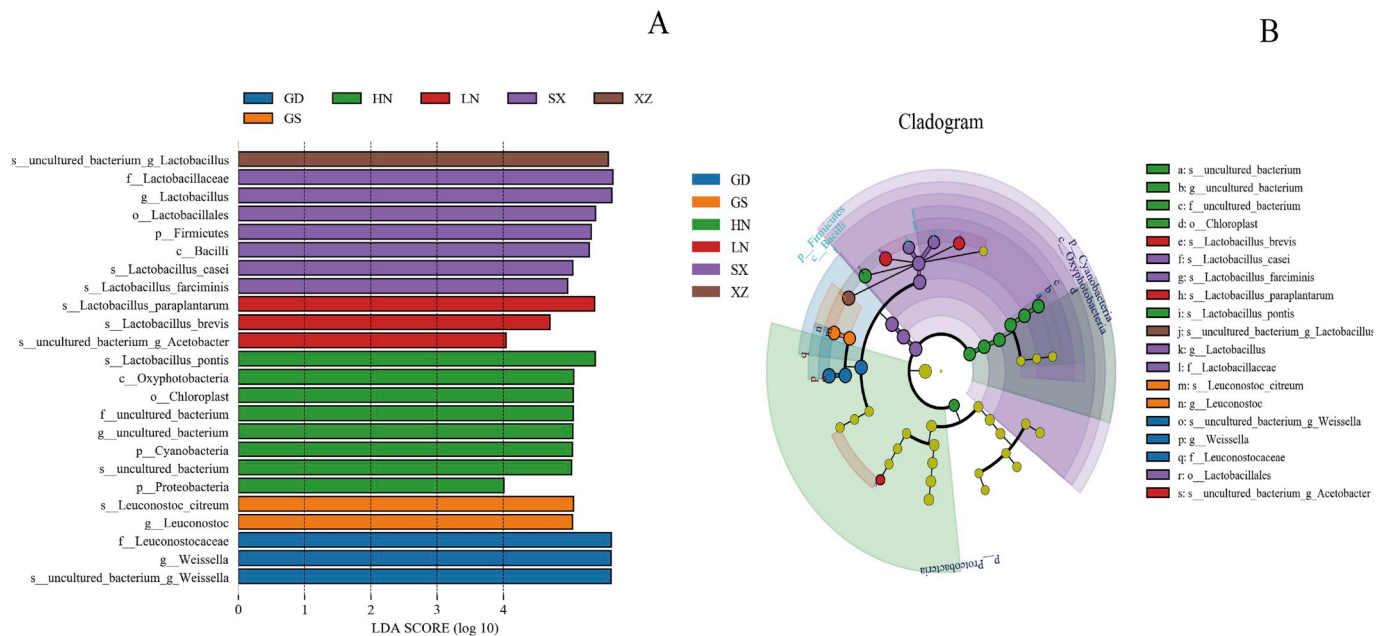


Fig. 3. (A) Histogram of LDA value distribution with a threshold value of 4. (B) Lefse analysis of bacterial abundance between different samples. The species with no significant difference marked with yellow. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article).

et al., 2018). *Klebsiella* (genus), *Enterobacteriales* (order), *Pantoea* (genus) and *Proteobacteria* (phylum) displayed abundant advantages in HN samples. In summary, in genus level, *Lactobacillus*, *Leuconostoc*, *Weissella* were shown as biomarker of SX, GS and GD samples, respectively. But *Leuconostoc* accounted for 27% of the population in GS samples. There were more biomarker were found in HN and LN samples. Despite the existence of these biomarkers, they do not accounted for a large proportion in population of each sample. And the dominant strains in different samples were still basically the same (Zhao et al., 2019). LefSe analysis indicated that the predominant bacterial in 15/18 samples was *Lactobacillus*, while *Weissella* which accounted for 95% in population of GD samples. Consistent with previous study that lactic acid bacterial became predominant after long time sourdough fermentation (Menezes et al., 2019).

3.3. Relationship between bacterial community and environmental factors

Linear regression, Pearson, and Redundancy analysis (RDA) were performed to reveal the correlation between bacterial community in sourdough samples and environmental factors. The dependent and independent variable were environmental factor and alpha diversity indices. In Linear regression, a P value of less than 0.05 means that the dependent variable varies significantly with the independent variable. As shown in Fig. 4A and B, Shannon diversity index was negatively related to annual average temperature (AT) and annual rainfall (AR) (linear regression: $R^2 = 0.3659$, $P = 0.0078$; $R^2 = 0.7741$, $P < 0.0001$). In addition, the relationship between Shannon index and altitude (AD) showed that extreme altitudes conditions have a negative impact on bacterial diversity in sourdough (Fig. 4C $P < 0.05$). Fig. 4D and E showed that Chao index was positively related to annual rainfall (AR) ($R^2 = 0.2455$, $P = 0.0365$), while negatively related to sunshine duration (SD) ($R^2 = 0.2649$, $P = 0.0289$). Similar results were also found in Pearson analysis, of which AR (AT) and SD were negatively related to Shannon and Chao indices, while AR was positively related to Chao values (pearson correlation analysis: $r = -0.880$, $P = 0.000$; $r = -0.605$, $P = 0.008$; $r = -0.515$, $P = 0.029$; $r = 0.495$, $P = 0.037$, respectively). The thin air and low concentration of atmospheric suspended particulate matter in the plateau region lead to less attenuation of solar ultraviolet (UV) radiation reaching the plateau surface. The

structure of ribonucleotides and deoxyribonucleotides in microorganisms can be damaged by higher UV molecules, resulting in lower microbial diversity in plateau region (Wu et al., 2013). AR is the main influencing factors in bacterial diversity of sourdough, compared to AT, AD and SD. Previous studies have shown that, in the air system, the microorganism counts declined with relative humidity increasing (Li, Liu, Xu, & Pu, 2012; Li, Liu, Zhu, Liu, & Wang, 2010). Furthermore, microorganisms in the environment were less likely to colonize the sourdough and thus reduce the bacterial diversity of sourdough. The annual rainfall value of GS sample was minimum, which might be a reason for the higher bacterial diversity. Those results indicated that environmental factors have potential effect on bacterial diversity and richness.

RDA were carried to explore the effects of environmental factors on the top ten dominant bacteria (in genus level) in sourdough (Fig. 4F). The points of different shapes were used to show clustering pattern in bacterial structure among samples. Consistent with UPGMA analysis, GD and HN samples were significantly separated from the others. As shown in RDA results, acute angles emerged among AR and AT, implying that the two variables had a synergistic effect on the microbial composition. Otherwise, AR (AT) and SD showed obtuse relationships, indicating that they had an opposite impact on the microbial community. According with linear regression and Pearson analysis, most of the ten dominant genera were negatively correlated with the annual rainfall and annual average temperature, implying that lower annual rainfall and annual average temperature will increase the complexity of microbial community in sourdough.

3.4. Effects of selected LAB treatment on allergenicity of wheat protein extracts

Several LAB strains were identified by molecular method and 16S rRNA gene sequencing results (Table S2) were partially shown as follows (Fig. 5): *Lactobacillus plantarum*LN5 (LN5), *Pediococcus pentosaceus*GD4 (GD4), *Pediococcus acidilactici*XZ31 (XZ31), *Lactobacillus plantarum*SX1 (SX1) and *Lactobacillus sakei*GS6 (GS6) were isolated from LN, GD, XZ, SX and GS samples, respectively. Additionally, *Enterococcus faecium*AH1 (AH1) and *Lactobacillus paracasei*AH2 (AH2) isolated from sourdough from Anhui province were also used in further

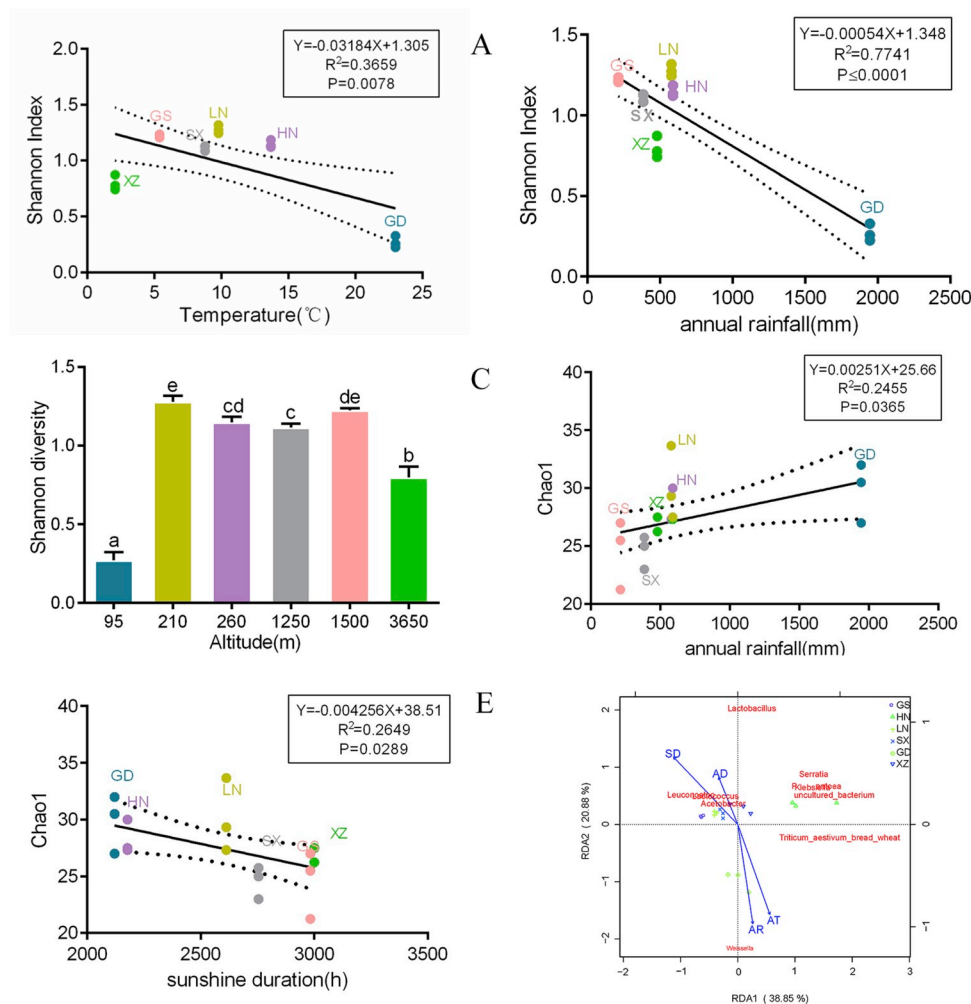


Fig. 4. The correlation analysis of environmental factors with alpha diversity indices and different colours represent different samples. Dashed lines in (A), (B), (D) and (E) are the 95% confidence intervals of linear models (solid lines). Different letters in (C) represent significant difference between ordinate values ($P < 0.05$). (F) Redundancy analysis which reflects the relationship between dominant bacteria and environmental factors. AD, altitude annual. AT, average temperature. AR, annual rainfall. SD, sunshine duration. Top ten genus in abundance were showed in red fonts. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article).

analysis. We selected those species, which have been commonly identified and reported to have the ability to hydrolyze the wheat protein, for further research (Hervé, Valérie, & Catherine, 2009; Lucilla et al., 2009). We isolated *Pediococcus* from XZ and GD samples, which could be inconsistent with the previous assumptions that *Pediococcus* are the indigenous microflora of the Qinghai Tibet plateau (X. Liu et al., 2018). However, it further confirms the results of high-throughput analysis that environmental factors are mainly related to the diversity and richness of bacteria in sourdough.

Western blots results have shown that, in accordance with other

studies (Battais et al., 2010), bands around 15–25 KDa, 25–35 KDa (prolamins), 45–60 KDa (prolamins and LMW-GS) were more highly sensitized to human serum we chosen (Fig. 6A and B). By SDS-PAGE analysis, for some of lanes, the levels of peptide degradation increased with time. The appearance of diffused bands around 40–100 KDa may be due to the antigen concentration was high and the peptide profile was very diverse. There was no significant difference between 0 h and 12 h in control. After 12 h's hydrolyzing, the most sensitized bands around 15–25 KDa were very weak in GD4, XZ31 and GS6 lanes (Fig. S). And that may be one reason for lower IgG-immunoreactivity of GD4,

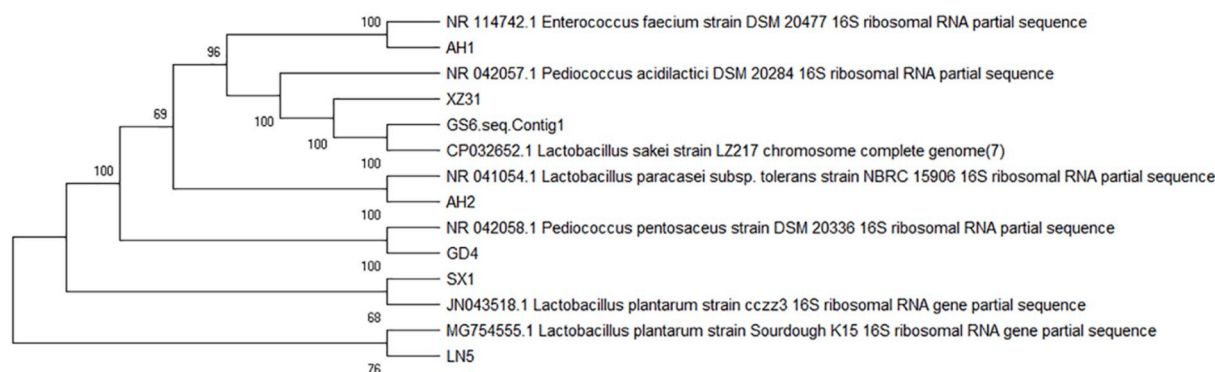


Fig. 5. Phylogenetic trees of seven strains isolated from sourdough. LN5, *Lactobacillus plantarum*LN5. GD4, *Pediococcus pentosaceus*GD4. XZ31, *Pediococcus acidilactici*XZ31. SX1, *Lactobacillus plantarum*SX1. GS6, *Lactobacillus sakei*GS6. AH1, *Enterococcus faecium*AH1. AH2, *Lactobacillus paracasei*AH2.

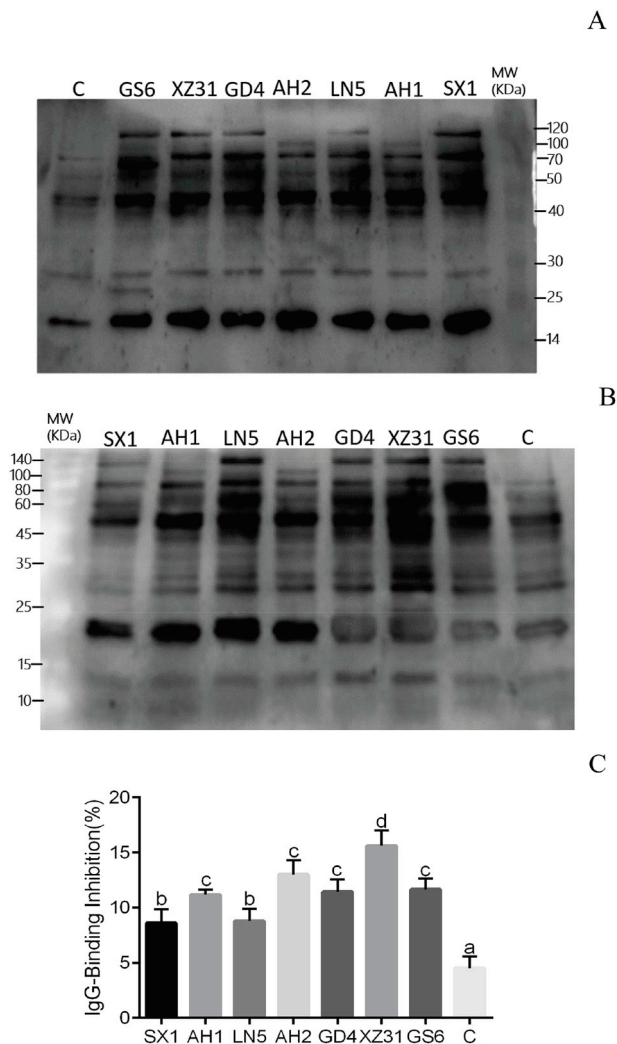


Fig. 6. (A) IgG-immunoblotting of wheat protein treated with different strains in 0 h. (B) IgG-immunoblotting of wheat protein treated with different strains for 12 h. (C) IgG-binding of wheat protein treated with different strains. Control was untreated wheat protein hydrolysate.

XZ31 and GS6 (Fig. 6B). To compare selected seven strains capabilities of reducing allergenicity, ELISA analysis was also used to test IgG-binding capabilities. The results were evaluated by IgG-binding inhibition and negatively correlated with the allergenicity. As shown in Fig. 6C, all strains' capabilities of reducing allergenicity were significantly different with control group. XZ31 was the most effective strain, followed by AH2, GS6, GD4, AH1 in reducing the immunoreactivity of wheat proteins. Antibodies were bound with linear epitope in Western blots, while conformational epitope in ELISA. That may give reasons for different results in AH1 and AH2 by two methods. By Western blots and ELISA analysis, XZ31, GD4, GS6 were identified as potential strains in reducing allergenicity of wheat proteins. Furthermore, as shown in Fig. 5, XZ31 and GS6 have closer phylogenetic distance. Interestingly, those strains were all isolated from sourdoughs which have shown distinct features in some environmental factors. Protein utilization by lactic acid bacteria contain three steps and performed by cell-envelope proteinase (PrpP, PrpH, PrpR, PrpS and PrpB), oligopeptide transport systems and peptidases, respectively. Cell-envelope proteinase play an important role in protein degrading process, and then the protein will be transformed into oligopeptides which can be taken up by cells. *Lactobacillus sakei*, *Lactobacillus paracasei* have been shown to be equipped with cell-envelope proteinase (Savijoki, K., Ingmer, H., & Varmanen, P., 2006), which might result in higher

capabilities of reducing allergenicity. In this study, *Pediococcus acidilactici*XZ31 and *Pediococcus pentosaceus*GD4 were selected as the efficient strains to reduce wheat protein allergenicity, while there are no systematic research in proteolytic systems of *Pediococcus*. Apparently, further research focused on the proteolytic systems is needed to reveal the mechanism of reducing allergenicity.

4. Conclusions

Previous studies have showed the bacterial composition differences between western, northern and southern sourdough samples in China. However, the relationship or connections among region and bacterial flora were unclear. The data presented here indicated that environmental factors have potential effects on community diversity and richness, while no influence on type of dominant bacteria. Moderate altitude conditions are conducive to better bacterial diversity in sourdough ($P < 0.05$). Within a certain range, annual average temperature and annual rainfall have negative effects on bacterial diversity. Annual rainfall, sunshine duration were shown as positive and negative effect factors of bacterial richness. Moreover, sourdough fermentation, as an emerging technique to produce hypoallergenic product, need further study on selection of efficient strains. The preliminary screening of seven predominant LAB from sourdough showed *Pediococcus acidilactici*XZ31 (XZ31), *Pediococcus pentosaceus*GD4 (GD4), *Lactobacillus sakei*GS6 (GS6) have superiority over the others in reducing allergenicity of wheat proteins, which serves as a reference for producing of the hypoallergenic product.

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.lwt.2019.108669>.

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Table S1

Alpha diversity parameters

Sample ID	ACE	Chao	Simpson	Shannon	Coverage
GS01	26.9083	25.5	0.3336	1.236	1
GS02	27.5604	27	0.3447	1.2252	0.9999
GS03	22.1895	21.25	0.3413	1.2073	1
SX01	27.8793	25	0.393	1.0854	0.9999
SX02	23.3523	23	0.3805	1.13	1
SX03	26.1431	25.75	0.3748	1.1262	0.9999
GD01	30.2874	30.5	0.9224	0.2246	0.9999
GD02	27.4351	27	0.9077	0.2574	0.9999
GD03	31.6254	32	0.8724	0.3282	0.9999
XZ01	28.2836	27.5	0.6026	0.8737	1
XZ02	27.6576	27.5	0.643	0.7771	1
XZ03	26.8593	26.25	0.6544	0.7423	1
HN01	27.8156	27.3333	0.3975	1.1218	1
HN02	28.8338	30	0.3869	1.134	1
HN03	28.424	27.5	0.3687	1.1865	1
LN01	30.0952	27.3333	0.4003	1.2446	0.9999
LN02	37.6154	33.6667	0.3904	1.2739	0.9998
LN03	35.4378	29.3333	0.3714	1.3182	0.9999

Table S2

Blast results of 16S rRNA genes of the bacteria isolated from sourdough in different regions

Strain No	Blast results	Similarity	Gene Bank NO
GS1	<i>Lactobacillus sakei</i>	100%	NR 113821.1
GS2	<i>Lactobacillus plantarum</i>	100%	NR 115605.1
GS3	<i>Lactobacillus sakei</i>	99.27%	NR 113821.1
GS5	<i>Lactobacillus sakei</i>	99.09%	NR 113821.1
GS6	<i>Lactobacillus sakei</i>	100%	CP 032652.1
SX1	<i>Lactobacillus plantarum</i>	100%	JN 043518.1
SX2	<i>Lactobacillus plantarum</i>	100%	NR 117813.1
HN2	<i>Lactobacillus plantarum</i>	99.54%	NR 117813.1
GD1	<i>Pediococcus pentosaceus</i>	99.78%	NR 042058.1
GD2	<i>Pediococcus pentosaceus</i>	99%	NR 042058.1
GD4	<i>Pediococcus pentosaceus</i>	98.96%	NR 042058.1
GD7	<i>Pediococcus pentosaceus</i>	98.96%	NR 042058.1
XZ2	<i>Pediococcus acidilactici</i>	99.27%	NR 042057.1
XZ3	<i>Pediococcus acidilactici</i>	99.52%	NR 042057.1
LN2	<i>Lactobacillus sp.</i>	100%	NR 117813.1
LN5	<i>Lactobacillus plantarum</i>	99.64%	MG 754555.1
AH1	<i>Enterococcus faecium</i>	99.43%	NR 114742.1
AH2	<i>Lactobacillus paracasei</i>	98.47%	NR 041054.1

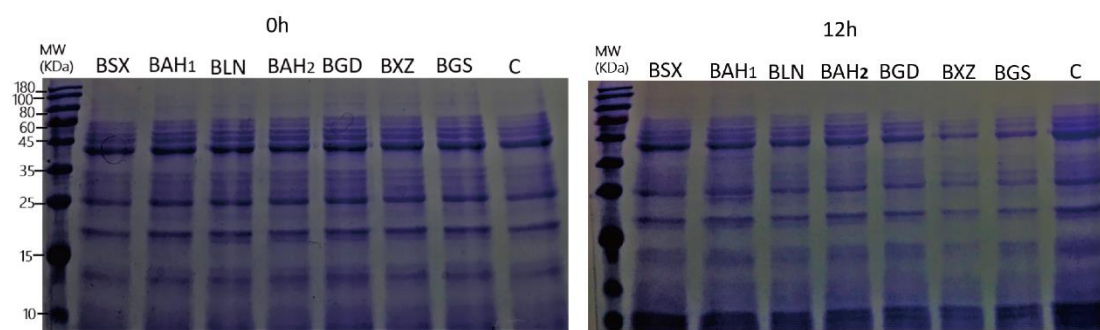


Fig. S. SDS-PAGE of LAB treatment wheat protein extracts. Control was mixture without cellular suspension and incubated at the same conditions.