Fungal Deterioration of the Bagasse Storage from the Harvested Sugarcane

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Abstract

Background

Sugarcane is an essential crop for sugar and ethanol production. Immediate processing of sugarcane is necessary after harvesting because of rapid sucrose losses and deterioration of stalks. This study was conducted to fill the knowledge gap regarding the exploration of fungal communities in harvested deteriorating sugarcane. Experiments were performed on simulating production at 30 ℃ and 40 ℃ after 0, 12, and 60 hours (h) of sugarcane harvesting and powder-processing.

Results

Both pH and sugar content declined significantly within 12 h. Fungal taxa were unraveled using ITS amplicon sequencing. With the increasing temperature, the diversity of the fungal community decreased over time. The fungal community structure significantly changed within 12 h of bagasse storage. Before storing, the dominant genus (species) in bagasse was *Wickerhamomyces (W. anomalus)*. Following storage, at 30 ℃ and 40°C, *Kazachstania* (*K. humilis*) and *Saccharomyces* (*S. cerevisiae*) gradually grew, becoming abundant fungi. We found that the bagasse at different temperatures had a similar pattern after storage for the same intervals, indicating that the temperature was the primary cause for the variation of core features. Moreover, the impact of dominant fungal species on sugar content and pH of stored sugarcane juice was investigated. Also, the correlation between top fungal genera and significant environmental factors was assessed.

Conclusions

The study highlighted the importance of timeliness to refine sugar as soon as possible after harvesting the sugarcane crop. The lessons learned from this research are vital for sugarcane growers and the sugar industry for minimizing postharvest losses.

Background

Sugarcane is one of the most important crops for sugar and bioethanol production (Goldemberg et al., 2008). The estimated annual gross output of sugar from sugarcane is valued as high as $76 billion (Hector et al., 2015). Sugarcane is a perishable commodity and must be processed into sugar quickly after harvesting. The postharvest deterioration of sugarcane is a severe problem for the sugar industry, causing 20–30 percent sucrose losses in cane-producing countries (Eggleston, 2002; Solomon, 2009; Saxena, 2010; Hector et al., 2015). Many factors are associated with the deterioration of the harvested cane, including cane cultivar and its maturity, mechanical or manual harvesting, exposure to microbes, cut-to-crush delay, and storage (Solomon, 2009; Solomon et al., 2011; Hector et al., 2015).

In many countries, the harvested cane is kept in the field for 3–5 days due to the flawed field transport system and 1–3 days in the factory storage under undesirable conditions (Solomon, 2009; Hector et al., 2015). Sucrose inversions in the deterioration of harvested cane result from the chemical (acid) and enzymic inversion and microbial activity. In the first 14 hours (h) of cane juice deterioration, 93.0% of sucrose losses are caused by microbial, 5.7% by enzymic, and 1.3% by chemical changes (acid degradation) (Eggleston, 2002). Hence, microbial proliferation is the primary reason for sucrose loss and acidification after harvesting. The high sugar concentration within the mature internodes provides a favorable environment for microbial thriving.

The microbial communities enter the harvested stalk through wounds or cut ends (Solomon et al., 2011). Also, leaf-sheaths and growth cracks provide excellent sites for microbial growth. Over 400 species of actinomycetes, bacteria, and fungi are associated with sugarcane and its products (Stevenson and Rands, 1938). After milling, *Penicillium, Lactobacillus, Leuconostoc*, and yeast invade the stored sugarcane (Hector et al., 2015; Misra et al., 2017; Misra et al., 2019). These acid-producing microorganisms are the principal cause of deterioration, which lowers the sucrose content, juice purity, and pH, especially under anaerobic conditions such as mud-coated canes and the cane stored in large piles with poor ventilation (Solomon, 2009; Misra et al., 2017). The glucose and fructose are converted to organic acids and mannitol by the enzymes secreted by these microorganisms. Besides these external microbes, the endophytic microbial flora *viz*., *Acetobacter, Enterobacter, Pseudomonas, Aeromonas, Vibrio, Bacillus*, and *lactic acid group* are also responsible for the deterioration of juice quality during staling (Suman et al., 2000; Misra et al., 2017).

It is crucial to identify the key players in sugarcane deterioration and improve early detection strategies of degradation-causing microbes. In this reference, the sucrose-related dynamics of fungi in stored sugarcane bagasse remain unexplored. Thereby, the fungal community variations in the stored bagasse were analyzed for assessing the core fungi and specific biodegradation biomarkers using high-throughput sequencing. Moreover, the study also explored the impact of different cane storage durations and temperatures on sugarcane deterioration and dissected these parameters associated with the fungal richness and diversity.

Results

Sucrose and pH

In the first 12 h, the steepest decline was observed in the bagasse stored at 30°C. The sucrose reduced by 53.9% within the first 12 h and then completely decomposed during the initial 36 h for all the treated bagasse. Contrary to sucrose, the glucose and fructose of stem powder increased in the first 12 h and then fell to zero by 60 h (Fig. 1b, 1c). However, the glucose and fructose increase in the bagasse stored at 40 °C was higher than those at 30 °C. The pH of all the bagasse reduced significantly in the first 12 h (one-way ANOVA, $p < 0.001$) and then maintained at around 3.6–3.8 (Fig. 1).

Diversity of fungal communities

For determining the fungi prevailing in biodegrading sugarcane, community DNA was extracted and amplified using the ITS2 primer pair. Subsequently, the amplicons were sequenced on the Illumina MiSeq platform. A total of 962,640 reads were recovered from all samples after quality filtering (Table S1 in the supplemental material), representing 31 fungal Operational Taxonomic Units (OTUs) at 97% sequence similarity. The rarefaction curves confirmed that all the samples reached the plateau phase (Fig. S1). The richness and diversity of fungal OTUs rose in the first 12 h in all the samples. The fungal richness and diversity were significantly lower in the bagasse stored at 40 ℃ for 60 h than those stored at 30 ℃ ($p \leq 0.05$) (Fig. 2). The temperature was significant in the β-diversity by Principal Components Analysis (PCA). The storage temperature alone explained 34.66% of the microbial community variation (PERMANOVA, $p = 0.020$), while the storage duration explained 73.65% of the variation (PERMANOVA, $p < 0.001$) (Fig. 3).

Only *Ascomycota* was identified at the phylum level. Four classes were identified at the class level, including *Saccharomycetes, Dothideomycetes, Sordariomycetes*, and *Eurotiomycetes*, of which *Saccharomycetes* accounted for 99.7%. Five orders were identified, including *Saccharomycetales*, *Pleosporales*, *Hypocreales*, *Chaetothyriales*, and *Eurotiales*, of which *Saccharomycetales* alone accounted for 99.7%. At the family level, ten families were identified, of which *Saccharomycetaceae* accounted for 64.1%, followed by *Phaffomycetaceae* (35.3%). Further, at the genus level, a total of 14 fungal genera were identified. *Saccharomyces*, *Torulaspora*, *Hanseniaspora*, and *Curvulari* were exclusively present in the bagasse stored at 40°C while *Kazachstania*, *Zygosaccharomyces*, and *Hanseniaspora* were solely identified in bagasse stored at 30°C. The dominant genus in CK bagasse was *Wickerhamomyces* (92%).

When the bagasses were stored at 30°C, *Kazachstania* gradually replaced *Wickerhamomyces* as the most abundant fungus (60% at 12 h and 80% at 60 h). However, the bagasses stored at 40°C for 12 h had two highly abundant genera of *Saccharomyces* (50%) and *Wickerhamomyces* (50%). The relative abundance of *Saccharomyces* increased up to 90% after 60 h in these bagasses (Fig. 4a). At the species level, both *Saccharomyces* (*S. cerevisiae*) or *non- Saccharomyces* yeast (*K. humilis* and *W. anomalous*) were predominant and varied with the increase in temperature and duration of the storage. Before temperature treatment (CK), *W. anomalous* accounted for 92.4% of all tested OTUs. When stored at 30℃, *K. humilis* was predominant, and its abundance increased from 3.5% (before treatment) to 68.8% after 12 h and 82.9% after 60 h. On the other hand, *W. anomalus* sharply decreased to 8.6% after 60 h of treatment, while *S. cerevisiae* increased to 7.2%. *S. cerevisiae* dominated at 40℃, accounting for 49.9% after 12 h and 99.6% after 60 h, whereas *W. anomalous* sharply reduced from 44.8% after 12 h to an undetectable level after 60 h. Only 2.4% of *K. humilis* was detected at 40℃ after 12 h of storage (Fig. 4b).

The primary driver for fungal community composition

The redundancy analysis (RDA) was used to determine how the environmental physicochemical parameters affected the fungal community composition (Fig. 5). Among environmental factors, sucrose content (F-ratio = 77.53, $p = 0.002$) and storage time (F-ratio = 65.5, $p = 0.003$) are significantly associated

with the fungal communities. The storage temperature (F-ratio = 30.67) and pH (F-ratio = 37.33) also played an essential role in the variation of fungal communities. Hence, RDA showed that the fungal communities varied with the physicochemical parameters.

The correlation heatmap of the relationships among the top 14 genera and six selected environmental factors (storage temperature, storage time, sucrose content, fructose content, glucose content, and pH) was presented in Fig. 6. The storage temperature was significantly negatively correlated with pH, *i.e.*, the higher the storage temperature, the lower the pH. This association supported higher growth of *Saccharomyces* and negatively impacted the richness of any other fungal genera. The storage time had a significant influence on the conversion of sucrose to glucose and fructose. Fructose and glucose content showed a slight correlation with the fungal abundance, while sucrose significantly affected *Wickerhamomyces* and *Torulapora*.

Functional verification of isolated strains

Five fungal genera were isolated by plate culture. Further, the utilization of sucrose was assessed by inoculating fungal solution in the sucrose medium. *Saccharomyces*, *Wickerhamomyces*, and *Torulaspora* had a solid ability to degrade sucrose at 30℃, consuming sucrose entirely within 36 h (Fig. 7). This phenomenon also yielded different reducing sugar byproducts in different quantifies (Fig. S2). However, at 40℃, only *Saccharomyces* maintained the same activity, and the sucrose in its culture medium was completely degraded within 36 h. All other fungi were sensitive to temperature. Corresponding to change in sucrose contents, the pH of the media culturing the strains dropped. At 30℃, the pH dropped to 3.6–3.8 after 36 h. The pH of the media having *Saccharomyces* and *Wickerhamomyces* reduced faster (Fig. 8). At 40℃, only the pH of the *Saccharomyces* medium decreased significantly.

Discussion

Sugarcane staling is one of the significant issues of the sugar industry, and it causes significant economic losses because of cane and sucrose deterioration during storage and transportation (Solomon, 2009; Khan et al., 2020). The postharvest sugarcane biodegradation necessitates the abating of harvest to crush delays. Microbial communities thriving the harvested sugarcane play a crucial role in this deterioration. Moreover, other factors, such as storage time, temperature, and environmental conditions, are also important (Solomon, 2009; Solomon et al., 2011; Hector et al., 2015). As per our knowledge, the sucrose-related dynamics of fungi in stored sugarcane remain unexplored. Therefore, this study was conducted to investigate the diversity and abundance of fungal communities in harvested sugarcane. Further, we also examined the impact of different storage temperatures and duration on deterioration processes and fungal abundance.

The pH and sucrose sharply reduced in the first 12 h and then remained stable over 36 h. However, both fructose and glucose increased in the first 12 h, followed by a sharp decrease. The richness and diversity of fungal OTUs declined with the increase in storage temperature and duration. *Wickerhamomyces (W.*

anomalus), *Kazachstania* (*K. humilis*), and *Saccharomyces* (*S. cerevisiae*) were the most abundant fungal genera (species) in the deterioration of harvested sugarcane. *S. cerevisiae* dominated in the bagasses stored at 40°C, accounting for more than 49.9% after 12 h and 99.6% after 60 h. At 30°C, *K. humilis* enriched from 3.5% before treatment to 82.9% after 60 h of treatment. *W. anomalus* was more prevalent in the pre-treatment bagasses and decreased from 92.4–8.6% (after 60 h) at 30°C and to an undetectable level under 40°C after 60 h.

W. anomalus exhibits wide metabolic and physiological diversity, which makes it assimilate sucrose, lactose, and starch, and adapt to a wide range of growth conditions in terms of temperature (3–37°C), pH (2–12), sucrose levels, and osmolarity (Fredlund et al., 2002; Walker, 2011). As a classical killer yeast, *W. anomalus* has antifungal activity. It produces protein toxins or low molecular mass glycoproteins that are lethal to other susceptible yeasts and filamentous fungi (*Aspergillus*, *Botrytis*, *Penicillium*, *Fusarium*) (Masih et al., 2000; Sun et al., 2012; Guo et al., 2013; Oro et al., 2014; Aloui et al., 2015; Oro et al., 2018; Czarnecka et al., 2019). The antifungal properties of *W. anomalus* could be the reason that richness and diversity were very low in our bagasse samples. Only 31 OTUs were assembled from 962,640 reads of our high-throughput sequencing. *W. anomalus* is sensitive to oxygen. Its respiratory growth is favored under aerobic conditions, while alcoholic fermentation is induced under limited oxygen conditions (Walker, 2011; Padilla et al., 2018). *W. anomalus* exhibited growth rates of 0.22 and 0.056 h^{-1} and biomass yields of 0.59 and 0.11 g/g glucose under aerobic and anaerobic conditions, respectively (Fredlund et al., 2004). Therefore, the changes observed in the fungal community structure in our study could be attributed to the fact that since oxygen would have run out in the Ziplock bags during the storage, the fungal communities changed from predominantly respiratory to fermentative. Hence, *Kazachstania* and *Saccharomyces* replaced *Wickerhamomyces* as the most abundant fungi during storage treatment.

Kazachstania and *Saccharomyces* are both recognized as typical acid-tolerant yeast; they grow well at pH as low as 3.5 (Guerzoni et al., 2013). Most species of *Kazachstania* can assimilate sucrose, and several species have similar growth rates and ethanol yields as *Saccharomyces* (Hagman et al., 2013). The dominance of *S. cerevisiae* over other microbial competitors during fermentation has been traditionally ascribed to its high fermentative power and aptitude to cope with the harsh environmental conditions, *i.e.*, high levels of ethanol and organic acids, low pH, scarce oxygen availability, and depletion of certain nutrients (Bauer and Pretorius, 2000; Hansen et al., 2001; Albergaria and Arneborg, 2016). However, *K. humilis* optimally grows in the temperature range of 25–30°C and does not grow at 37°C. Therefore, *Kazachstania* was the top genus at 30°C, while *Saccharomyces* was the dominant genus at 40°C (Fig. 4). The fungal community's richness and diversity remained low due to the high sucrose content, acidic pH, and killer toxins.

The differences in storage temperature could be the primary cause of the variation of core features in this study. Moreover, storage time also played a significant role in influencing community structure and abundance. Further, the RDA analysis revealed the pH as another critical environmental factor. In agreement with our study, the temperature was evaluated as a critical factor in shaping the sugar beets' microbial community (Liebe et al., 2016). We observed that sucrose reduced by 53.9% in the first 12 h.

Solomon et al. (2011) also documented similar findings of rapid deterioration in harvested sugarcane. Average losses in the untreated billets and whole cane were 0.735 and 0.502 units per day, respectively, which indicated that deterioration occurred more rapidly and violently nearby wounds and cut ends (Solomon et al., 2011). Cutting the sugarcane stalk in harvesting disrupts the physiology of the plant. The altered balance among the plant functions leads to many undesirable changes in the composition of the stalk. These adverse effects are worsened by the increased duration of the postharvest storage period between harvest and mill processing and high ambient temperatures (Verma et al., 2012). Due to respiration, an increase in temperature within the heap is likely to have significant consequences for the postharvest stalk rage and juice quality. Only approximately 33% of the energy released in sucrose's oxidative catabolism is captured in ATP's chemical bonds (Siedow and Day, 2000). The remaining energy degenerates as heat that significantly contributes to the warming of the storage piles (2006; Verma et al., 2012).

This study presented here showed that the reduction in sucrose percent in the harvested and stored sugarcane increased by the cut-to-crush delay and high atmospheric temperature. Therefore, appropriate measures should be adopted to avoid wounds, cut ends on canes, and complete the crushing in a shorter time, especially for the mechanically harvested crop. pH was also found to be related to sucrose conversion, and it declined ahead of sucrose. Similar results have been documented by previous studies as well (Singh et al., 2006). A rapid decrease in pH and a rise in titratable acidity during storage; both of these changes increased at higher temperatures (Mao et al., 2006). Since pH is very easy to measure, it can be used as a suitable physio-biochemical indicator of postharvest deterioration of sugarcane.

Conclusions

This study was conducted to investigate the variation in fungal communities and changes in relevant physio-biochemical factors in postharvest deterioration of sugarcane. A very low richness and diversity of the fungal community, primarily dominated by yeast, was observed during the cane storage. *Wickerhamomyces (W. anomalus)*, *Kazachstania* (*K. humilis*), and *Saccharomyces* (*S. cerevisiae*) were the most abundant fungal genera (species) in the deteriorating cane. The differences in the storage temperature were observed to be the primary cause of variation of core features. Moreover, storage time also played a significant role in shaping the fungal community by deciding the relative abundance. The identified top genera in deteriorating sugarcane can be used as a decision tool. Cane degradation occurs rapidly within cuts and wounds. Therefore, measures should be adopted to limit wounds and cut ends on canes during harvesting. Furthermore, the cane should be transported and crushed as early as possible. pH can be used as an easy and accessible physio-biochemical indicator to estimate cane deterioration.

Methods

Sugarcane preservation after harvest

A total of 30 healthy sugarcane stalks were sampled from the same field in Fusui, China. Six clean harvested stalks without leaf sheath were surface-sterilized and randomly crushed into bagasse using DM540-CPS. This system rapidly smashes sugarcane with a high-speed rotating blade without pressing or producing cane juice, which the stalks were immediately shredded using DM540 (IRBI Machines & Equipment Ltd, Brazil), blended, and transmitted by CPS (Cane presentation system, Bruker Optik GmbH, Germany). The cane bagasse of five groups was packed in 50 zip lock bags (500 g each), 45 of which were randomly separated as four groups stored at 30°C and 40°C for four days. Sampling from each bag was performed at 0 h, 12 h, 36 h, 60 h, and 84 h. A total of 100 g bagasse was blended in cold ddH_2O using a juicer. The blended solution was filtered through eight layers of sterile bandage and centrifuged at 5,000 rpm for 10 min at 4°C. The supernatant was stored at -20°C, while pellet, deemed to have epiphytic stalk microbial communities, was stored at − 80°C. The bagasse filtrates' pH was determined using a pH meter (Seven Compact, Mettler Toledo).

Isolation and functional verification of strains

The sugarcane juice was diluted and coated on separation plates. After culturing, different colonies were selected according to colony characteristics for pure culture and molecular identification. Three kinds of separation plates (PDA, YPD, and WL) were used to obtain isolated fungal genera. The isolated strains were inoculated in 1 ml PDW medium, cultured at 30 ℃, and 220 rpm for 6 h. Then, 100 µl of the fungal solution was inoculated in 100 ml sucrose medium, cultured at 30 ℃ and 40 ℃ for 0 h, 12 h, 24 h, and 36 h. The culture medium was stored at -20 ℃ to determine sucrose, glucose and fructose contents, and pH. The sucrose medium consists of 20 % sucrose, 0.1 % glucose, 0.1 % fructose, 1 %NaCl, and 1 % yeast extract by stimulating sugarcane juice composition.

Soluble carbohydrates quantification by HPAEC-PAD

Soluble carbohydrates (sucrose, glucose, and fructose) were quantified by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC–PAD) in a 40 mm × 250 mm CarboPac PA-10 column on an ICS 5000 Dionex System (Thermo Scientific, Waltham, MA, USA). Diluted sample solutions (diluted 1000 times in Mili-Q water) were filtered through a 0.22 µm membrane before injection. D-Lactose monohydrate (Aladdin D1902045, Shanghai, China) was used as an internal standard. The gradient was established by mixing 60 % eluant A (H_2O) with 40 % eluant B (500 mmol L^{-1} NaOH) for 5 minutes, using a flow rate of 1 ml min^{-1} through the column (Aldrete-Herrera et al., 2019; Vasic et al., 2020).

DNA extraction and sequencing

The community DNA was extracted by the ALFA-SEQ Soil DNA kit (mCHIP, R0911) and quantified by Qubit 3.0 Fluorometer (Life Technologies, Carlsbad, CA, USA)(Cui et al., 2019). For amplicon library construction, we used the ITS2 primer pair [ITS21F (5’-CTTGGTCATTTAGAGGAAGTAA-3’) and ITS22R (5’-GCTGCGTTCTTCATCGATGC-3’)]. The PCR mixture contained 5 × TransStart FastPfu buffer (4 µL), 2.5 mM dNTPs (2 µL), forward primer (5 µM, 0.8 µL), reverse primer (5 µM, 0.8 µL), TransStart FastPfu DNA

Polymerase (0.4 μL), template DNA (10 ng), and ddH_2O (up to 20 μL). The PCR program was as follows: 95°C for 3 min, 35 cycles at 95°C for 30 s, 55°C for 30 s, and 72°C for 45 s with a final extension at 72°C for 10 min. The PCR products were purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA). According to the standard protocols, the purified amplicons were pooled in equimolar and paired-end sequenced (2 ×300) on an Illumina MiSeq platform (Illumina, San Diego, USA) by Majorbio Bio-Pharm Technology Co. Ltd., Shanghai, China.

Statistical analysis

The data of pH and sugar concentration were processed using Microsoft Excel and Origin 2016 software and analyzed using SPSS statistical software (SPSS incorporation, USA).

The raw ITS2 gene sequencing reads were quality-filtered in the BMKCloud platform. Operational Taxonomic Units (OTUs) were clustered at a 97 % similarity cutoff. We plotted and calculated a rarefaction curve and rank abundance curve on the BMKCloud platform. Alpha diversity indices (Chao 1, Shannon, and Simpson index) were determined through Microbiome Analyst, a web-based tool. Principal Components Analysis (PCA) was performed to explore the fungal community by image GP platform (http://www.ehbio.com/ImageGP). The variance explained by parameters was analyzed using a PERMANOVA test in R software.

The significant differences in relative abundance (average value of three replicates) of fungal genera among treatment groups were determined through a one-way analysis of variance ($p \leq 0.05$). R software's corrplot package was used to analyze the correlation between the top fourteen genera and six selected environmental factors (temperature, storage time, sugar content, and pH). Further, a redundancy analysis (RDA) was performed using the OTU table to investigate the taxa-environment relationships.

Declarations

Authors’ contributions

NP and ZY completed the major experiment, analyzed the data. ZW and JH participated in the microbiome analysis sugar detection. MZ designed the project, supervised the experiments, interpreted the data, and finalized the manuscript. MTK and BC revised the manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

The datasets supporting the conclusions of this article are included within the article and its Additional files.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

References

Albergaria H, Arneborg N. (2016). Dominance of Saccharomyces cerevisiae in alcoholic fermentation process: role of physiological fitness and microbial interactions. Appl Microbiol Biotechnol 100. doi:10.1007/s00253-015-7255-0.

Aldrete-Herrera PI, Lopez MG, Medina-Torres L, Ragazzo-Sanchez JA, Calderon-Santoyo M, Gonzalez-Avila M, et al. (2019). Physicochemical Composition and Apparent Degree of Polymerization of Fructans in Five Wild Agave Varieties: Potential Industrial Use. Foods 8(9). doi:10.3390/foods8090404.

Aloui H, Licciardello F, Khwaldia K, Hamdi M, Restuccia C. Physical properties and antifungal activity of bioactive films containing Wickerhamomyces anomalus killer yeast and their application for preservation of oranges and control of postharvest green mold caused by Penicillium digitatum. Int J Food Microbiol. 2015;200:22–30. doi:10.1016/j.ijfoodmicro.2015.01.015.

Bauer F, Pretorius I. Yeast stress response and fermentation efficiency: how to survive the making of wine. South African Journal for Enology Viticulture. 2000;21:27.

Cui P, Chen Z, Zhao Q, Yu Z, Yi Z, Liao H, et al. Hyperthermophilic composting significantly decreases N2O emissions by regulating N2O-related functional genes. Bioresour Technol. 2019;272:433–41. doi:10.1016/j.biortech.2018.10.044.

Czarnecka M, Zarowska B, Polomska X, Restuccia C, Cirvilleri G. Role of biocontrol yeasts Debaryomyces hansenii and Wickerhamomyces anomalus in plants' defense mechanisms against Monilinia fructicola in apple fruits. Food Microbiol. 2019;83:1–8. doi:10.1016/j.fm.2019.04.004.

de Souza RS, Okura VK, Armanhi JS, Jorrin B, Lozano N, da Silva MJ, et al. Unlocking the bacterial and fungal communities assemblages of sugarcane microbiome. Sci Rep. 2016;6:28774.

doi:10.1038/srep28774.

Eggleston G. Deterioration of cane juice - sources and indicators. Food Chem. 2002;78(1):95–103. doi:10.1016/s0308-8146(01)00390-9.

Fredlund E, Blank LM, Schnürer J, Sauer U, Passoth V. Oxygen- and Glucose-Dependent Regulation of Central Carbon Metabolism in Pichia anomala. Appl Environ Microbiol. 2004;70(10):5905–11. doi:10.1128/AEM.70.10.5905-5911.2004.

Fredlund E, Druvefors U, Boysen M, Lingsten K-J, Schnürer J. Physiological characteristics of the biocontrol yeast Pichia anomala J121. FEMS yeast research. 2002;2:395–402. doi:10.1016/S1567-1356(02)00098-3.

Goldemberg J, Coelho ST, Guardabassi P. The sustainability of ethanol production from sugarcane. Energy Policy. 2008;36(6):2086–97. doi:10.1016/j.enpol.2008.02.028.

Guerzoni ME, Serrazanetti DI, Vernocchi P, Gianotti A. (2013). *Physiology and Biochemistry of Sourdough Yeasts.* Springer US.

Guo FJ, Ma Y, Xu HM, Wang XH, Chi ZM. A novel killer toxin produced by the marine-derived yeast Wickerhamomyces anomalus YF07b. Antonie Van Leeuwenhoek. 2013;103(4):737–46. doi:10.1007/s10482-012-9855-3.

Hagman A, Säll T, Compagno C, Piskur J. Yeast “Make-Accumulate-Consume” Life Strategy Evolved as a Multi-Step Process That Predates the Whole Genome Duplication. PLOS ONE. 2013;8(7):e68734. doi:10.1371/journal.pone.0068734.

Hansen E, Nissen P, Sommer P, Nielsen JC, Arneborg N. The effect of oxygen on the survival of non-Saccharomyces yeasts during mixed culture fermentation of grape juice with Saccharomyces cerevisiae. J Appl Microbiol. 2001;91:541–7. doi:10.1046/j.1365-2672.2001.01426.x.

Hector S, Willard K, Bauer R, Mulako I, Slabbert E, Kossmann J, et al. Diverse Exopolysaccharide Producing Bacteria Isolated from Milled Sugarcane: Implications for Cane Spoilage and Sucrose Yield. PLoS One. 2015;10(12):e0145487. doi:10.1371/journal.pone.0145487.

Khan MT, Yasmeen S, Botany, I.A.K.J.P.J.o.. (2020). Comparative analysis of sugarcane genotypes for postharvest deterioration under natural conditions. Pak J Bot 52(4): DOI:http://dx.doi.org/10.30848/PJB2020-4(26).

Liebe S, Wibberg D, Winkler A, Puhler A, Schluter A, Varrelmann M. (2016). Taxonomic analysis of the microbial community in stored sugar beets using high-throughput sequencing of different marker genes. FEMS Microbiol Ecol 92(2). doi:10.1093/femsec/fiw004.

Mao L, Que F, Wang G. Sugar metabolism and involvement of enzymes in sugarcane (*Saccharum officinarum* L.) stem during storage. Food Chem. 2006;98(2):338–42. doi:10.1016/j.foodchem.2005.05.076.

Masih EI, Alie I, Paul B. Can the grey mold disease of the grape-vine be controlled by yeast? FEMS Microbiol Lett. 2000;189(2):233–7. doi:10.1111/j.1574-6968.2000.tb09236.x.

Misra V, Mall AK, Pathak AD, Solomon S, Kishor R. Microorganisms affecting Post-Harvest Sucrose Losses in Sugarcane. International Journal of Current Microbiology Applied Sciences. 2017;6(7):2554–66. doi:10.20546/ijcmas.2017.607.361.

Misra V, Mall AK, Shrivastava AK, Solomon S, Shukla SP, Ansari MI. Assessment of Leuconostoc spp. invasion in standing sugarcane with cracks internode. J Environ Biol. 2019;40(3):316–21. doi:10.22438/jeb/40/3/MRN-650.

Oro L, Feliziani E, Ciani M, Romanazzi G, Comitini F. Biocontrol of postharvest brown rot of sweet cherries by Saccharomyces cerevisiae Disva 599, Metschnikowia pulcherrima Disva 267, and Wickerhamomyces anomalus Disva 2 strains. Postharvest Biol Technol. 2014;96:64–8. doi:10.1016/j.postharvbio.2014.05.011.

Oro L, Feliziani E, Ciani M, Romanazzi G, Comitini F. Volatile organic compounds from Wickerhamomyces anomalus, Metschnikowia pulcherrima, and Saccharomyces cerevisiae inhibit the growth of decay-causing fungi and control postharvest diseases of strawberries. Int J Food Microbiol. 2018;265,:18–22. doi:10.1016/j.ijfoodmicro.2017.10.027.

Padilla B, Gil J, Manzanares PJF. Challenges of the Non-Conventional Yeast Wickerhamomyces anomalus in Winemaking. Fermentation. 2018;4:68. doi:10.3390/fermentation4030068.

Saxena PS, RP and Sharma ML. Impact of Cut to Crush Delay and Bio-chemical Changes in Sugarcane. Aust J Crop Sci. 2010;4(9):692–9.

Siedow J, Day D. (2000). Respiration and photorespiration. *Biochemistry and molecular biology of plants*, 676–728.

Singh I, Solomon S, Shrivastava AK, Singh RK, Singh J. Postharvest quality deterioration of cane juice: physio-biochemical indicators. Sugar Tech. 2006;8(2):128–31. doi:10.1007/bf02943646.

Solomon S. Postharvest deterioration of sugarcane. Sugar Tech. 2009;11(2):109–23. doi:10.1007/s12355-009-0018-4.

Solomon S, Singh P, Shrivastava AK, Singh P, Chandra A, Jain R, et al. Physico-Chemical Method of Preserving Sucrose in Harvested Sugarcane at High Ambient Temperature in a Sub-Tropical Climate. Sugar Tech. 2011;13(1):60–7. doi:10.1007/s12355-011-0075-3.

Stevenson JA, Rands RD. (1938). An annotated list of the fungi and bacteria associated with sugarcane and its products. *Hawaiian planters' record* 1938 v.42 no.4(no. 4), pp. 247–240.

Suman A, Solomon S, Yadav DV, Gaur A, Singh M. Postharvest loss in sugarcane quality due to endophytic microorganisms. Sugar Tech. 2000;2(4):21–5. doi:10.1007/BF02995581.

Sun H-Y, Wang K, Chi Z, Xu H-M, Chi Z-M. Simultaneous production of single-cell protein and killer toxin by Wickerhamomyces anomalus HN1-2 isolated from mangrove ecosystem. Process Biochem. 2012;47(2):251–6. doi:10.1016/j.procbio.2011.10.040.

Vasic V, Durdic S, Tosti T, Radoicic A, Lusic D, Milojkovic-Opsenica D, et al. Two aspects of honeydew honey authenticity: Application of advance analytical methods and chemometrics. Food Chem. 2020;305:125457. doi:10.1016/j.foodchem.2019.125457.

Verma AK, Singh SB, Agarwal AK, Solomon S. Influence of postharvest storage temperature, time, and invertase enzyme activity on sucrose and weight loss in sugarcane. Postharvest Biol Technol. 2012;73:14–21. doi:10.1016/j.postharvbio.2012.04.014.

Walker GMJAVL. (2011). Pichia anomala: cell physiology and biotechnology relative to other yeasts. 99(1), 25–34.

Figures

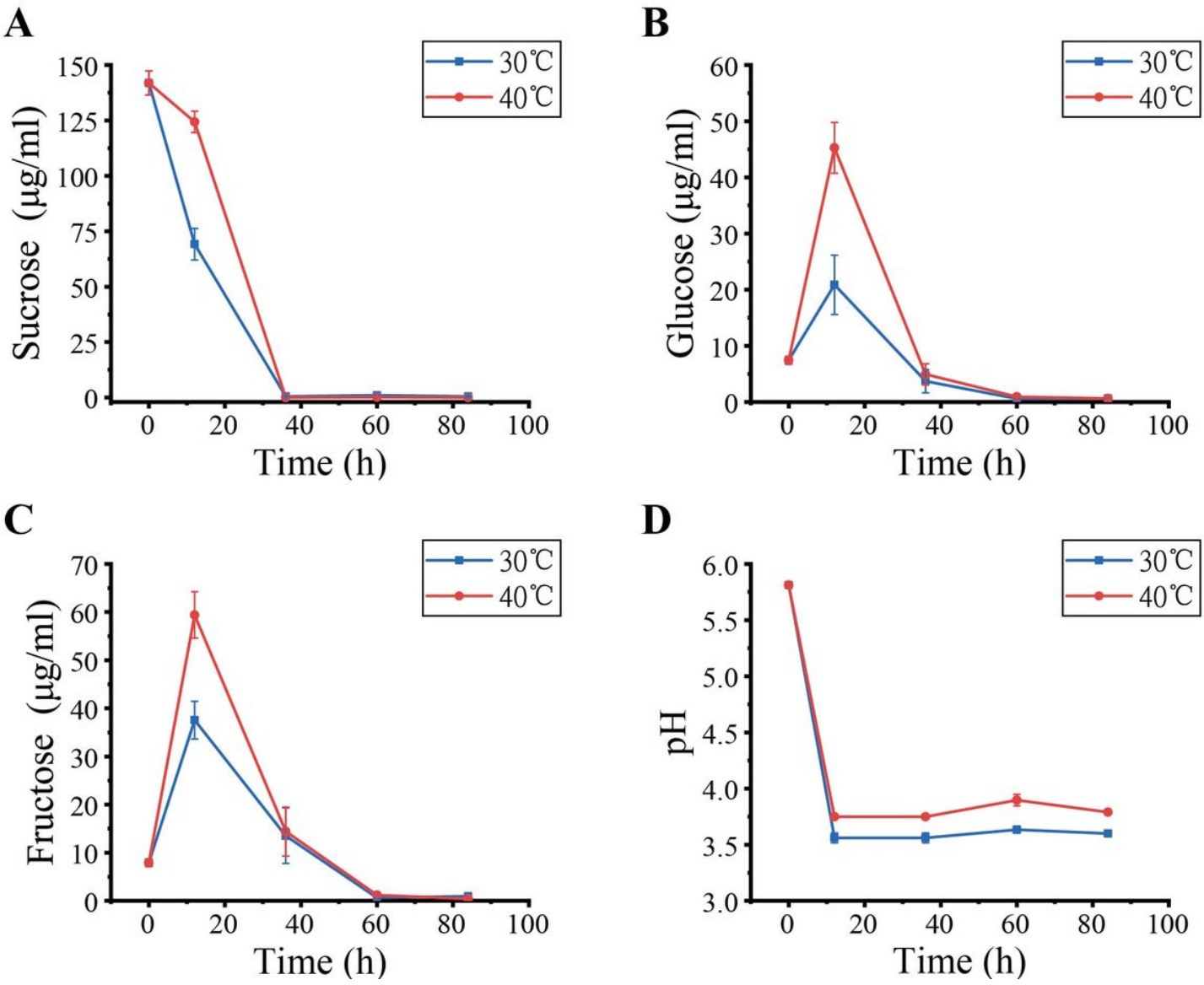

Figure 1

Properties of sugarcane stem samples. Sucrose (A), glucose (B), fructose (C), and pH (D) profiles in the stem powder recorded during the storage period. Data are presented as the mean ± standard deviation.

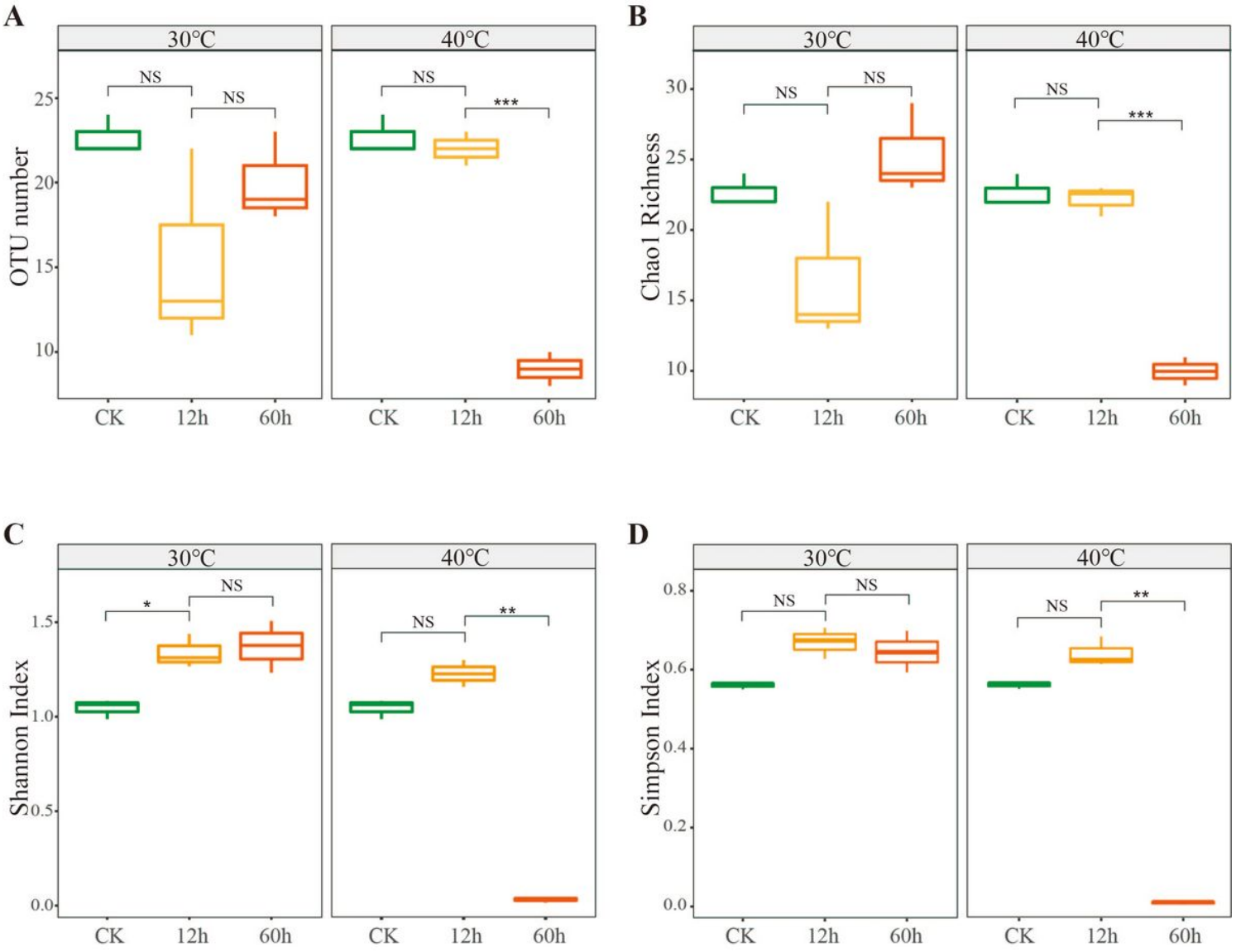

Figure 2

Sequences rarefaction curve and fungal diversity of sugarcane stem samples. Observed OTUs numbers (A), Chao richness index (B), Shannon diversity index (C), and Simpson diversity index (D). Each sample was determined in triplicate (n = 3). Data were shown as average ± SD. * indicates significant correlation at $p < 0.05$, ** indicates significant correlation at $p < 0.01$, and *** indicates significant correlation at $p < 0.001$.

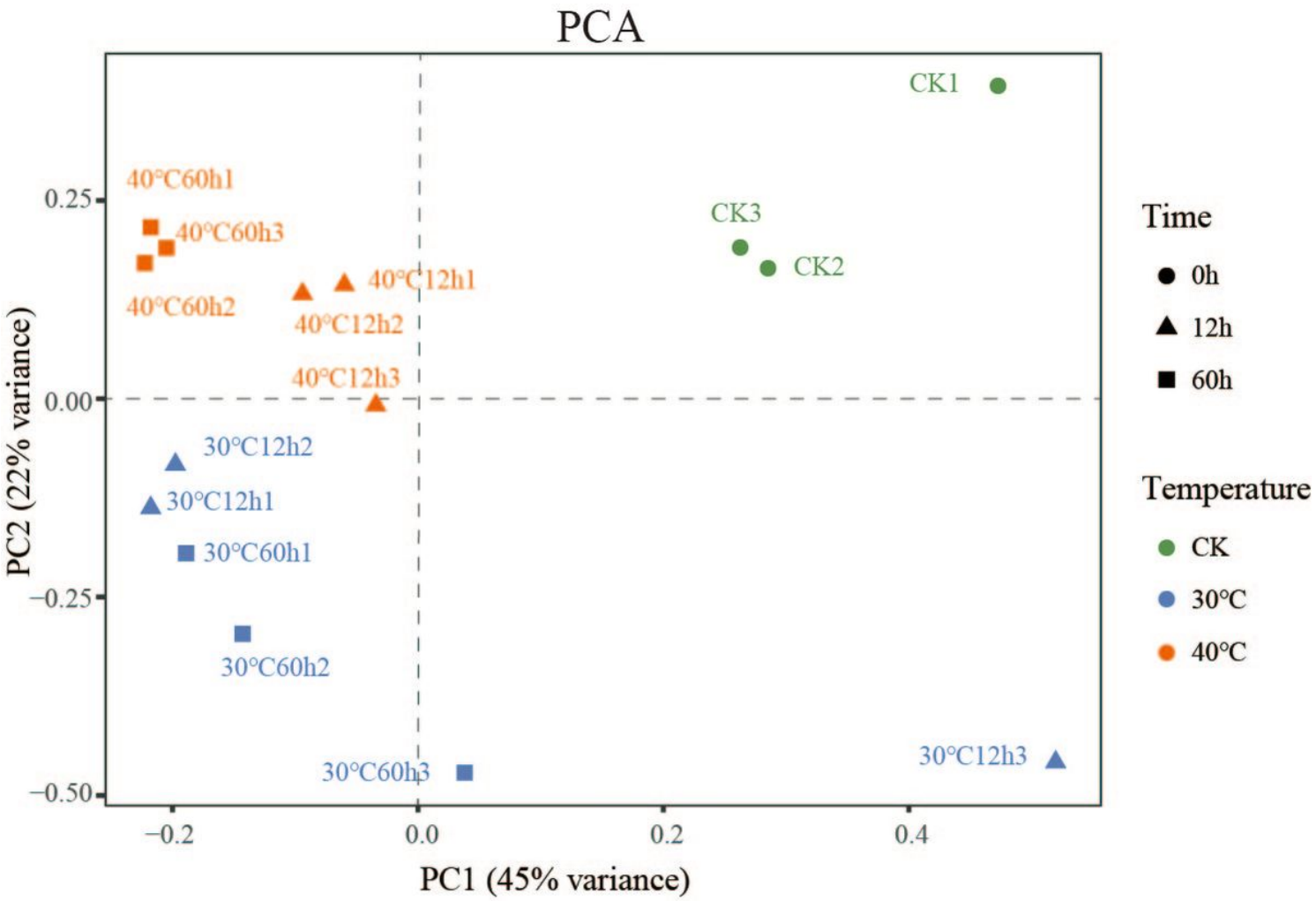

Figure 3

Fungal community structure in sugarcane stalk. The figure presents Principal Coordinate Analysis (PCoA) of ITS2 sequences diversity in the stored stem at the disparate temperatures used in the study. The different colors indicate the different temperatures of the storage and fungal communities from the sugarcane stem.

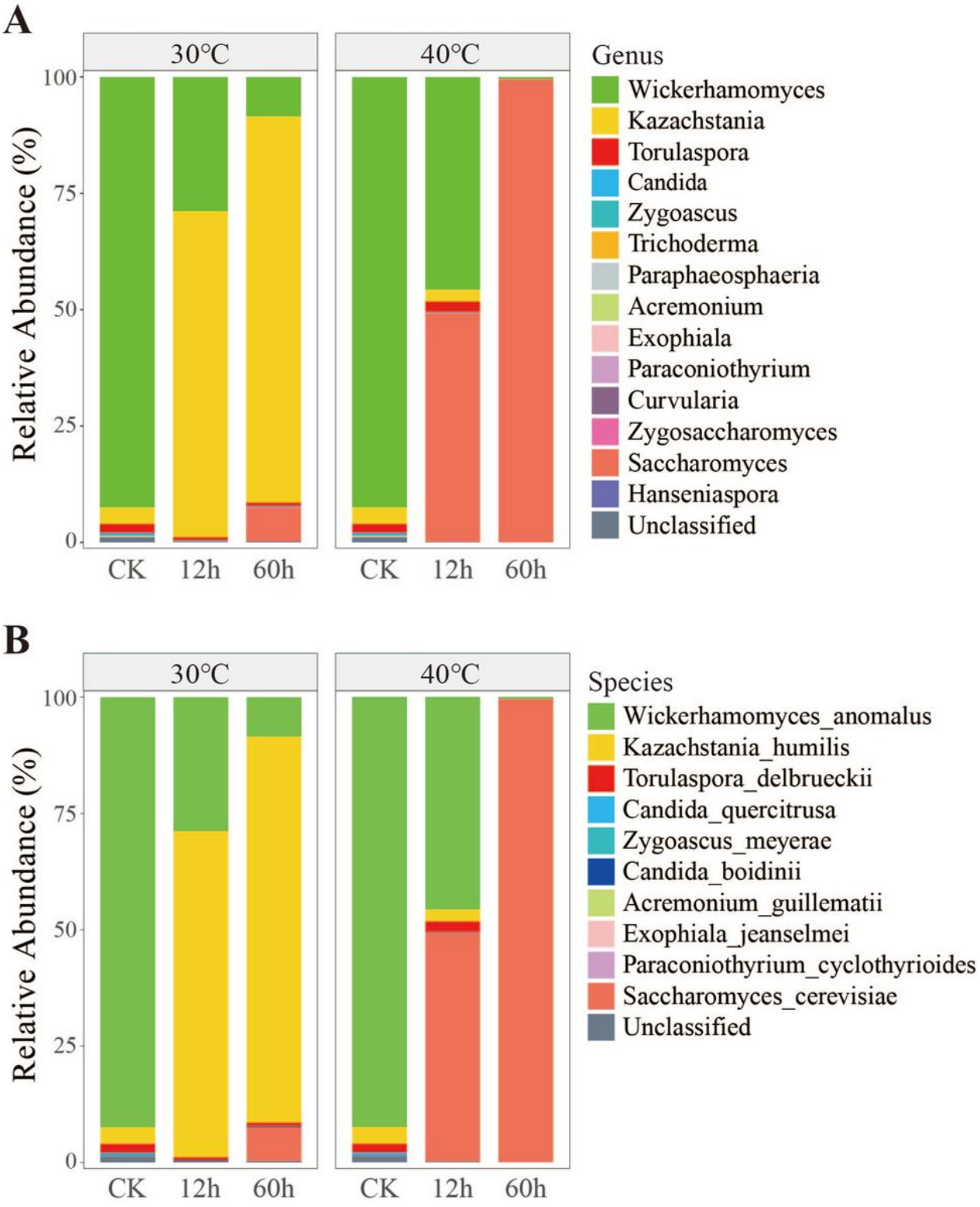

Figure 4

Composition and abundance of microbial communities at the genus (A) and species (B).

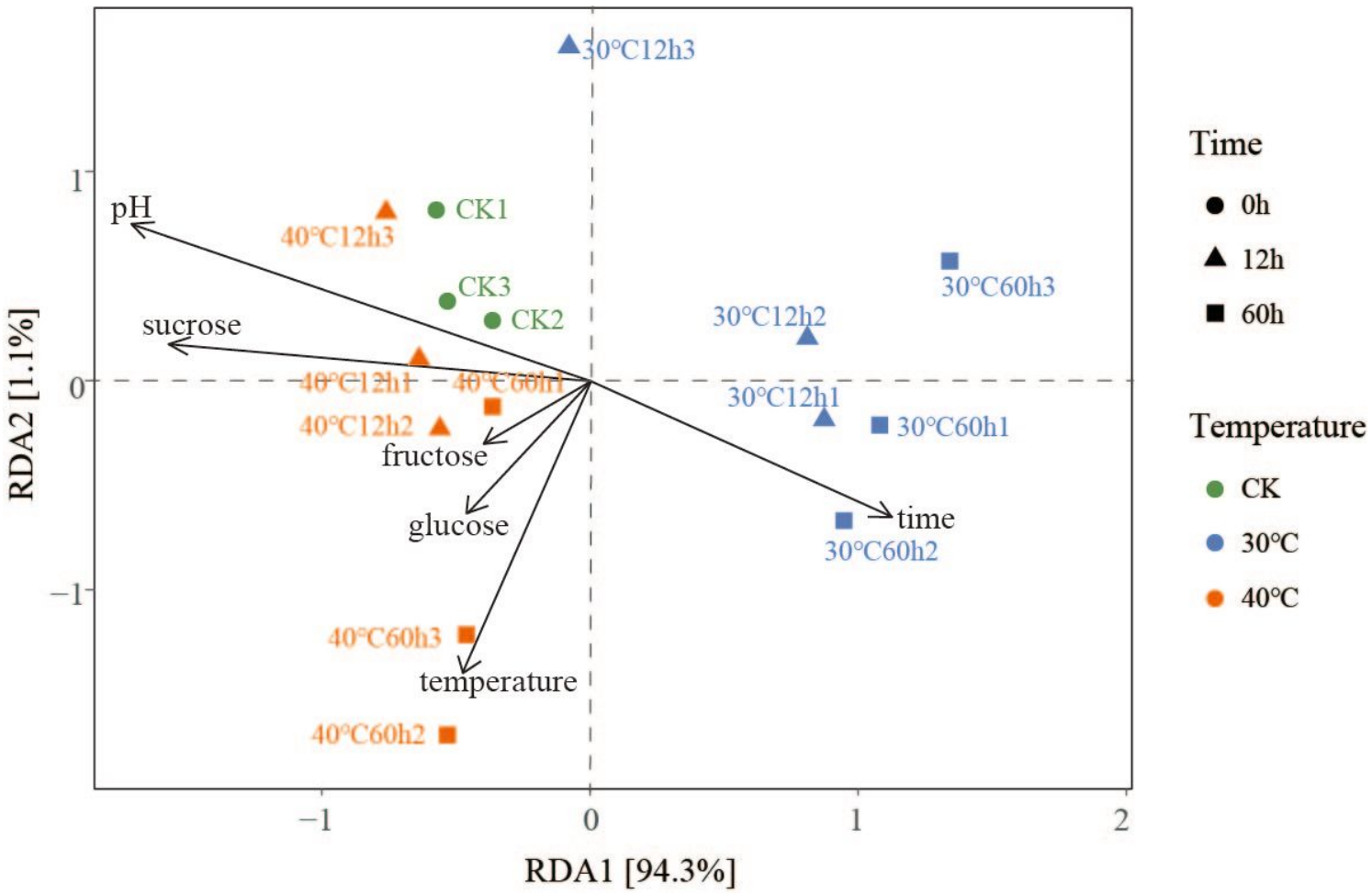

Figure 5

Redundancy analysis (RDA) for the correlation between the fungal community and environmental factors.

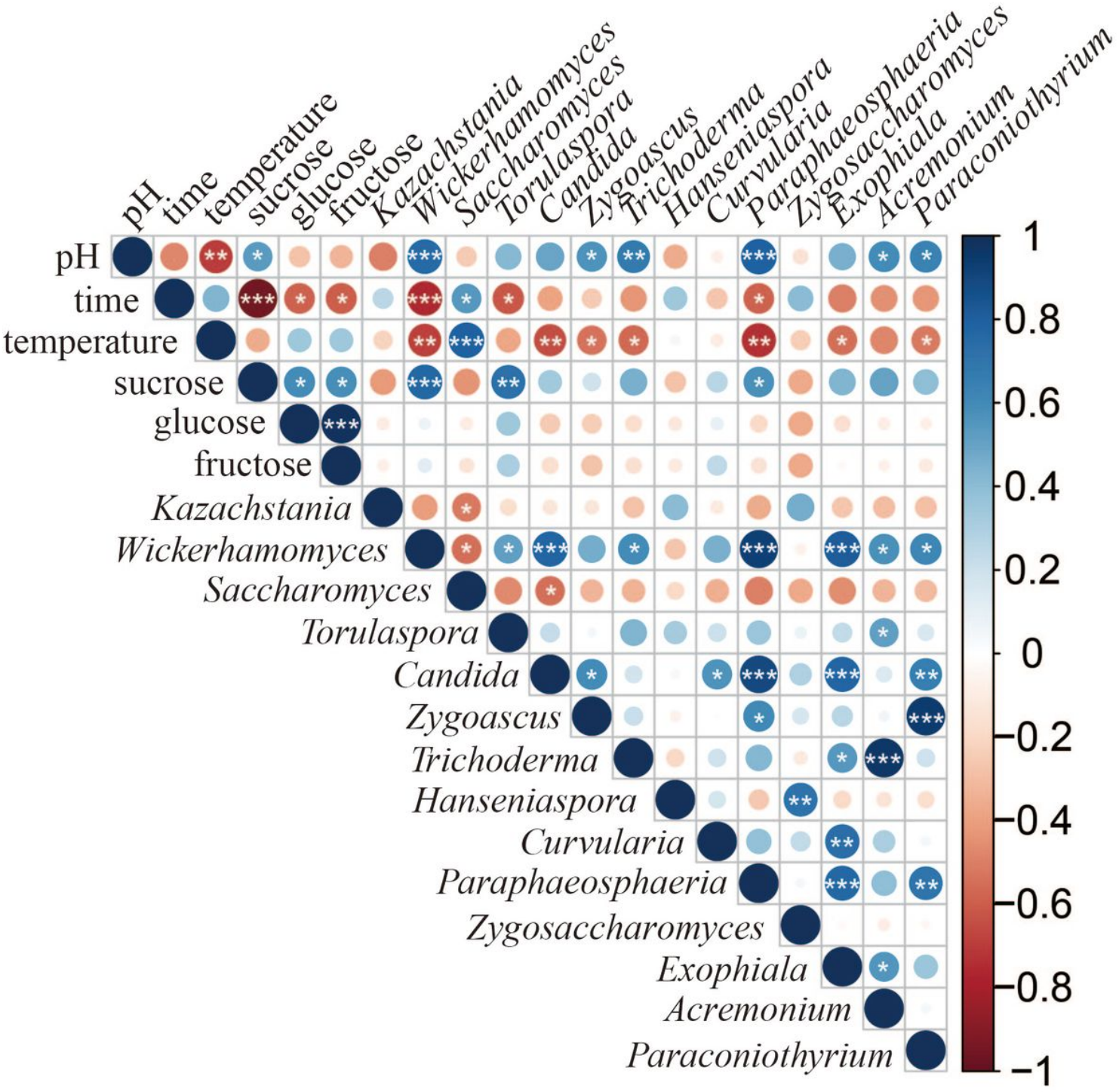

Figure 6

The correlation heatmap of the relationships among the top 14 genera and six selected environmental factors. Minus value of Spearman's correlation index denotes a negative correlation, while a positive value denotes a positive correlation. * indicates significant correlation at $p < 0.05$, ** indicates significant correlation at $p < 0.01$, and *** indicates significantly correlation at $p < 0.001$.

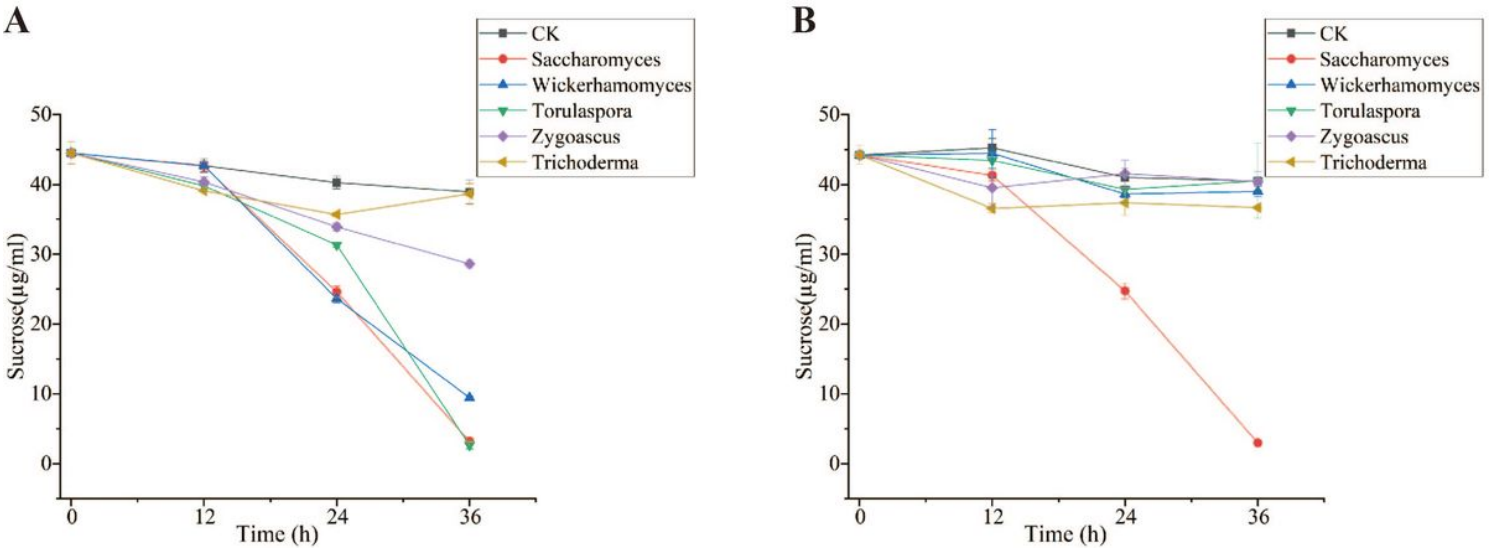

Figure 7

Sucrose profiles in sucrose medium of the isolated strains cultured at 30°C (A) and 40°C (B). Data are presented as the mean ± standard deviation.

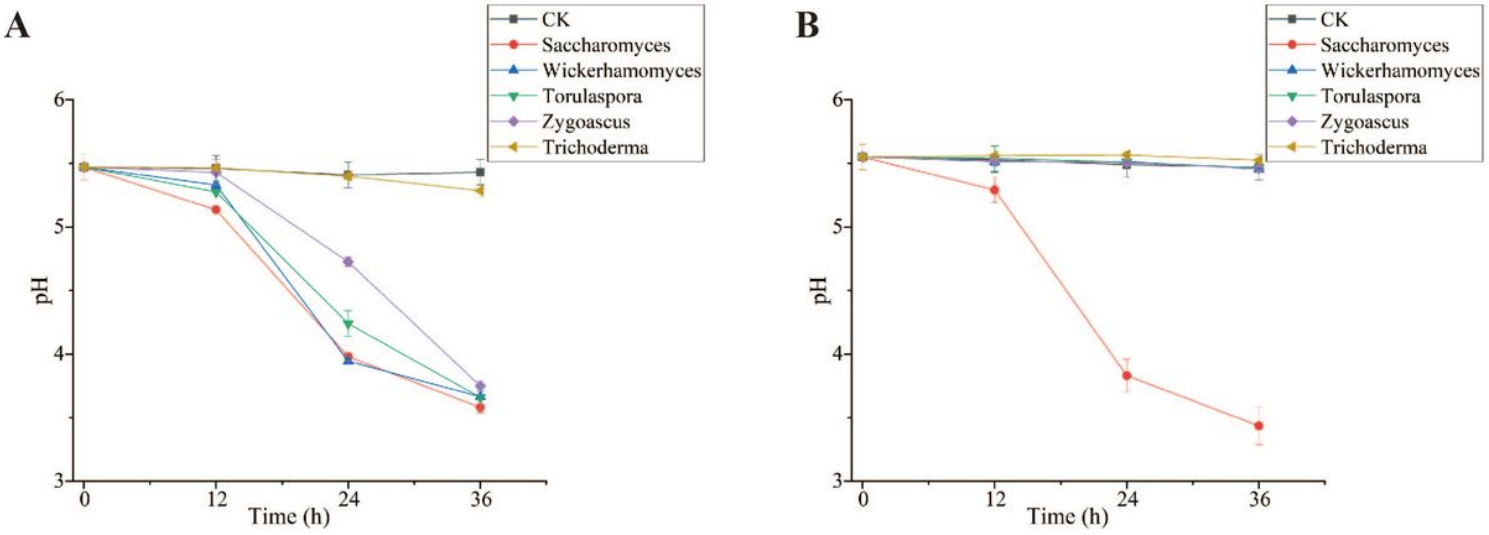

Figure 8

The pH profiles in the sucrose medium of the isolated strains cultured at 30°C (A) and 40°C (B). Data are presented as the mean ± standard deviation.

Supplementary Files

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