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Diversity and pathogenicity of microbial communities causing grape sour rot in eastern coastal areas of China

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Background As a polymicrobial disease, grape sour rot can lead to the decrease in the yield of grape berries and wine quality. The diversity of microbial communities in sour rot-infected grapes depends on the planting location of grapes and the identified methods. The east coast of China is one of the most important grape and wine regions in China and even in the world.

Methods To identify the pathogenic microorganism s causing sour rot in table grapes of eastern coastal areas of China, the diversity and abundance of the bacteria and fungi were assessed based on two methods, including traditional culture-methods, and 16S rRNA and ITS gene high-throughput sequencing . Then the pathogenicity of cultivable microorganisms was determined in laboratory.

Results Based on traditional culture-methods, we identified 15 cultivable bacterial species and 10 fungal species from sour rot-infected grapes. The p athogenicity assay confirmed five cultivated fungi species (three *Aspergillus* species, *Alternaria tenuissima*, and *Fusarium proliferatum*), and four bacteria species (two *Cronobacter* species, *Serratia marcescens* and *Lysinibacillus fusiformis*) as mainly pathogenic on grape. *A. tenuissima*, and *F. proliferatum* were the firstly discovered as pathogens on harvesting grape. Moreover, high-throughput sequencing revealed the OTUs numbers of bacteria and fungi were 1343.33 and 1038.67 respectively. Proteobacteria (72.15%) and Firmicutes (26.83%) were dominant phylums among the 19 bacterial phyla identified, while Ascomycota (93.86%) was the dominant fungal phylum. Then, bacteria such as *Acetobacter* sp., *Gluconobacter* sp., *Bacillus* sp., and *Lactococcus* sp. and fungi such as *Incertae sedis* sp., *Issatchenkia terricola*, *Colletotrichum viniferum*, *Hanseniaspora vineae*, *Saprochaete gigas*, and *Candida diversa* took the vast majority ofmicrobial species in sour rot-infected grapes. Therefore, more accurate and abundant microbial communities in sour rot-infected grapes could be identified using the traditional culture-methods and high-throughput sequencing.

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Abstract

Background

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Introduction

Grape sour rot is a polymicrobial disease characterized by disaggregation of the internal tissues of berries, detachment of the rotten berry from the pedicel, and a strong ethyl acetate smell. It often causes millions of dollars revenue loss per year due to decrease in quality of the berries (Barata et al., 2011; Steel, Blackman & Schmidtke, 2013). A number of microorganisms such as ascomycota yeasts, acetic acid bacteria (AAB), and filamentous fungi, infecting ripe and thin-skinned grape berries (Nally et al., 2013), are often considered as the causes of grape sour rot. However, microorganisms in sour rot-infected grapes depends on the planting location and varieties of grapes.

Studies have analyzed the frequency and density of yeast species associated with sour rot in different wine grape cultivars. The most frequent ascomycetous species recovered from rotten wine grapes include *Candida krusei*, *Kloeckera apiculata*, and *Metschnikowia pulcheryima* and a less frequent species *Issatchenkia occidentalis*

(Guerzpni & Marchetti, 1987). Barata et al. (2008) reported *Candida vanderwaltii*, *Hanseniaspora uvarum*, and *Zygoascus hellenicus* as the most frequent species in rotten grapes (Trincadeira Preta red grape variety). Moreover, the proportion of these microorganisms depends on the ripening stage and the availability of nutrients. Intact grape berries are dominated by basidiomycetous yeasts and the yeast-like fungus *Aureobasidium pullulans*. However, ascomycetes with higher fermentative activity like *Pichia* spp. and *Zygoascus hellenicus*, wine spoilage yeasts, and AAB in rotten grape samples were more than those found in healthy grapes (Barata, Malfeito-Ferreira & Loureiro, 2012a; Barata, Malfeito-Ferreira & Loureiro, 2012b).

Expect for widespread *Hanseniaspora uvarum* in sour rot wine grape and table grape, the non-*saccharomyces* yeast (NSY) and acetic acid bacteria (AAB) could also be identified in sour rot table grape. Pinto et al. (2017) proved that among all NSY-AAB associations, the yeast-bacterium association composed of *Candida zemplinina* CBS 9494and *Acetobacter syzygii* LMG 21419 showed the highest prevalence. However, studies on the microbial diversity of bacteria and fungi in rotten grapes are limited, especially for the eastern coast of China, which is a very important grape growing region in China. Pathogenicity of bacteria and fungi associated with grape sour rot is ambiguous and controversial.

At least 99% of microbes in the natural environment is uncultivable in the laboratory (Kamagata & Tamaki, 2005). With the development of molecular biology techniques

and its application in microbial ecology, metagenomics has facilitated microbial community analysis for the diversity of microorganism. Not relying on our traditional culture methods, this technique excludes the limitations and biases from non-cultivable bacteria in the samples (Andreote, Azevedo & Araújo, 2009). In view of this, a great progress has been gained about the research on microbial diversity in plant disease. For example, Huang et al. (2017) reported the influence of a bioorganic fertilizer, used to control cucumber Fusarium wilt, on the soil microbial community. This study was based on high-throughput sequencing of 16S rRNA, 18S rRNA, and ITS genes. Recent study on bacterial wilt of banana revealed a significant difference in the diversity of microbiota associated with symptomatic and asymptomatic plants using metagenomic16S rRNA sequencing (Shen et al., 2018). A recent metagenomic study revealed that a microbiome consisting predominantly of *Brenneria goodwinii*, *Gibbsiella quercinecans*, and *Rahnella victoriana* caused necrotic lesions in acute oak decline (Brady et al., 2017). Therefore, a combination of traditional culture methods and metagenomic analysis was used in the present study and ~~may analyze provide~~ more precise information for the microbial diversity in sour rot-~~in~~fected grapes.

Yantai, a city of Shandong Province, located in the eastern coastal part of China, is an important grape growing region. The objective of this study was to determine: (1) the diversity and abundance of bacteria and fungi in sour rot-~~in~~fected table grapes collected from Yantai city based on traditional culture methods; (2) the pathogenicity of bacteria

and fungi associated with grape sour rot; and (3) the diversity and abundance of bacteria and fungi in sour rot-infected table grapes based on 16S rRNA and ITS high-throughput sequencing analysis.

Materials & Methods

Sour rot-infected grapes

Sour rot-infected grapes infested with fruit flies were collected from the vineyards in Yantai (N36°27′, E117°10′), Shandong Province, China. Approximately 1.0 g of the fruit tissue was ~~sliced from 100 sour rot-infected grapes~~, and collected together in a 50 ml sterile centrifuge tube. Three replicates were maintained used for separation and identification of bacteria and fungi. Another three replicates were stored at -80℃ for 16S rDNA and ITS high-throughput sequencing.

Diversity of cultivable microorganisms in sour rot-infected grapes

The samples were suspended in phosphate buffered saline (PBS, 0.2 M, pH 7.2) and diluted (10^3, $10^4$and 10^5 times) using PBS. The suspension (200 μL; different concentrations) was inoculated in nutrient agar medium and potato dextrose agar medium with three replicates for each. After culturing for 48h in nutrient broth and for seven days in potato dextrose agar medium in an incubator (25℃inRXZ-328A, Ningbo), single colonies ~~was~~ purified from microorganism ~~medium the using~~ primary medium.

(1) *Identification of cultivated bacteria*

We analyzed the physiological and biochemical characteristics of each bacterium according to the methods by Dong & Cai (2010). We performed Gram staining, spore staining, bacterial motility test, catalase reaction, methyl red test, starch hydrolysis,

benzpyrole test, V-P test, malonic acid test, gelatin test, H_2S test, citrate test, ammonia production test, litmus milk test, and urease test.

We extracted DNA of single colony of each bacterium using the Bacterial DNA Kit (OMEGA, USA) and purified using the DNA Clean-Up Kit (OMEGA, USA). The 16S rDNA was amplified for each DNA template using the instrument of Bio-Rad 1000-Series Thermal Cycler PCR (Bio-Rad, US). The thermal profile is as follows: an initial denaturation at 95°C for 3 min, quantification for 35 cycles (95°C for 15 sec followed by 52°C for 30 sec and 72°C for 1min), and a final extension at 72°C for 5min. Sequences of the primers used are as follows: 16S rDNA-27F: 5'-AGAGT TTGATCCTGGCTCAG-3'; 16S rDNA-1492R: 5'-TACGGYTACCTTGTTACGACTT-3'.

(2) *Identification of cultivated fungi*

We analyzed the morphological features of each fungus using alight microscope (Olympus CX41RF; Olympus Corporation, TOKYO) according to the methods by Wei (1979), and Dai (1988). The mycelium of each purified fungus was collected in PDA medium. DNA was extracted using the Fungal DNA kit (OMEGA, USA) and purified using the DNA Clean-Up Kit (OMEGA, USA). ITS gene was amplified according to the following thermal profile: an initial denaturation at 95°C for 3 min, quantification for 35 cycles (95°C for 15 sec followed by 52°C for 30 sec and 72°C for 1min), and a final extension at 72°C for 5min. Sequences of the universal primer are as follows: ITS1: 5'-TCCGTAGGTGAACCTGCGG-3'; ITS4: 5'-TCCTCCGCTTATTGATATGC -3'.

(3) *Sequencing*

153 PCR products were purified using TaKaRa Mini BEST Agarose Gel DNA Extraction
154 kit (Takara, Japan), cloned into pEASY-T3vector (Takara, Japan), and transformed into
155 *Escherichia coli* JM109 cells. The positive clones were sequenced on anABI-3730 DNA
156 analyzer (Applied Biosystems, USA).The sequences obtained were analyzed using
157 BLAST (http://ncbi.nlm.nih.gov/). Phylogenetic trees of the bacteria and fungi were
158 separately constructed using neighbor-joining method (NJ; Saitou andNei 1987) with
159 MEGA 6.0 software (LynnonBiosoft, USA). The sequences of bacteria and fungi were
160 submitted to GenBank using SEQUIN software, and the accession numbers were
161 included in the phylogenetic trees.

162 **Pathogenicity assay of cultivated bacteria and fungi**

163 Isolated bacteria and fungi were tested for pathogenicity on grape berries. Healthy grape
164 berries of Midknight Beauty, a susceptible variety, were collected and surface sterilized
165 with 1% sodium hypochlorite (NaClO) solution for one minute. Excess NaClO was
166 removed by washing (twice) the berries in sterile distilled water. The experimental
167 berries were pricked 2–3mm deep using a dissecting needle. Bacterial suspension and
168 spore suspension of the cultivable microorganisms were prepared having approximately
169 1×10^6 conidia/ml in the suspension. This suspension (5 µl per berry) was used to
170 inoculate the wound of healthy grape berries. Sterile water was used instead of the
171 suspension that served as a negative control. Subsequently, the inoculated grape berries
172 were kept in a moisture chamber at 27/25℃(day/night) and 80% humidity, and

observations were made at 5th days to record the symptom. The bacterial and fungal species were reisolated from these artificially inoculated grape berries using NA medium and PDA medium, respectively. The culture obtained was compared with the original culture (Jenkins, 1933; Hyun et al., 2001).

Based on the ratio of infected area to total area, grading was done as follows (Rouxel et al., 2013; Zhou et al., 2014): ~~0, No disease spot; 1, less than 5.0% of the total area infected; 3, 5.1% to 25.0% of the total area infected; 5, 25.1% to 50.0% of the total area infected; 7, 50.1% to 75.0% of the total area infected; 9, 75.1% to100.0% of the total area infected.~~

$$\text{The morbidity} = 100 * \frac{\text{the number of diseased berries}}{\text{the number of all berries}} \quad (1)$$

$$\text{The disease index} = 100 * \sum_{k=0}^{n} \frac{k*x}{\text{N}*9} \quad (2)$$

Where, x is the representative value of each grade; n is the number of diseased berries at each level; and N is the total number of fruits investigated.

16S rDNA and ITS high-throughput sequencing analysis

(1) *DNA extraction and Illumina MiSeq sequencing of 16S rRNA and ITS genes*

DNA was extracted from each sample using the insect DNA kit (OMEGA, USA)and further purified using the MoBioPowerSoilkit. DNA (10 ng) was amplified by polymerase chain reaction (PCR) to create a cDNA library of V3+V4 region of 16S rRNA gene. The bacterial universal primers used were 341 F (5’ -CCTAC ACGACGCTCTTCCGATCTN (barcode) CCTACGG-GNGGCWGCAG-3’) and 805 R (5’ -GACTGGAGTTCCTTGGCACCCGAGAATTCCA (barcode) GACTA

194 CHVGGGTATCTAATCC-3'). Similarly, a cDNA library of ITS gene was also created
195 using 10 ng of DNA. The fungal universal primers used were ITS4 F (5' –
196 CCCTACACGACGCTCTTCCGATCTN (barcode) TCCTCCGCTTATTGATATG-3')
197 and ITS3 R (5' - GTGACTGGAGTTCCTTGG
198 CACCCGAGAATTCCAGCATCGATGAAGAACGCAGC -3'). In the primer
199 sequences, the barcode was used to sort the groups in a single run. The cDNA library
200 was sequenced on an Illumina Miseq platform (Hiseq 2000; PE250) (Illumina, USA).
201 After removal of low-quality reads containing primer/adaptor sequences and cleaning
202 the reads using SeqClean, high-quality reads (clean data) were generated that were used
203 for further analysis.
204 (2) *Alpha diversity analysis*
205 Sequences were clustered into operational taxonomic units (OTUs) using the 97%
206 identity threshold (3% dissimilarity level). According to the number of OTUs, Shannon
207 and Simpson diversity index were calculated to indicate the microbial diversity among
208 these OTUs of microorganism, and Chao1 and ACE indices were calculated to indicate
209 the microbial richness using Mothur software. All the OTUs were analyzed using
210 BLASTN and the 16S rDNA database and ITS database (http://ncbi.nlm.nih.gov/). The
211 best results (similarity >90% and coverage>90%) were used for the next classification.
212 The sequences that did not satisfy these criteria were defined as "unclassified". We
213 measured the species richness and relative abundance. The pie graph were used to
214 depict the microbial community structure of microorganism.
215 (3) *Functional Analysis*
216 According to the microbial community structure generated by16S rDNA sequencing,
217 The annotation and composition of the functional genes were speculated based on COG

(clusters of orthologous groups) and KEGG (kyoto encyclopedia of genes and genomes) using PICRUSt software.

Results

Diversity of cultivable microorganisms in sour rot-infected grapes

15 bacterial species were identified from sour rot-infected grapes infested by fruit flies (Table 1). We identified Firmicutes as the dominant phylum (60%) with nine species such as *Staphylococcus saprophyticus*, *Lactococcus garvieae*, *Lactobacillus plantarum*, two *Lysinibacillus* species, and four *Bacillus* species. Six bacterial species of Proteobacteria phylum were also identified. The physiological and biochemical characteristics of bacteria are shown in Table 2. All were gram-positive bacteria and presented positive results in catalase reaction, gelatin test, H_2S test, and ammonia production; however, they were methyl red negative. Moreover, *Cronobacter malonaticus*, *Cronobacter sakazakii*, and *Klebsiella pneumoniae* presented negative results in the biochemical tests.

Among ten cultivable fungi identified from sour rot-infected grapes, five were Deuteromycotina fungi including *Cladosporium oxysporum*, *Alternaria tenuissima*, *Geotrichum gigas*, *Fusarium proliferatum*, and *Nigrospora sp.* (Table 1). The characteristics of fungal colony, hyphae, and spores are shown in Fig. 1. *C. oxysporum* with bottle-green colonies developed into conidia through asexual reproduction. *A. tenuissima* colonies with white front side and brown reverse side developed into conidia in the form of a chain lattice. The hyphae of *Saprochaetegigas* or *Geotrichumgigas* with white colonies developed into arthrospores through asexual reproduction. *F.*

241 *proliferatum* with red colonies had branched conidiophores and sickle or long column-
242 shaped conidia. *Nigrospora sp.* had irregular colonies, branched conidiophores, and
243 ball-shaped conidia. Moreover, five species including *Penicillium citrinum*, *P.*
244 *georgiense*, *Aspergillusniger*, *A. aculeatus*, *A. oryzae*, belonged to Ascomycotina. The
245 sporophores of *P. citrinum* and *P. georgiense* grew from hyphae and developed into
246 brush-like structures. However, these two *Penicillium* species differed in colony color.
247 The conidia of *A. niger*, *A. aculeatus*, and *A. oryzae* were black, green, and yellow,
248 respectively.
249 These 15 bacterial species and 10 fungal species were identical with the species in
250 NCBI (97-100% identity). The phylogenetic trees of the bacteria and fungi are shown in
251 Fig. 2, and their GenBank accession numbers are shown in Table 1.

252 **Pathogenicity of cultivable bacteria and fungi for grape sour rot**
253 Each Bacterial and fungal suspensions was inoculated on healthy grape berries of the
254 susceptible variety Midknight Beauty. All the 15 bacterial species and 10 fungal species
255 demonstrated pathogenicity in grapes with different degrees of damage (Fig. 3). Most of
256 the microorganisms caused ~~cracking in grapes~~ except for *B. amyloliquefaciens*, which
257 was similar to the sour rot symptom in the field. The bacterial species and the fungal
258 species reisolated from these diseased grapes using NA medium and PDA medium were
259 confirmed as the original microorganisms in Table 1.
260 The morbidity and disease index of 15 bacterial species and 10 fungal species were
261 significantly different from the control such as, sterile water and LB medium
262 (Morbidity: $F=10.439$, $P<0.01$; disease index: $F=43.277$, $P<0.01$; Fig. 4). Fungal
263 isolates demonstrated stronger pathogenicity in the grape berries with a morbidity of

more than 75%. Except for *C. oxysporum* and *P. citrinum*, the disease index of all other fungi was more than fifty, which was more than that of bacteria. Three *Aspergillus* species and *P. georgiense* with a morbidity of 100% recorded the highest disease index. Healthy grapes were also highly sensitive to *A. tenuissima* and *F. proliferatum*, which had the high disease index with 51.57±0.57 and 49.57±2.15, respectively. Among the bacteria, the morbidity and disease index of two *Cronobacter species*, *Serratia marcescens* and *Lysinibacillus fusiformis* was higher than the other bacteria. *B. amyloliquefaciens* and *B. cereus* led to less serious sour rot than other bacteria (Fig. 3, then Fig. 4).

Sequencing and alpha diversity analysis

We performed Illumina MiSeq sequencing of 16S rRNA and ITS genes of sour rot-infected grapes (Table 3). The information of raw data and clean sequences of 16S rDNA of bacteria were more than that of the ITS genes of fungi. However, the mean length of ITS gene of fungi was 279.37 ± 4.52bp, which was shorter than that of 16S rDNA of bacteria (412.86 ± 2.76bp). The OTUs numbers of bacteria and fungi were 1343.33±282.94 and 1038.67±386.36 respectively (Table 3).

Phylogenetic trees were constructed for the top 50 OTUs of bacteria and fungi according to the number of sequences (Fig. 5, then Fig. 6). For bacteria, 15 OTUs were classified in Firmicutes phylum, Bacilli class. Among other 35 Proteobacteria OTUs, 21 belonged to Alphaproteobacteria class, three to Betaproteobacteria class, and 11 to Gammaproteobacteria class. In the top 50 fungal OTUs, one belonged to Basidiomycota phylum and eight were not identified in the ITS database. Among other 41 Ascomycota

OTUs, 29 belonged to Saccharomycetes class, nine to Sordariomycetes class, and two to Dothideomycetes class.

The diversity indices of OTUs of bacteria and fungi are shown in Table 3. The microbial diversity and richness were higher for bacteria.

Microbial taxonomy analysis

The bacterial community structure(phylum and genus) in sour rot-infected grapes is shown in Fig. 7. Proteobacteria (72.15%) and Firmicutes (26.83%) were dominant among the 19 phyla identified (Fig. 7A). The proportion of other bacteria was less than 1.00%. The dominant genera in sour rot-infected grapes were *Acetobacter* (37.62%), *Gluconobacter* (23.64%), *Bacillus* (12.38%), and *Lactococcus* (Fig. 7B).

The fungal community structure (phylum and species) in sour rot-infected grapes is shown in Fig. 8. Ascomycota (93.86%) was the dominant phylum identified (Fig. 8A). The dominant species identified in sour rot-infected grapes were *Incertaesedis* sp. (32.40%), *Issatchenkia terricola* (17.57%), *Colletotrichum viniferum* (13.43%), *Hanseniaspora vineae* (13.40%), *Saprochaete gigas* (4.44%), and *Candida diversa* (3.94%) (Fig. 8B).

The COG function of bacteria OTUs in sour rot-infected grapes is shown in Fig. 9A. In the COG functional classification, seven categories were dominant except for “the general function prediction only” (1137145) such as “function unknown” (894042), “amino acid transport and metabolism” (882372),“cell wall/membrane/envelope biogenetic” (729731), “transcription” (694607), “carbohydrate transport and metabolism” (645873), and “energy production and conversion” (634499). Moreover, “transport and metabolism of inorganic ion, coenzyme and lipid” (1301247)and “secondary metabolites biosynthesis, transport, and catabolism” (217135) were the

310 important functions of bacteria in sour rot-infected grapes. In the KEGG functional
311 classification (Fig. 9B), four main categories were dominant such as “amino acid
312 metabolism” (1179865), “carbohydrate metabolism” (1177863), “membrane transport”
313 (1176255), and “replication and repair” (935634).
314

315 ## Discussion

316 As a serious and polymicrobial disease in grapes during the ripening stage, yeasts and
317 acetic acid bacteria (AAB) are usually recognized as the pathogens causing sour rot. For
318 example, AAB such as *Acetobacter leaniensis*, *A. syzygii*, *A. malorum*,
319 *Gluconacetobacter hansenii*, and *G. intermedius* were recovered from sour rot-
320 infected grapes (Barata, Malfeito-Ferreira & Loureiro, 2012b). Mateo et al. (2014)
321 identified AAB including four species of *Gluconobacter* genus, two of *Asaia*, and one
322 of *Acetobacter* from rot-affected grapes collected from three vineyards of Adelaide
323 Hills (South Australia) through molecular typing and identification methods. In the
324 present study, *Acetobacter* sp. was also recovered from sour rot-infected grapes.
325 Moreover, 14 other cultivable species of bacteria were identified in the samples
326 including nine species from Firmicutes and six species from Proteobacteria. However,
327 the microbial taxonomy analysis by high-throughput sequencing revealed that the
328 Proteobacteria phylum was predominant. AAB such as *Acetobacter* sp. (37.62%) and
329 *Gluconobacter* sp. (23.64%) alone constituted 61.26% of the bacteria which is
330 consistent with previous studies.
331 Barata, Malfeito-Ferreira & Loureiro (2012b) also recovered yeast species such as
332 *Issatchenkia occidentalis*, *Zygoascus hellenicus*, *Zygosacchar omycesbailii* from

sour rot-infected grapes[7]. However, the fungi excluding yeasts could also contribute the sour rot disease of grapes. For instance, ~~*Aspergillus*~~ species were predominantly isolated from sour rot-infected table grapes and wine-producing grape cultivars. *A. niger* and *A. carbonarius* were firstly recovered from the grapes on the island of Rhodes, Greece (Tjamos et al., 2004). *A. carbonarius* could causing sour rot of table grapes (*Vitisvinifera*) in California (Rooney-Latham et al., 2008). In present study, we also identified and cultured three *Aspergillus* fungi (*A. niger*, *A. aculeatus*, and *A. oryzae*) in eastern coastal areas of China. Moreover, *Issatchenkia occidentalis*, *Hanseniaspor auvarum*, and *Candida vanderwaltii* were recovered from damaged grapes (Trincadeira Preta red grape variety) (Guerzoni & Marchetti, 1987; Barata et al. 2008). According to the microbial taxonomy analysis by high-throughput sequencing in present study, 34.91% of the fungi were *Issatchenkia terricola*, *Hanseniaspora vineae*, and *Candida diversa*. However, *Incertaesedis* sp. (the new species), *Colletotrichum viniferum*, and *Saprochaete gigas* with a total proportion of 50.27% were still dominant in sour rot-infected grapes. Although the cultivated bacteria and fungi did not identify the overall microorganism, it was necessary to analyze the pathogenicity of the identified microbes to determine the pathogenic species of sour rot-infected grapes. Fungal isolates demonstrated greater pathogenicity in the grape berries. Except for three *Aspergillus* species with the higher disease index, healthy grapes were also sensitive to the pathogenic fungi (*A. tenuissima* and *F. proliferatum*) of common grape diseases. As the most common species in the cosmopolitan genus *Alternaria*, *A.tenuissima* was found on a broad range of fruit products and caused diseases like post harvest black rot of fruit (Logrieco, Moretti & Solfrizzo, 2009). Bakshi, Sztejnberg & Yarden (2001) reported that *F. proliferatum* could also cause the rot of corn, rice, and lily. Therefore,

Aspergillus species, *A. tenuissima* and *F. proliferatum* were the main cultivated pathogenic fungi causing sour rot of grapes. Moreover, among the bacterial isolates, *B. amyloliquefaciens* and *B. cereus* led to the less serious sour rot in this study. It is possibly due to the antibacterial active substances generated by *B. amyloliquefaciens* and *B. cereus*, which have been used as biological agents (Risoen, Ronning & Hegna, 2004, Wang et al., 2014). Although *L. fusiformis* restricts biofilm formation of some pathogenic bacteria, it caused the serious rot of grape berries (Fig. 3). Moreover, healthy grapes were sensitive to *Cronobacter* sp. and *S. marcescens*, which are opportunistic pathogens (Healy et al., 2010). Therefore, the pathogenicity assay confirmed *Cronobacter* species, *S. marcescens*, and *L. fusiformis* as the main cultivated pathogenic bacteria causing sour rot of grapes.

It's worth mentioning that insects lay eggs on the grape berries and spread the disease to other healthy grape berries. Insects involved in this process include ~~*Drosophila melanogaster* and *D. suzukii*,~~ which survive feeding yeasts and other microorganisms (Barata et al., 2012c). The disease increases grape attractiveness to ovipositing *D. melanogaster* females and oviposition by *D. suzukii* facilitates sour rot development (Rombaut et al., 2017; Ioriatti et al., 2018). Additionally, as a relevant player in the sour rot microbial ecology of vineyards, the paper wasp *Polistes dominula*, could facilitate sour rot diseases though increasing host susceptibility and transmitting these microbial communities to healthy grapes (Madden et al., 2017). Due to the multiple species associated with sour rot in grapes including bacteria, fungi, and insects, the control of the disease is difficult. Calvo-Garrido et al. (2013) revealed that Candida sake CPA-1

379 strain isolated from the surface of apples was an effective control agent against grape
380 bunch rot. Phyllosphere yeast *Aureobasidium pullulans* Y-1 isolated from the leaves of
381 vine (*Vitis vinifera* L.) was also effective to control *Aspergillus carbonarius* present on
382 sour rot-infected berries at harvest (Dimakopoulou et al., 2008). In the terminal storage
383 stage, cold ozonation used as an effective approach to control the development of
384 pathogenic bacteria such as *Acetobacter syzygii* and *Candida zemplinina* extended the
385 shelf-life of table grapes (Pinto *et al*. 2017). To conclude, identification and analysis of
386 pathogenic bacteria and fungi is necessary for the prevention and treatment of plant
387 diseases.
388

389 ## Conclusions

390 This study identified more ~~pathogenic~~ species in sour rot-infected grapes of China using
391 the traditional culture-methods combined with high-throughput sequencing, which
392 would provide comprehensive information on targets for the control of the disease. *A.*
393 *tenuissima*, and *F. proliferatum* were the firstly discovered as pathogens on harvesting
394 grape. We need to continue to find the effective prevention and control method for the
395 new pathogenic bacteria found in this study. However, the insects, such as *D.*
396 *melanogaster*, *D. suzukii* females and paper wasp, all could facilitate sour rot
397 development. More comprehensive analysis of nosogenesis based on the research of
398 relationship among insects, microorganism and grapes in our future study.

399

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403

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517

518 **Figure legends**

519 Figure 1 Colony morphology and the light mophology of the fungi in sour rot-infected
520 grapes. A: Colony morphology; *r* indicates the reverse side of colony; *f* indicates
521 the front side of colony; B: Light morphology of the fungi in sour rot-infected
522 grapes

523 Figure 2 The phylogenetic trees of bacteria (A) and fungi (B) in sour rot-infected grapes.
524 Phylogenetic trees were constructed using neighbor-joining method(NJ) with Mega
525 6.0 software

526 Figure 3 The pathogenicity of fungi in healthy grape berries.

527 Figure 4 The morbidity (A) and disease index (B)of 15 bacterial species and 10 fungal
528 species. Different letters in each figure (A and B) indicate significant difference
529 between adults and larvae (One-way ANOVA; $\alpha = 0.05$).

530 Figure 5 The first 50 OTUs of the bacteria by high-throughput sequencing

531 Figure 6 The first 50 OTUs of the fungi by high-throughput sequencing

532 Figure 7 The bacterial community structure based on phylum (A) and genus (B) in sour
533 rot-infected grapes based on 16S rDNA high-throughput sequencing

534 Figure 8 The fungal community structure based on phylum (A) and genus (B) in sour
535 rot-infected grapes based on ITS high-throughput sequencing

536 Figure 9 The COG (A) and KEGG (B) functional categories of bacterial OTUs in sour
537 rot-infected grapes.

538

Table 1 The cultivable microorganism in the sour rotted grapes

Microognism	Phylum	Species	Accession numbers
Bacterium	Proteobacteria	*Cronobacter malonaticus*	MK743990
		Cronobacter sakazakii	MK743989
		Klebsiella pneumoniae	MK743987
		Acetobacter sp.	MK743980
		Serratia marcescens	MK743984
		Enterobacter hormaechei	MK743988
	Firmicutes	*Staphylococcus saprophyticus*	MK743982
		Lactococcus garvieae	MK743983
		Lactobacillus plantarum	MK743986
		Lysinibacillus fusiformis	MK753026
		Lysinibacillus sp.	MK743985
		Bacillus amyloliquefaciens	MK743994
		Bacillus cereus	MK743993
		Bacillus sp.-1	MK743992
		Bacillus sp.-2	MK743991
Fungus	Deuteromycotina	*Cladosporium oxysporum*	MK748311
		Alternaria tenuissima	MK748314
		Saprochaete gigas or Geotrichum gigas	
		Fusarium proliferatum	MK748309
		Nigrospora sp.	MK748317
	Ascomycotina	*Penicillium citrinum*	MK748316
		Penicillium georgiense	MK748315
		Aspergillus niger	MK748313
		Aspergillus oryzae	MK748312
		Aspergillus aculeatus	MK748310

539

Table 2 The physiological and biochemical characteristic of bacterium in sour rotted grape

Bacterium	Gram staining	Spore staining	Bacterial motility	Catalase reaction	Methyl red test	Starch hydrolysis test	Benzpyrole test	V-P test
Cronobacter malonaticus	-		-	+	-	-	-	+
Cronobacter sakazakii	-		-	+	-	-	-	+
Klebsiella pneumoniae	-		-	+	-	-	-	+
Acetobacter sp.	-		+	+	-	-	-	+
Serratia marcescens	-		+	+	-	+	-	+
Enterobacter hormaechei	-		+	+	-	+	-	+
Staphylococcus saprophyticus	+		+	+	-	+	-	+
Lactococcus garvieae	+		-	+	-	+	-	+
Lactobacillus plantarum	+		-	+	-	+	-	+
Lysinibacillus fusiformis	+	purple	+	+	-	+	-	-
Lysinibacillus sp.	+	purple	+	+	-	+	-	+
Bacillus amyloliquefaciens	+	pink	+	+	-	+	-	-
Bacillus cereus	+	purple	+	+	-	+	-	+
Bacillus sp.-1		purple	+	+	-	+	-	+
Bacillus sp.-2	+	purple	+	+	-	+	-	+

Bacterium	Malonic acid test	Gelatin test	H_2S test	Citrate test	Ammonia production test	Litmus milk test	Urease test
Cronobacter malonaticus	-	+	+	+	+	+	-
Cronobacter sakazakii	+	+	+	-	+	+	-
Klebsiella pneumoniae	+	+	+	+	+	-	+
Acetobacter sp.	+	+	+	+	+	+	+
Serratia marcescens	+	+	+	-	+	+	-

Enterobacter hormaechei	+	+	+	+	+	+	-
Staphylococcus saprophyticus	-	+	+	+	+	+	-
Lactococcus garvieae	-	+	+	-	+	+	-
Lactobacillus plantarum	+	+	+	+	+	+	-
Lysinibacillus fusiformis	+	+	+	-	+	-	-
Lysinibacillus sp.	+	+	+	+	+	-	-
Bacillus amyloliquefaciens	-	+	+	+	+	+	+
Bacillus cereus	-	+	+	+	+	+	-
Bacillus sp.-1	+	+	+	+	+	+	+
Bacillus sp.-2	-	+	+	+	+	+	+

1

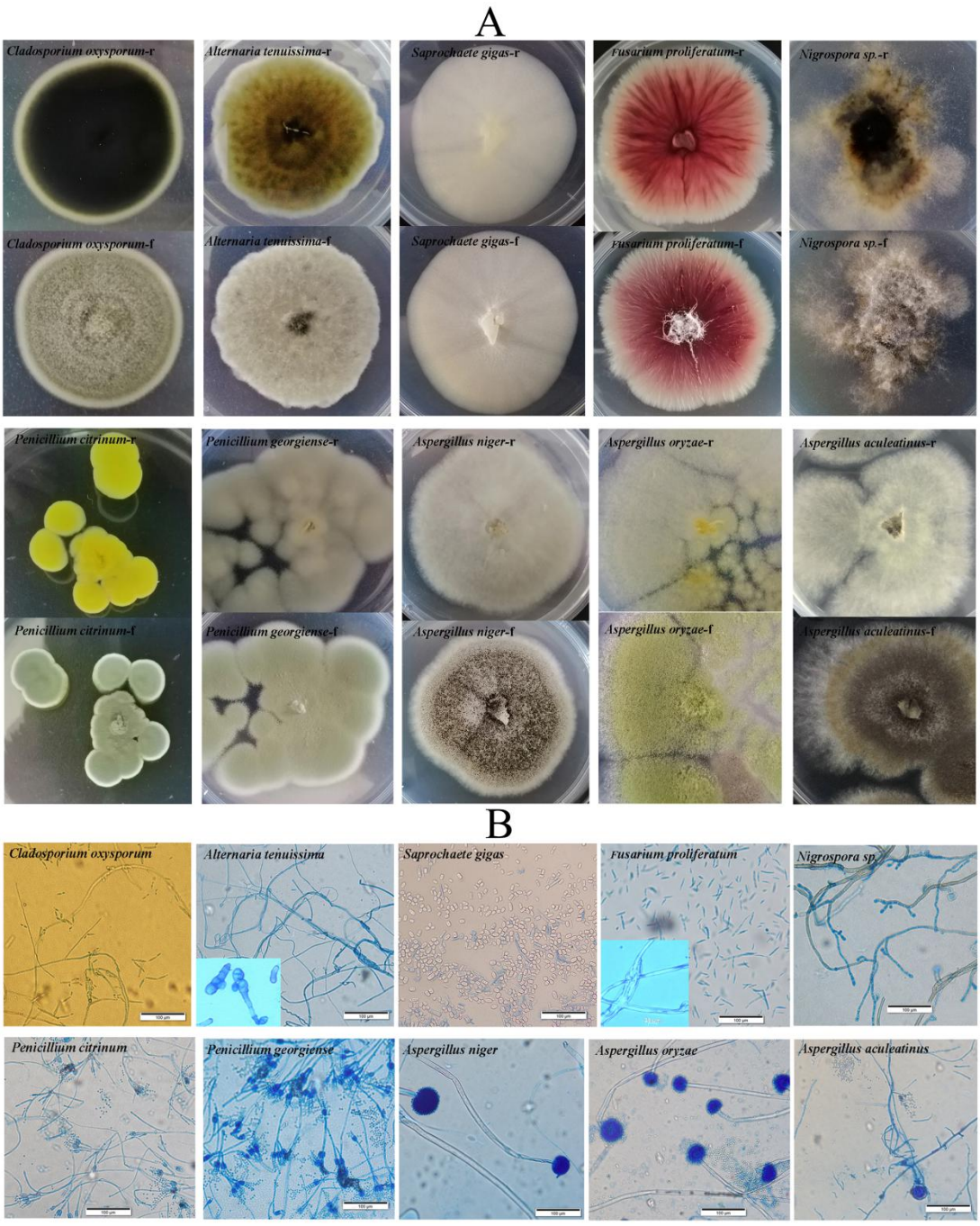
Table 3 Sequence information and OTUs diversity, number of bacterium and fungi

Parameters	Parameters	Bacterium	Fungi
Sequence information	Raw number	57503.67±3213.31	76211±2711.94
	Mean length (bp)	451.07±3.32	322.36±4.66
	Clean number	55895.33±3088.35	76113.67±2703.97
	Mean length (bp)	412.86±2.76	279.37±4.52
Diversity indices	Shannon	3.26±0.13	2.20±0.17
	ACE	22033.53±2927.20	32667.47±1384.78
	Chao1	9744.84±1429.46	10778.66±1475.59
	Simpson	0.10±0.02	0.21±0.04
OTUs number		1343.33±282.94	1038.67±386.36

2

3

4 Fig.1

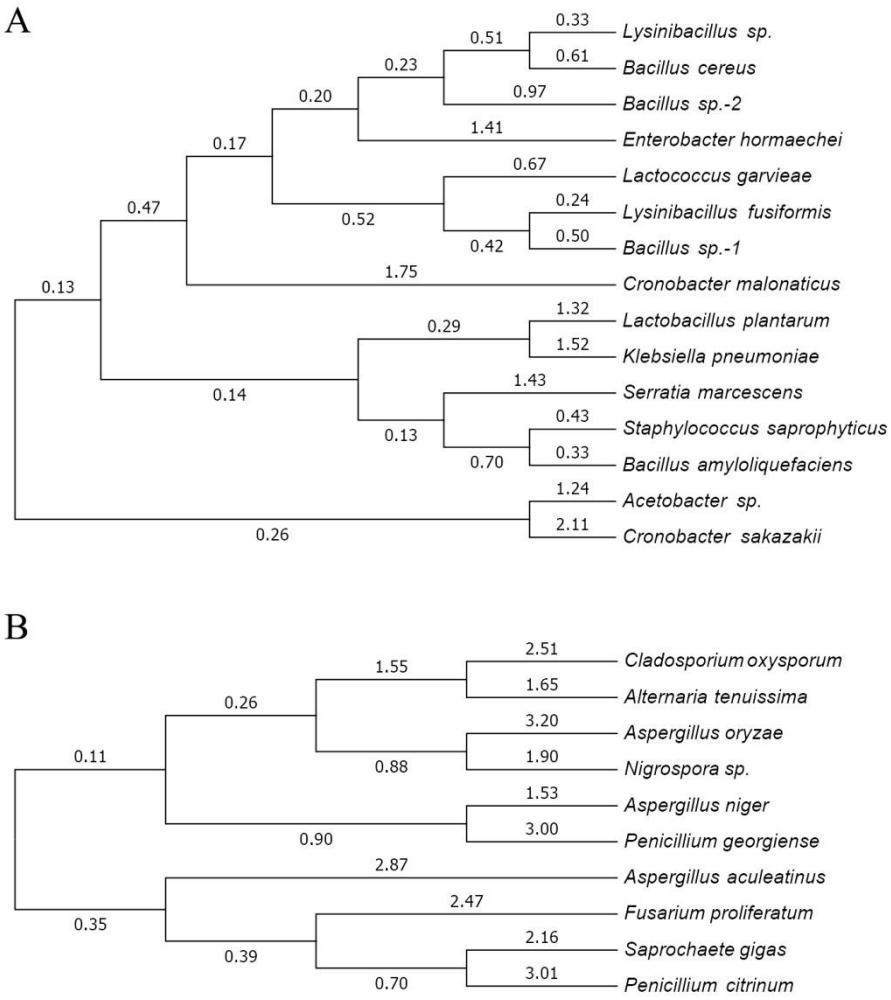

A
Cladosporium oxysporum-r
Alternaria tenuissima-r
Saprochaete gigas-r
Fusarium proliferatum-r
Nigrospora sp.-r
Cladosporium oxysporum-f
Alternaria tenuissima-f
Saprochaete gigas-f
Fusarium proliferatum-f
Nigrospora sp.-f
Penicillium citrinum-r
Penicillium georgiense-r
Aspergillus niger-r
Aspergillus oryzae-r
Aspergillus aculeatinus-r
Penicillium citrinum-f
Penicillium georgiense-f
Aspergillus niger-f
Aspergillus oryzae-f
Aspergillus aculeatinus-f
B
Cladosporium oxysporum
Alternaria tenuissima
Saprochaete gigas
Fusarium proliferatum
Nigrospora sp.
Penicillium citrinum
Penicillium georgiense
Aspergillus niger
Aspergillus oryzae
Aspergillus aculeatinus

5

6

7 Fig.2

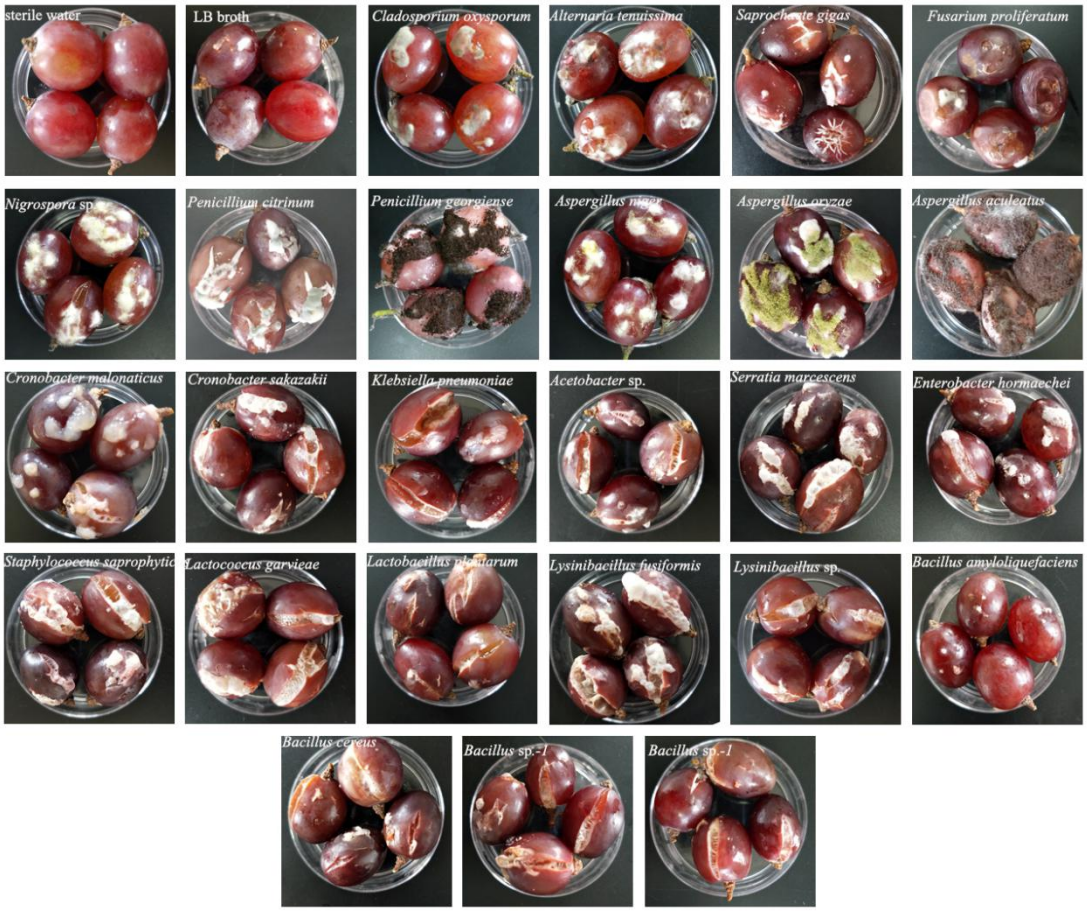
8


A
0.33
0.51
0.61
0.23
0.97
0.20
1.41
0.17
0.67
0.47
0.24
0.52
0.50
0.42
1.75
0.13
1.32
0.29
1.52
1.43
0.14
0.43
0.13
0.33
0.70
1.24
2.11
0.26
Lysinibacillus sp.
Bacillus cereus
Bacillus sp.-2
Enterobacter hormaechei
Lactococcus garvieae
Lysinibacillus fusiformis
Bacillus sp.-1
Cronobacter malonaticus
Lactobacillus plantarum
Klebsiella pneumoniae
Serratia marcescens
Staphylococcus saprophyticus
Bacillus amyloliquefaciens
Acetobacter sp.
Cronobacter sakazakii
B
2.51
1.55
1.65
0.26
3.20
0.11
1.90
0.88
1.53
3.00
0.90
2.87
2.47
0.35
2.16
0.39
3.01
0.70
Cladosporium oxysporum
Alternaria tenuissima
Aspergillus oryzae
Nigrospora sp.
Aspergillus niger
Penicillium georgiense
Aspergillus aculeatinus
Fusarium proliferatum
Saprochaete gigas
Penicillium citrinum

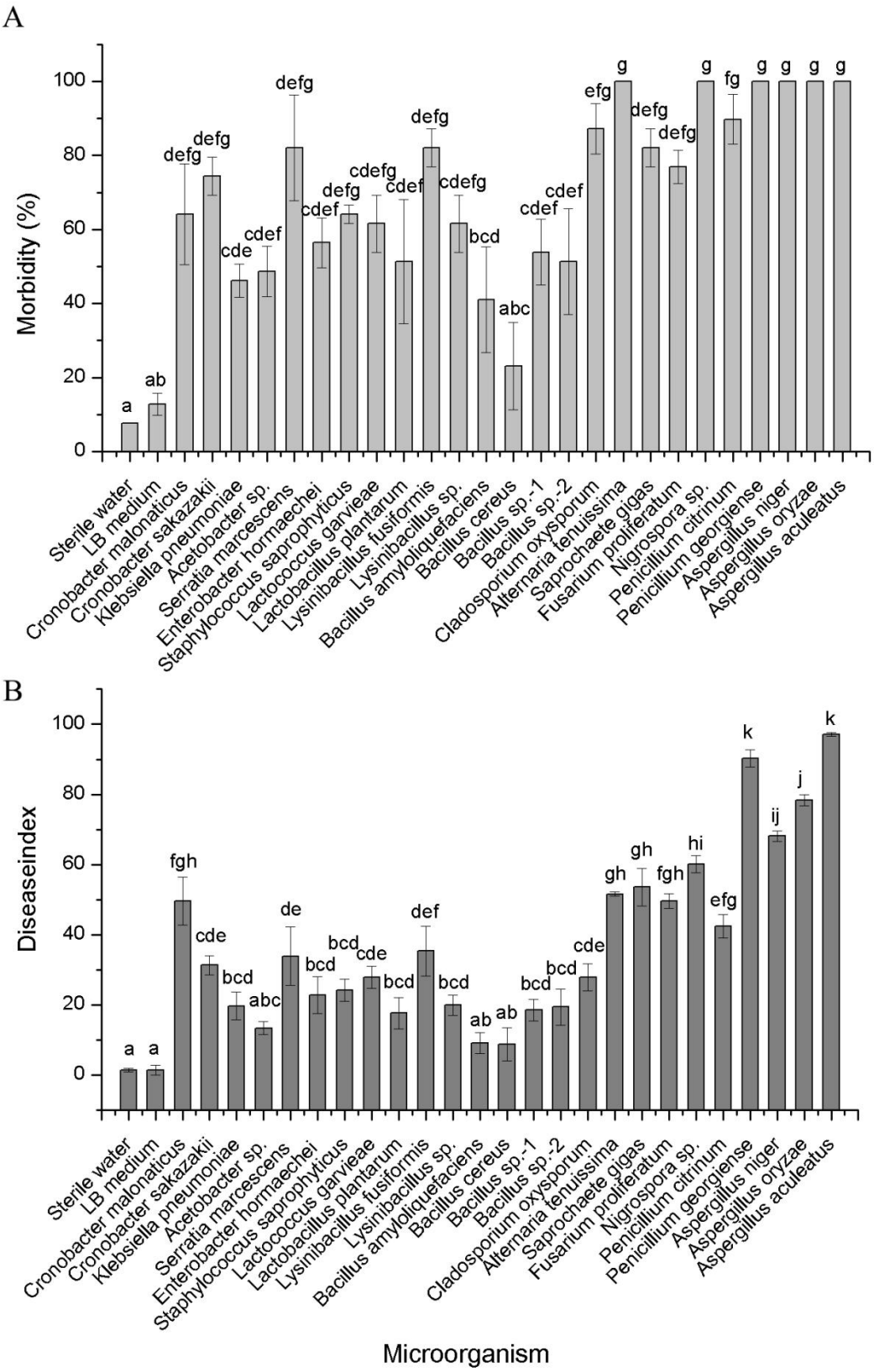
9

10

Fig.3


sterile water
LB broth
Cladosporium oxysporum
Alternaria tenuissima
Saprochaete gigas
Fusarium proliferatum
Nigrospora sp.
Penicillium citrinum
Penicillium georgiense
Aspergillus niger
Aspergillus oryzae
Aspergillus aculeatus
Cronobacter malonaticus
Cronobacter sakazakii
Klebsiella pneumoniae
Acetobacter sp.
Serratia marcescens
Enterobacter hormaechei
Staphylococcus saprophytic
Lactococcus garvieae
Lactobacillus plantarum
Lysinibacillus fusiformis
Lysinibacillus sp.
Bacillus amyloliquefaciens
Bacillus cereus
Bacillus sp.-1
Bacillus sp.-1

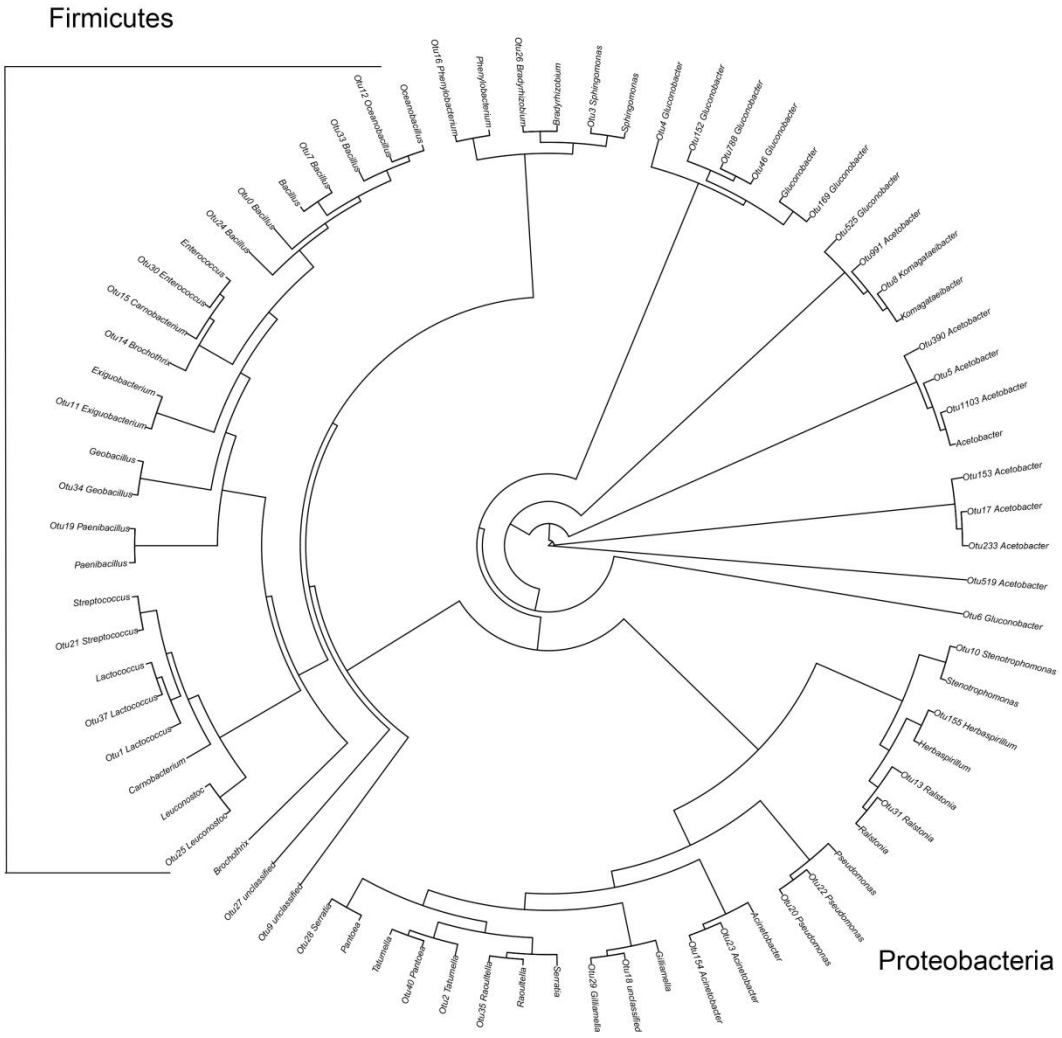
16 Fig.4


A
Morbidity (%)
0
20
40
60
80
100
a
ab
defg
defg
cde
cdef
defg
cdef
defg
cdefg
cdef
defg
cdefg
bcd
abc
cdef
cdef
efg
g
defg
defg
g
fg
g
g
g
g
Sterile water
LB medium
Cronobacter malonaticus
Cronobacter sakazakii
Klebsiella pneumoniae
Acetobacter sp.
Serratia marcescens
Enterobacter hormaechei
Staphylococcus saprophyticus
Lactococcus garvieae
Lactobacillus plantarum
Lysinibacillus fusiformis
Lysinibacillus sp.
Bacillus amyloliquefaciens
Bacillus cereus
Bacillus sp.-1
Bacillus sp.-2
Cladosporium oxysporum
Alternaria tenuissima
Saprochaete gigas
Fusarium proliferatum
Nigrospora sp.
Penicillium citrinum
Penicillium georgiense
Aspergillus niger
Aspergillus oryzae
Aspergillus aculeatus
B
Diseaseindex
0
20
40
60
80
100
a
a
fgh
cde
bcd
abc
de
bcd
bcd
cde
bcd
def
bcd
ab
ab
bcd
bcd
cde
gh
gh
fgh
hi
efg
k
ij
j
k
Sterile water
LB medium
Cronobacter malonaticus
Cronobacter sakazakii
Klebsiella pneumoniae
Acetobacter sp.
Serratia marcescens
Enterobacter hormaechei
Staphylococcus saprophyticus
Lactococcus garvieae
Lactobacillus plantarum
Lysinibacillus fusiformis
Lysinibacillus sp.
Bacillus amyloliquefaciens
Bacillus cereus
Bacillus sp.-1
Bacillus sp.-2
Cladosporium oxysporum
Alternaria tenuissima
Saprochaete gigas
Fusarium proliferatum
Nigrospora sp.
Penicillium citrinum
Penicillium georgiense
Aspergillus niger
Aspergillus oryzae
Aspergillus aculeatus
Microorganism

17

18

19 Fig.5

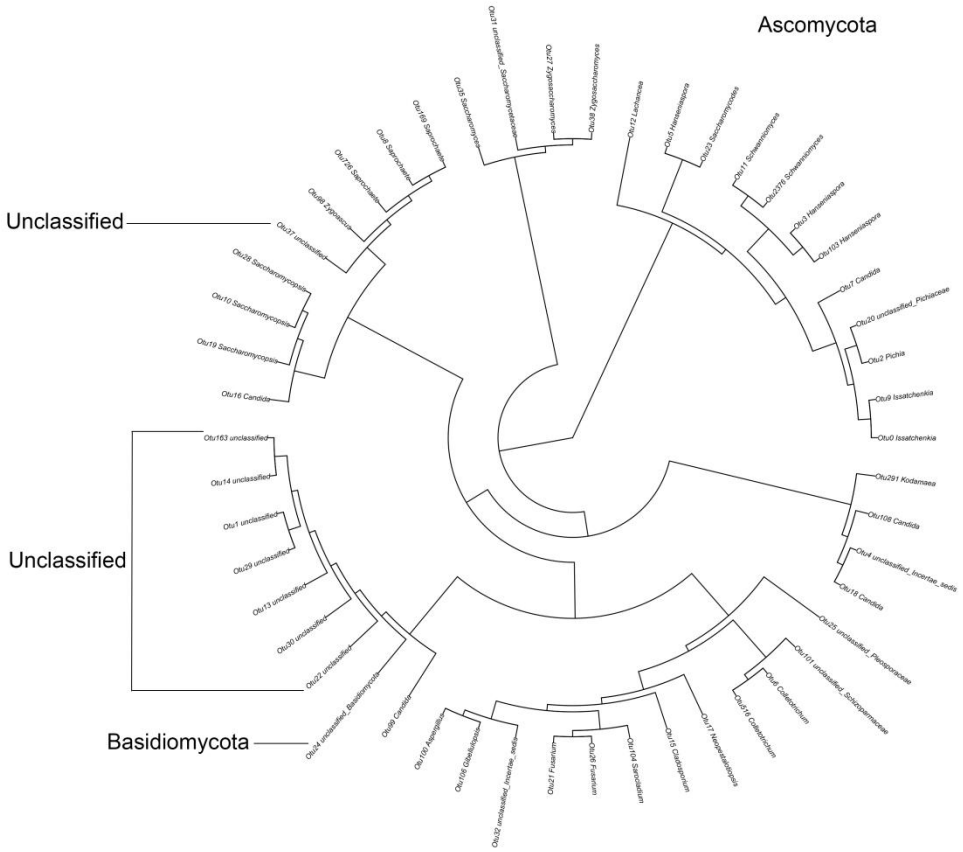

Firmicutes
Proteobacteria
Otu16 Phenylobacterium
Phenylobacterium
Otu26 Bradyrhizobium
Bradyrhizobium
Otu3 Sphingomonas
Sphingomonas
Otu4 Gluconobacter
Otu153 Gluconobacter
Otu789 Gluconobacter
Otu46 Gluconobacter
Gluconobacter
Otu189 Gluconobacter
Otu535 Gluconobacter
Otu991 Acetobacter
Otu8 Komagataeibacter
Komagataeibacter
Otu390 Acetobacter
Otu5 Acetobacter
Otu1103 Acetobacter
Acetobacter
Otu153 Acetobacter
Otu17 Acetobacter
Otu233 Acetobacter
Otu519 Acetobacter
Otu6 Gluconobacter
Otu10 Stenotrophomonas
Stenotrophomonas
Otu155 Herbaspirillum
Herbaspirillum
Otu13 Ralstonia
Otu31 Ralstonia
Ralstonia
Pseudomonas
Otu22 Pseudomonas
Otu20 Pseudomonas
Acinetobacter
Otu23 Acinetobacter
Otu154 Acinetobacter
Gillisiella
Otu19 unclassified
Otu29 Gillisiella
Serratia
Raoultella
Otu35 Raoultella
Otu2 Tatumella
Otu40 Pantoea
Tatumella
Pantoea
Otu28 Serratia
Otu8 unclassified
Otu27 unclassified
Brochothrix
Otu25 Leuconostoc
Leuconostoc
Carnobacterium
Otu1 Lactococcus
Otu37 Lactococcus
Lactococcus
Otu21 Streptococcus
Streptococcus
Otu19 Paenibacillus
Paenibacillus
Otu34 Geobacillus
Geobacillus
Otu11 Exiguobacterium
Exiguobacterium
Otu14 Brochothrix
Otu15 Carnobacterium
Otu30 Enterococcus
Enterococcus
Otu24 Bacillus
Otu0 Bacillus
Bacillus
Otu7 Bacillus
Otu33 Bacillus
Otu12 Oceanobacillus
Oceanobacillus

20

21

22 Fig.6

23



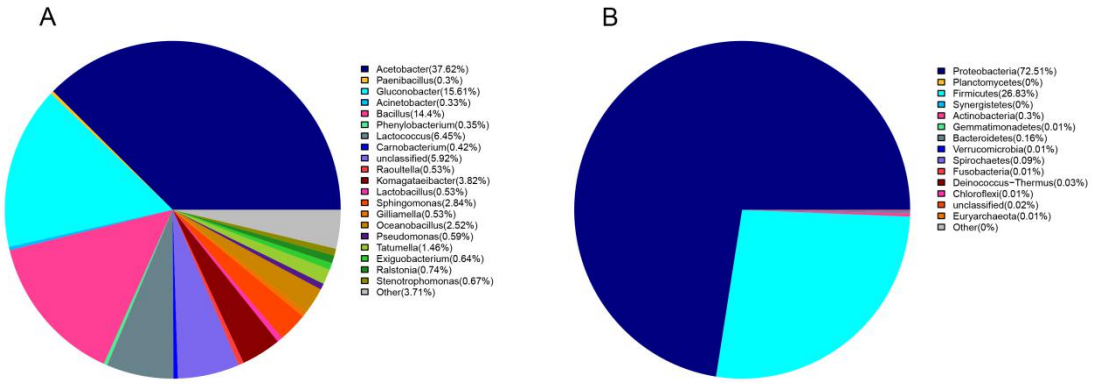
24

25

26

27 Fig. 7

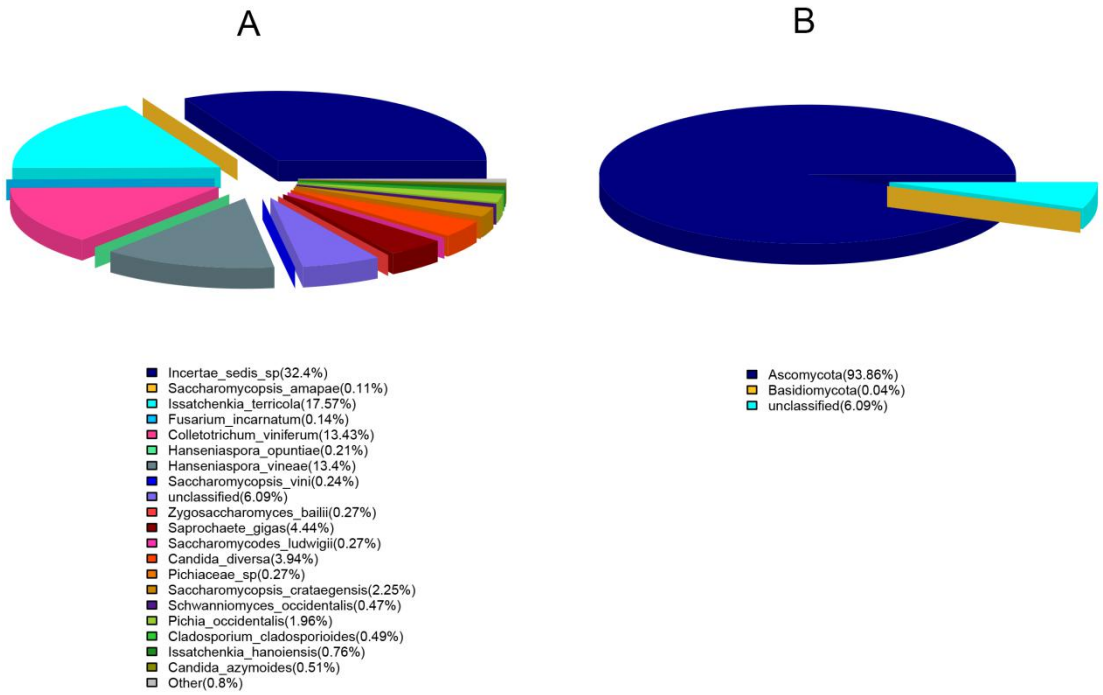
28



29

30

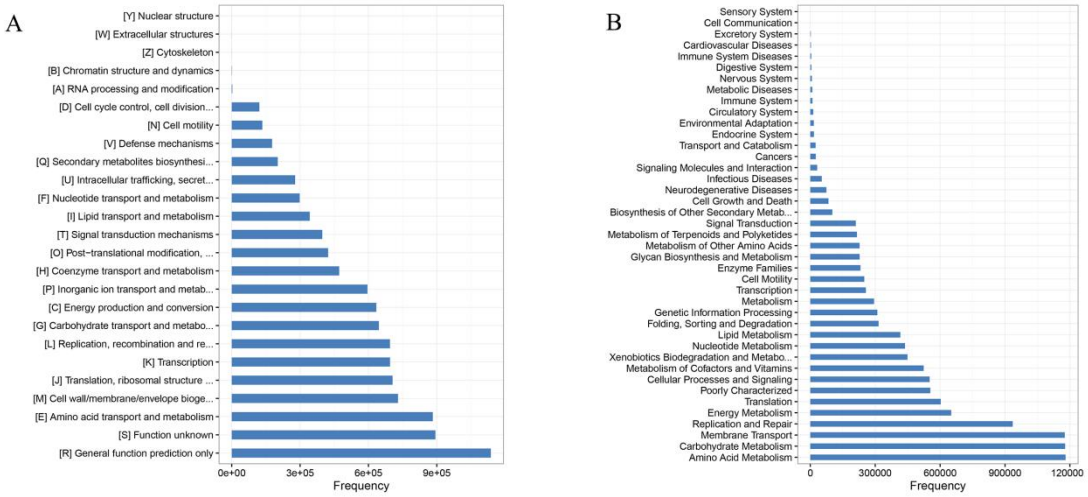
31 Fig.
32 8


A
B
Incertae_sedis_sp(32.4%)
Saccharomycopsis_amapae(0.11%)
Issatchenkia_terricola(17.57%)
Fusarium_incarnatum(0.14%)
Colletotrichum_viniferum(13.43%)
Hanseniaspora_opuntiae(0.21%)
Hanseniaspora_vineae(13.4%)
Saccharomycopsis_vini(0.24%)
unclassified(6.09%)
Zygosaccharomyces_bailii(0.27%)
Saprochaete_gigas(4.44%)
Saccharomycodes_ludwigii(0.27%)
Candida_diversa(3.94%)
Pichiaceae_sp(0.27%)
Saccharomycopsis_crataegensis(2.25%)
Schwanniomyces_occidentalis(0.47%)
Pichia_occidentalis(1.96%)
Cladosporium_cladosporioides(0.49%)
Issatchenkia_hanoiensis(0.76%)
Candida_azymoides(0.51%)
Other(0.8%)
Ascomycota(93.86%)
Basidiomycota(0.04%)
unclassified(6.09%)

33

34 Fig.9

35


A
[Y] Nuclear structure
[W] Extracellular structures
[Z] Cytoskeleton
[B] Chromatin structure and dynamics
[A] RNA processing and modification
[D] Cell cycle control, cell division...
[N] Cell motility
[V] Defense mechanisms
[Q] Secondary metabolites biosynthesi...
[U] Intracellular trafficking, secret...
[F] Nucleotide transport and metabolism
[I] Lipid transport and metabolism
[T] Signal transduction mechanisms
[O] Post−translational modification, ...
[H] Coenzyme transport and metabolism
[P] Inorganic ion transport and metab...
[C] Energy production and conversion
[G] Carbohydrate transport and metabo...
[L] Replication, recombination and re...
[K] Transcription
[J] Translation, ribosomal structure ...
[M] Cell wall/membrane/envelope bioge...
[E] Amino acid transport and metabolism
[S] Function unknown
[R] General function prediction only
0e+00
3e+05
6e+05
9e+05
Frequency
B
Sensory System
Cell Communication
Excretory System
Cardiovascular Diseases
Immune System Diseases
Digestive System
Nervous System
Metabolic Diseases
Immune System
Circulatory System
Environmental Adaptation
Endocrine System
Transport and Catabolism
Cancers
Signaling Molecules and Interaction
Infectious Diseases
Neurodegenerative Diseases
Cell Growth and Death
Biosynthesis of Other Secondary Metab...
Signal Transduction
Metabolism of Terpenoids and Polyketides
Metabolism of Other Amino Acids
Glycan Biosynthesis and Metabolism
Enzyme Families
Cell Motility
Transcription
Metabolism
Genetic Information Processing
Folding, Sorting and Degradation
Lipid Metabolism
Nucleotide Metabolism
Xenobiotics Biodegradation and Metabo...
Metabolism of Cofactors and Vitamins
Cellular Processes and Signaling
Poorly Characterized
Translation
Energy Metabolism
Replication and Repair
Membrane Transport
Carbohydrate Metabolism
Amino Acid Metabolism
0
300000
600000
900000
120000
Frequency

36

Table 1(on next page)

The cultivable microorganism in the sour rotted grapes

The cultivable microorganism in the sour rotted grapes

1

Table 1 The cultivable microorganism in the sour rotted grapes

Microognism	Phylum	Species	Accession numbers
Bacterium	Proteobacteria	*Cronobacter malonaticus*	MK743990
		Cronobacter sakazakii	MK743989
		Klebsiella pneumoniae	MK743987
		Acetobacter sp.	MK743980
		Serratia marcescens	MK743984
		Enterobacter hormaechei	MK743988
	Firmicutes	*Staphylococcus saprophyticus*	MK743982
		Lactococcus garvieae	MK743983
		Lactobacillus plantarum	MK743986
		Lysinibacillus fusiformis	MK753026
		Lysinibacillus sp.	MK743985
		Bacillus amyloliquefaciens	MK743994
		Bacillus cereus	MK743993
		Bacillus sp.-1	MK743992
		Bacillus sp.-2	MK743991
Fungus	Deuteromycotina	*Cladosporium oxysporum*	MK748311
		Alternaria tenuissima	MK748314
		Saprochaete gigas or Geotrichum gigas	
		Fusarium proliferatum	MK748309

	Nigrospora sp.	MK748317
	Penicillium citrinum	MK748316
	Penicillium georgiense	MK748315
Ascomycotina	*Aspergillus niger*	MK748313
	Aspergillus oryzae	MK748312
	Aspergillus aculeatus	MK748310

Table 2(on next page)

The physiological and biochemical characteristic of bacterium in sour rotted grape

The physiological and biochemical characteristic of bacterium in sour rotted grape

Table 2 The physiological and biochemical characteristic of bacterium in sour rotted grape

Bacterium	Gram staining	Spore staining	Bacterial motility	Catalase reaction	Methyl red test	Starch hydrolysis test	Benzpyrole test	V-P test
Cronobacter malonaticus	-		-	+	-	-	-	+
Cronobacter sakazakii	-		-	+	-	-	-	+
Klebsiella pneumoniae	-		-	+	-	-	-	+
Acetobacter sp.	-		+	+	-	-	-	+
Serratia marcescens	-		+	+	-	+	-	+
Enterobacter hormaechei	-		+	+	-	+	-	+
Staphylococcus saprophyticus	+		+	+	-	+	-	+
Lactococcus garvieae	+		-	+	-	+	-	+
Lactobacillus plantarum	+		-	+	-	+	-	+
Lysinibacillus fusiformis	+	purple	+	+	-	+	-	-
Lysinibacillus sp.	+	purple	+	+	-	+	-	+
Bacillus amyloliquefaciens	+	pink	+	+	-	+	-	-
Bacillus cereus	+	purple	+	+	-	+	-	+

Bacillus sp.-1		purple	+	+	-	+	-	+
Bacillus sp.-2	+	purple	+	+	-	+	-	+

Bacterium	Malonic acid test	Gelatin test	H_2S test	Citrate test	Ammonia production test	Litmus milk test	Urease test
Cronobacter malonaticus	-	+	+	+	+	+	-
Cronobacter sakazakii	+	+	+	-	+	+	-
Klebsiella pneumoniae	+	+	+	+	+	-	+
Acetobacter sp.	+	+	+	+	+	+	+
Serratia marcescens	+	+	+	-	+	+	-
Enterobacter hormaechei	+	+	+	+	+	+	-
Staphylococcus saprophyticus	-	+	+	+	+	+	-
Lactococcus garvieae	-	+	+	-	+	+	-
Lactobacillus plantarum	+	+	+	+	+	+	-
Lysinibacillus fusiformis	+	+	+	-	+	-	-
Lysinibacillus sp.	+	+	+	+	+	-	-
Bacillus amyloliquefaciens	-	+	+	+	+	+	+

Bacillus cereus	-	+	+	+	+	+	-
Bacillus sp.-1	+	+	+	+	+	+	+
Bacillus sp.-2	-	+	+	+	+	+	+

1

Table 3(on next page)

Sequence information and OTUs diversity, number of bacterium and fungi

Sequence information and OTUs diversity, number of bacterium and fungi

1

2

Table 3 Sequence information and OTUs diversity, number of bacterium and fungi

Parameters	Parameters	Bacterium	Fungi
Sequence information	Raw number	57503.67±3213.31	76211±2711.94
	Mean length (bp)	451.07±3.32	322.36±4.66
	Clean number	55895.33±3088.35	76113.67±2703.97
	Mean length (bp)	412.86±2.76	279.37±4.52
Diversity indices	Shannon	3.26±0.13	2.20±0.17
	ACE	22033.53±2927.20	32667.47±1384.78
	Chao1	9744.84±1429.46	10778.66±1475.59
	Simpson	0.10±0.02	0.21±0.04
OTUs number		1343.33±282.94	1038.67±386.36

3

Figure 1

Colony morphology and the light mophology of the fungi in sour rot-infected grapes

Colony morphology and the light mophology of the fungi in sour rot-infected grapes. A: Colony morphology; *r* indicates the reverse side of colony; *f* indicates the front side of colony; B: Light morphology of the fungi in sour rot-infected grapes

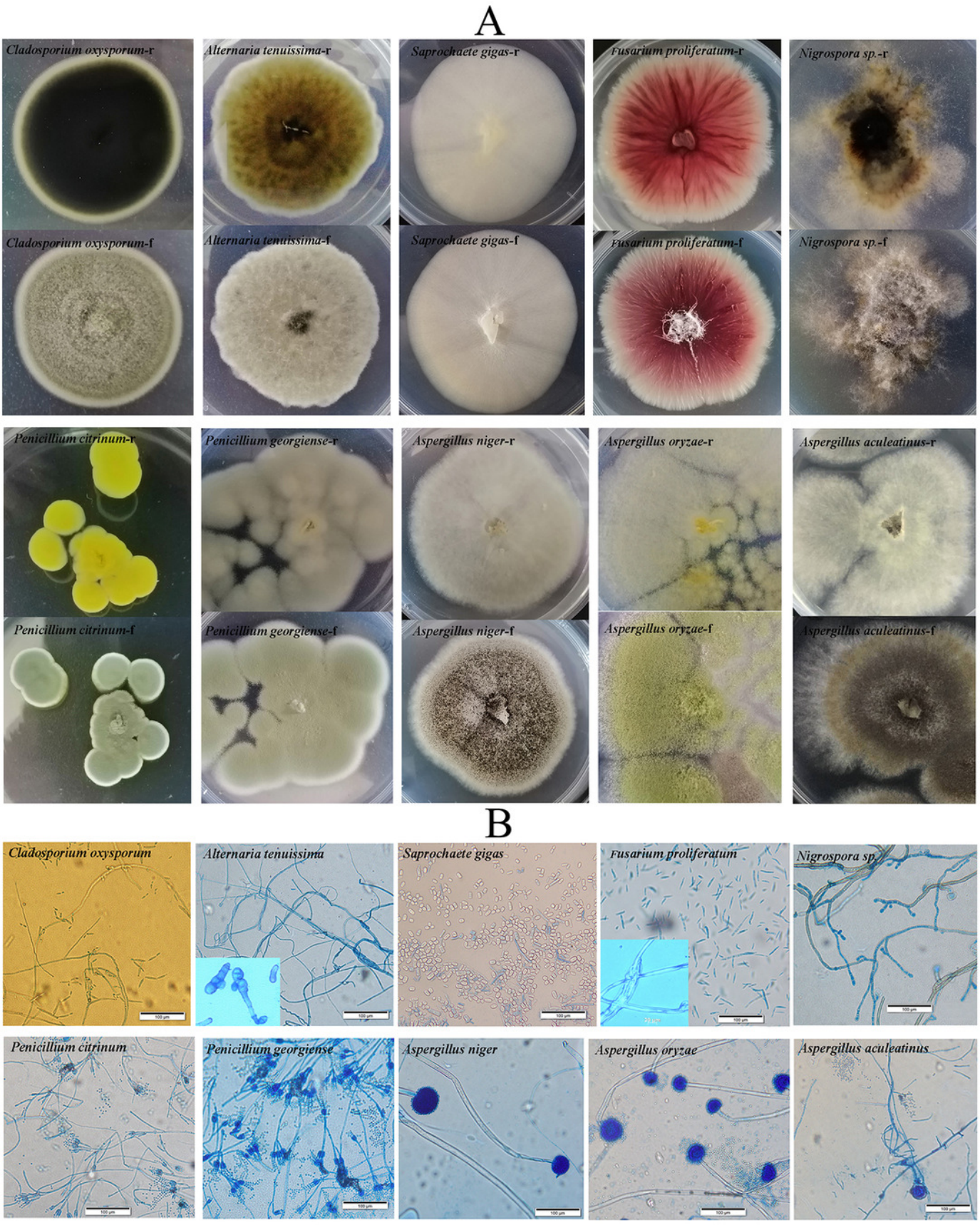

A
Cladosporium oxysporum-r
Alternaria tenuissima-r
Saprochaete gigas-r
Fusarium proliferatum-r
Nigrospora sp.-r
Cladosporium oxysporum-f
Alternaria tenuissima-f
Saprochaete gigas-f
Fusarium proliferatum-f
Nigrospora sp.-f
Penicillium citrinum-r
Penicillium georgiense-r
Aspergillus niger-r
Aspergillus oryzae-r
Aspergillus aculeatinus-r
Penicillium citrinum-f
Penicillium georgiense-f
Aspergillus niger-f
Aspergillus oryzae-f
Aspergillus aculeatinus-f
B
Cladosporium oxysporum
Alternaria tenuissima
Saprochaete gigas
Fusarium proliferatum
Nigrospora sp.
Penicillium citrinum
Penicillium georgiense
Aspergillus niger
Aspergillus oryzae
Aspergillus aculeatinus

Figure 2

The phylogenetic trees of bacteria (A) and fungi (B) in sour rot-infected grapes

The phylogenetic trees of bacteria (A) and fungi (B) in sour rot-infected grapes. Phylogenetic trees were constructed using neighbor-joining method(NJ) with Mega 6.0 software

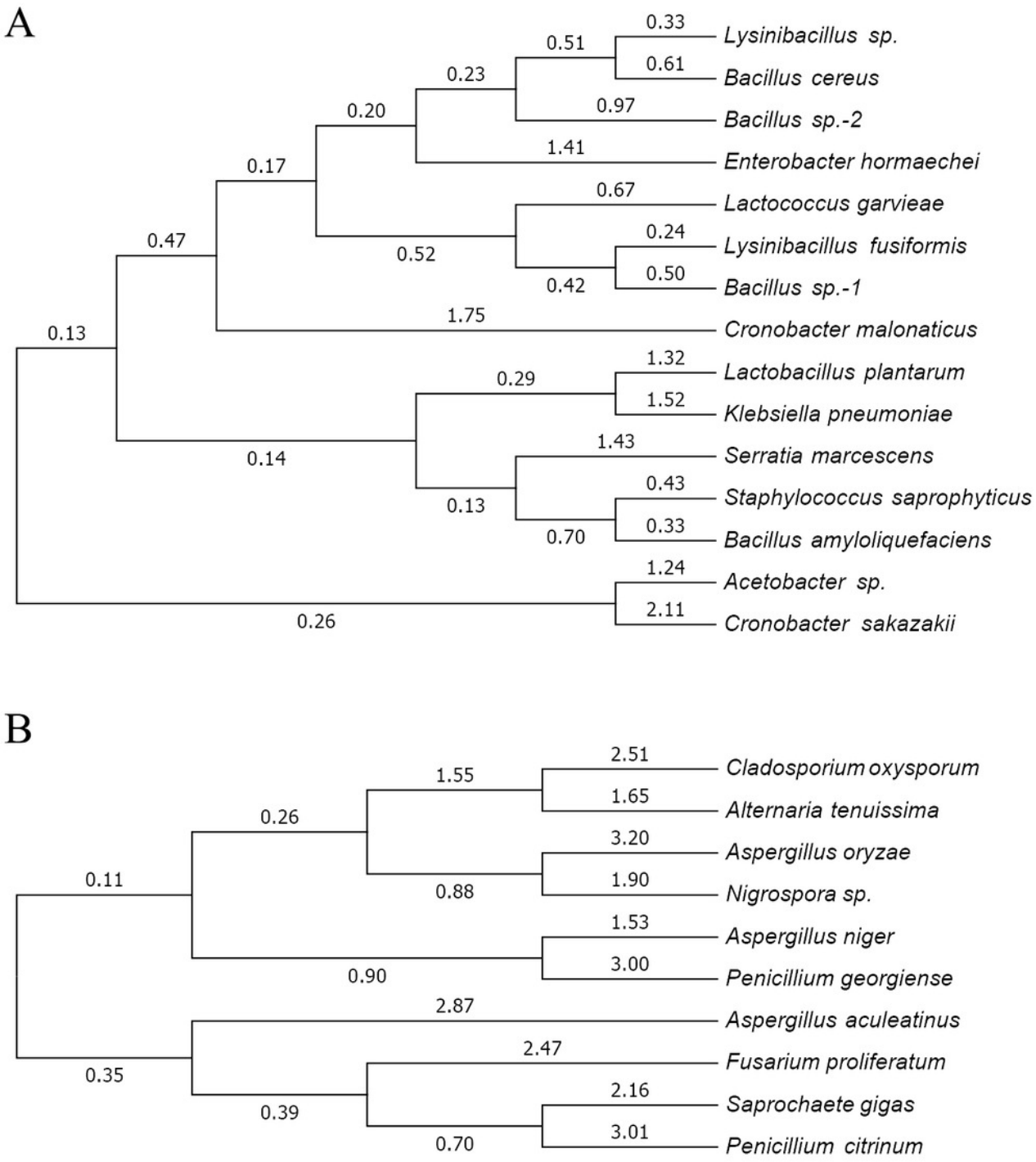

A
0.33
Lysinibacillus sp.
0.51
0.61
Bacillus cereus
0.23
0.97
Bacillus sp.-2
0.20
1.41
Enterobacter hormaechei
0.17
0.67
Lactococcus garvieae
0.47
0.24
Lysinibacillus fusiformis
0.52
0.50
Bacillus sp.-1
0.42
1.75
Cronobacter malonaticus
0.13
1.32
Lactobacillus plantarum
0.29
1.52
Klebsiella pneumoniae
1.43
Serratia marcescens
0.14
0.43
Staphylococcus saprophyticus
0.13
0.33
Bacillus amyloliquefaciens
0.70
1.24
Acetobacter sp.
2.11
Cronobacter sakazakii
0.26
B
2.51
Cladosporium oxysporum
1.55
1.65
Alternaria tenuissima
0.26
3.20
Aspergillus oryzae
0.11
0.88
1.90
Nigrospora sp.
1.53
Aspergillus niger
0.90
3.00
Penicillium georgiense
2.87
Aspergillus aculeatinus
2.47
Fusarium proliferatum
0.35
2.16
Saprochaete gigas
0.39
0.70
3.01
Penicillium citrinum

Figure 3

The pathogenicity of fungi in healthy grape berries

The pathogenicity of fungi in healthy grape berries

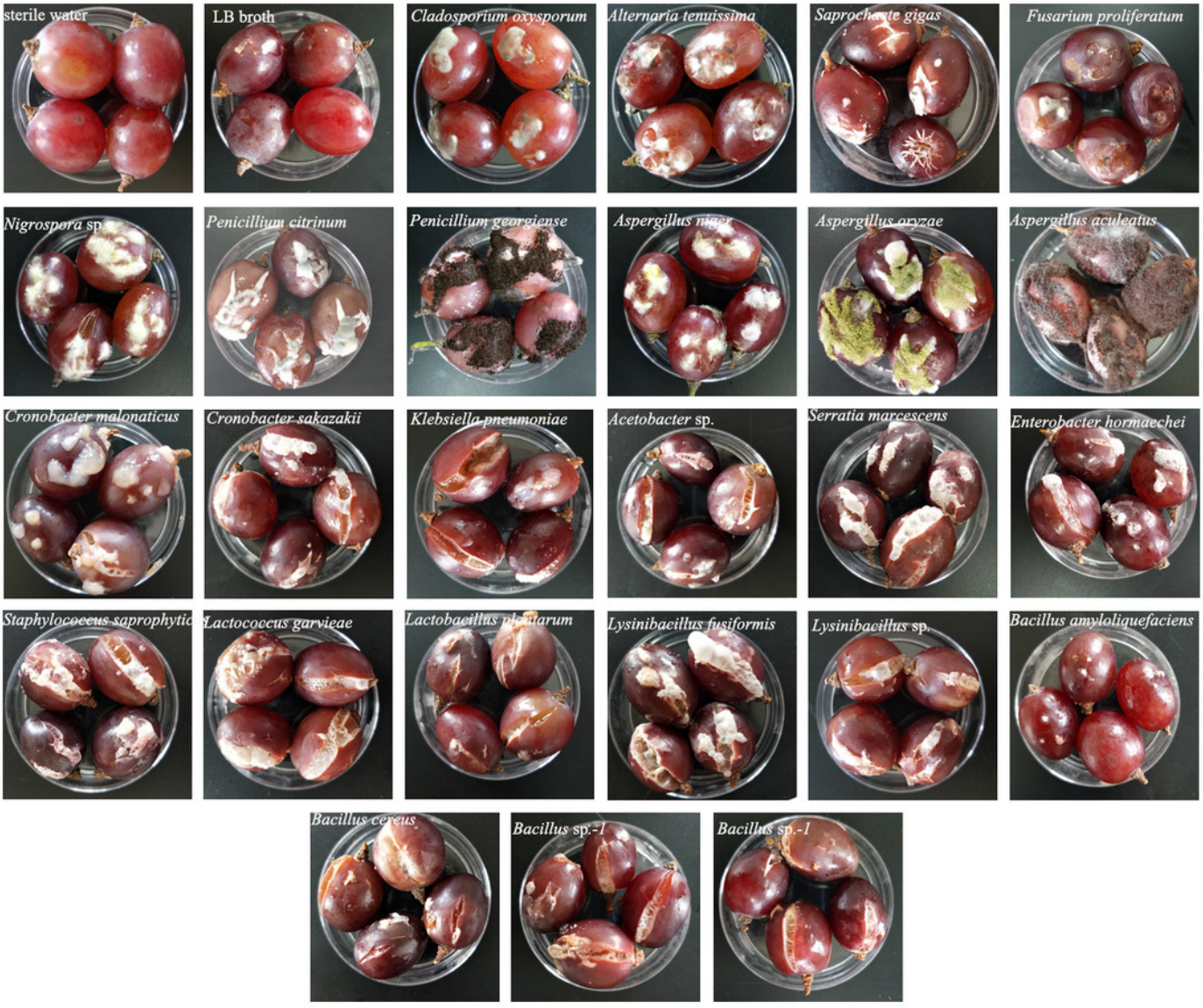


Figure 4

The morbidity (A) and disease index (B)of 15 bacterial species and 10 fungal species

The morbidity (A) and disease index (B)of 15 bacterial species and 10 fungal species. Different letters in each figure (A and B) indicate significant difference between adults and larvae (One-way ANOVA; $\alpha = 0.05$).

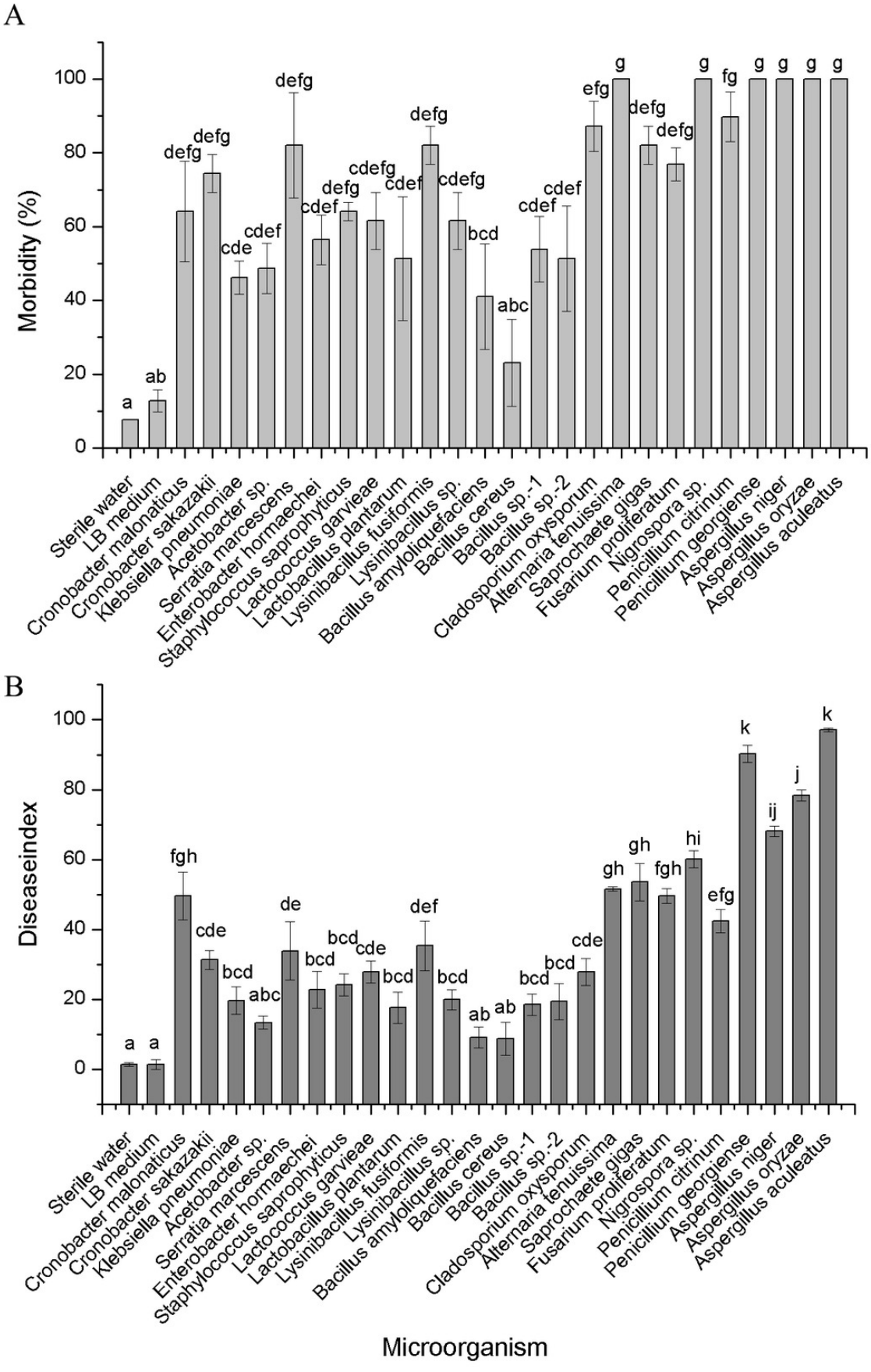

A
Morbidity (%)
0
20
40
60
80
100
a
ab
defg
defg
cde
cdef
defg
cdef
defg
cdefg
cdef
defg
cdefg
bcd
abc
cdef
cdef
efg
g
defg
defg
g
fg
g
g
g
g
Sterile water
LB medium
Cronobacter malonaticus
Cronobacter sakazakii
Klebsiella pneumoniae
Acetobacter sp.
Serratia marcescens
Enterobacter hormaechei
Staphylococcus saprophyticus
Lactococcus garvieae
Lactobacillus plantarum
Lysinibacillus fusiformis
Lysinibacillus sp.
Bacillus amyloliquefaciens
Bacillus cereus
Bacillus sp.-1
Bacillus sp.-2
Cladosporium oxysporum
Alternaria tenuissima
Saprochaete gigas
Fusarium proliferatum
Nigrospora sp.
Penicillium citrinum
Penicillium georgiense
Aspergillus niger
Aspergillus oryzae
Aspergillus aculeatus
B
Diseaseindex
0
20
40
60
80
100
a
a
fgh
cde
bcd
abc
de
bcd
bcd
cde
bcd
def
bcd
ab
ab
bcd
bcd
cde
gh
gh
fgh
hi
efg
k
ij
j
k
Sterile water
LB medium
Cronobacter malonaticus
Cronobacter sakazakii
Klebsiella pneumoniae
Acetobacter sp.
Serratia marcescens
Enterobacter hormaechei
Staphylococcus saprophyticus
Lactococcus garvieae
Lactobacillus plantarum
Lysinibacillus fusiformis
Lysinibacillus sp.
Bacillus amyloliquefaciens
Bacillus cereus
Bacillus sp.-1
Bacillus sp.-2
Cladosporium oxysporum
Alternaria tenuissima
Saprochaete gigas
Fusarium proliferatum
Nigrospora sp.
Penicillium citrinum
Penicillium georgiense
Aspergillus niger
Aspergillus oryzae
Aspergillus aculeatus
Microorganism

Figure 5

The first 50 OTUs of the bacteria by high-throughput sequencing

The first 50 OTUs of the bacteria by high-throughput sequencing

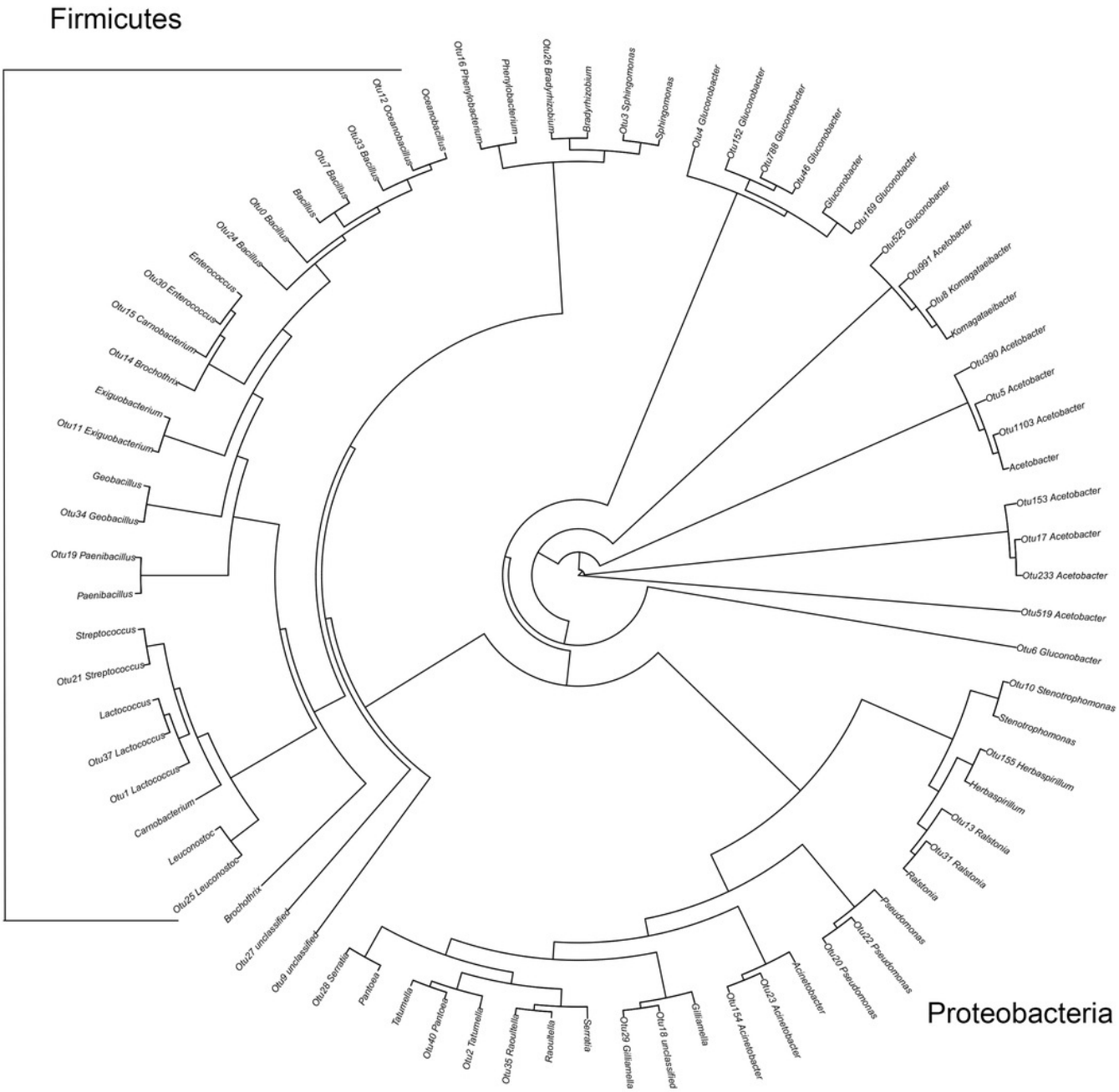


Figure 6

The first 50 OTUs of the fungi by high-throughput sequencing

The first 50 OTUs of the fungi by high-throughput sequencing

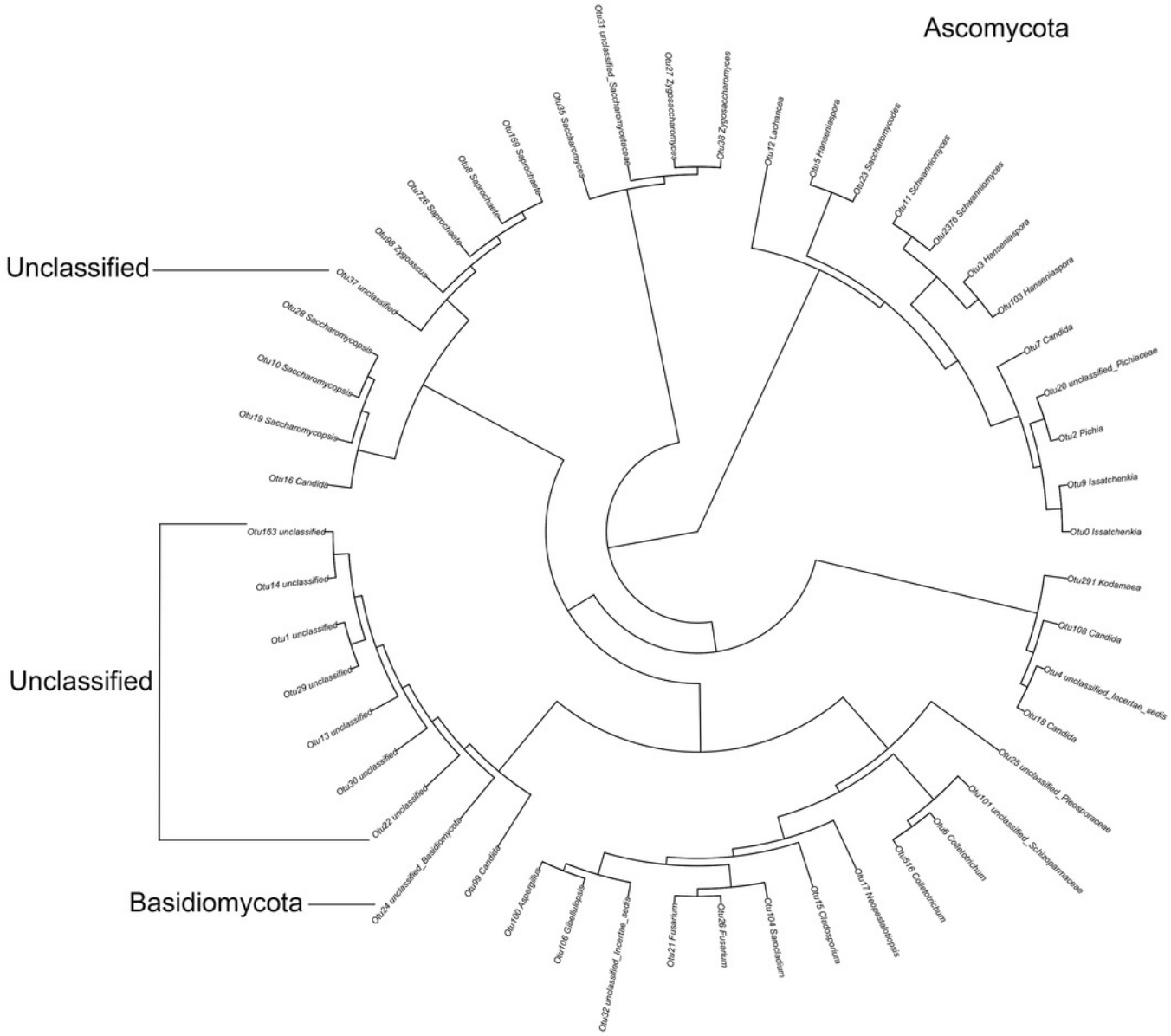


Figure 7

The bacterial community structure based on phylum (A) and genus (B) in sour rot-infected grapes based on 16S rDNA high-throughput sequencing

The bacterial community structure based on phylum (A) and genus (B) in sour rot-infected grapes based on 16S rDNA high-throughput sequencing

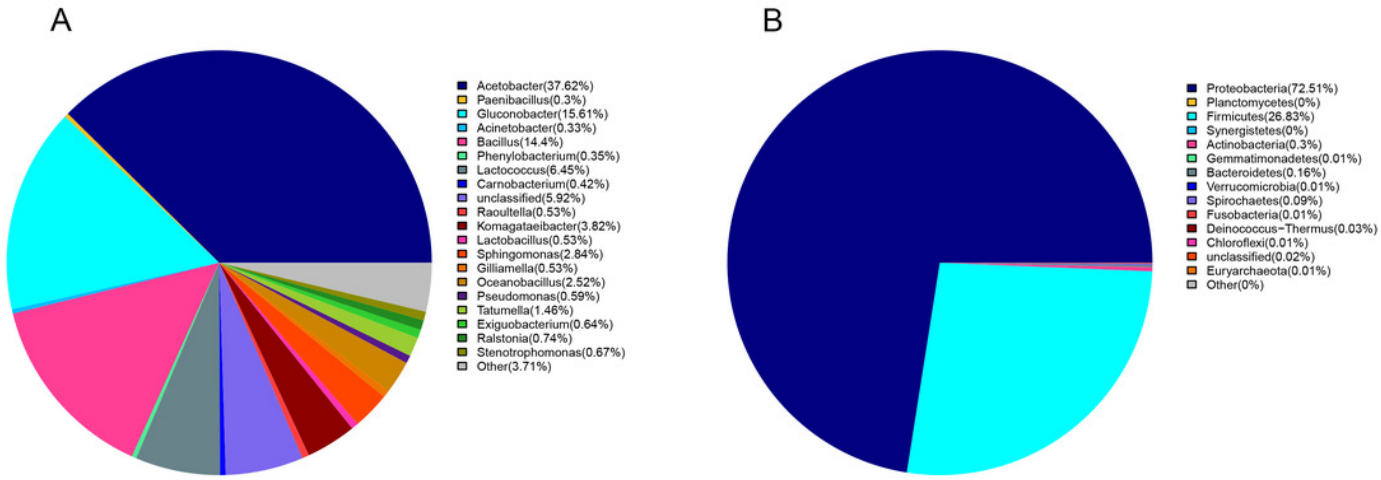


Figure 8

The fungal community structure based on phylum (A) and genus (B) in sour rot-infected grapes based on ITS high-throughput sequencing

The fungal community structure based on phylum (A) and genus (B) in sour rot-infected grapes based on ITS high-throughput sequencing

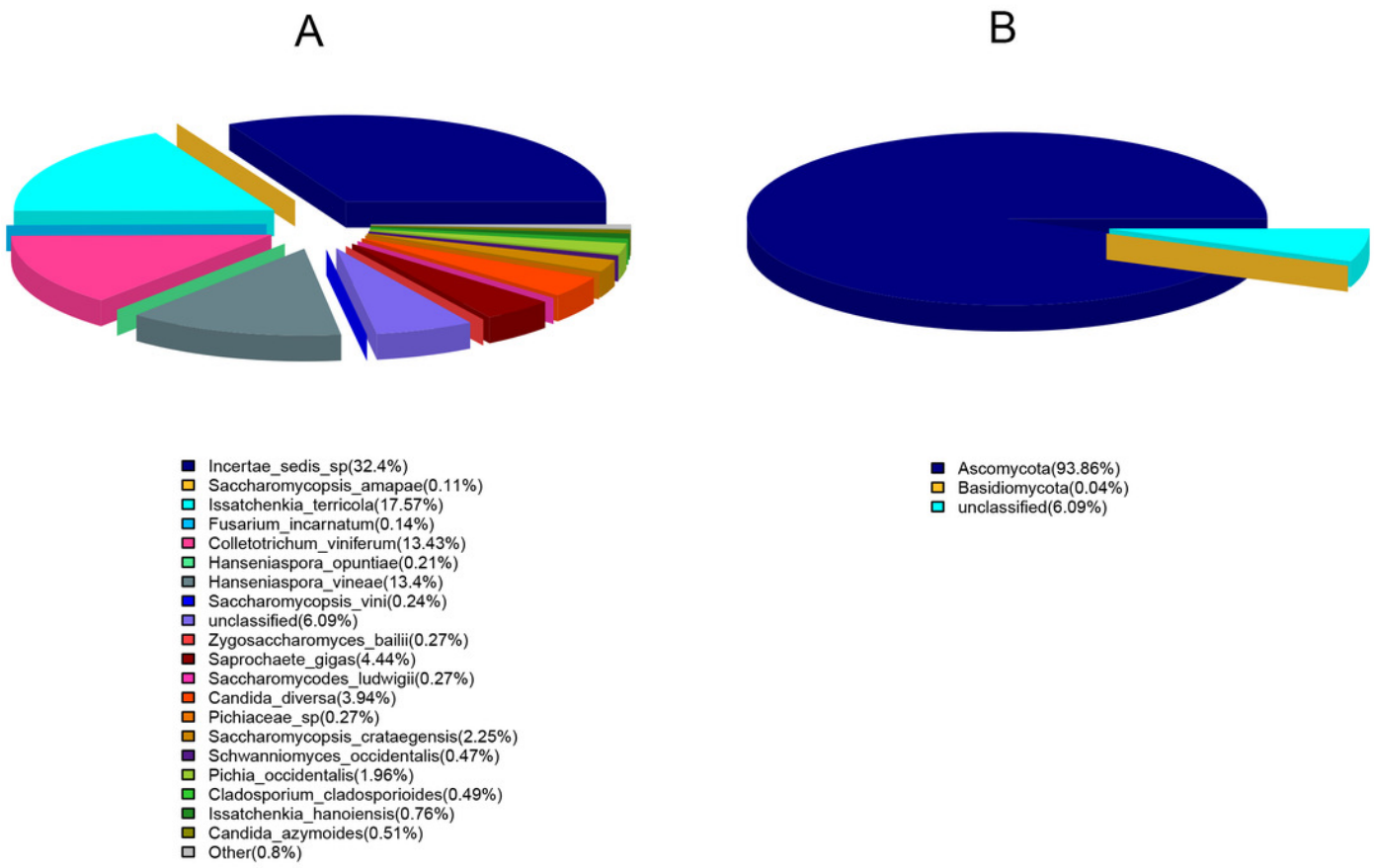


Figure 9

The COG (A) and KEGG (B) functional categories of bacterial OTUs in sour rot-infected grapes

The COG (A) and KEGG (B) functional categories of bacterial OTUs in sour rot-infected grapes