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Preliminary report on the study of postharvest fruit rot bacteria and yeasts in Lanzhou Lily (*Lilium davidii var. unicolor*) in China

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Abstract

Fruit rot is one of the most important factors affecting the postharvest quality and shelf life of Lanzhou Lily fruits. The aims of this study were to characterize different isolates from Lanzhou Lily using morphological and molecular approaches and to test their pathogenicity in Lanzhou Lily fruit. Different isolates were collected from decayed Lanzhou Lily fruits in Lanzhou in Gansu Province in China during 2016 and 2017. In total, four isolates were obtained and identified based on their DNA sequences of the 16S rDNA and 26S rDNA genes in combination with the morphological characteristics of the cultures and sporulation. Of the four isolates, one was identified as *Bacillus safensis*, one was *Stenotrophomonas maltophilia* and the other two isolates were *Metschnikowia pulcherrima*. The pathogenicity tests showed that all four isolates were pathogenic in Lanzhou Lily fruit. Previously *Fusarium tricinctum* has been reported to be the primary cause of fruit rot in Lanzhou Lily throughout the world. Our report is the first to show results that indicate that *B. safensis*, *S. maltophilia* and *M. pulcherrima* are pathogenic in Lanzhou Lily in China. This is the first report on the bacteria and yeast that infect Lanzhou Lily fruits.

KEYWORDS
fruit rot, Lanzhou Lily, postharvest pathogens, separation and identification

1 | INTRODUCTION

Postharvest diseases of fruit and vegetables cause significant losses worldwide, which are estimated at approximately 20% in industrialized regions and over 50% in regions with storage and transport limitations (Torres et al., 2011). Postharvest decays can be reduced by minimizing fruit injuries, maintaining the natural resistance of the host and delaying senescence (Shewfelt, 1986).

Losses due to postharvest disease may occur at any time during postharvest handling, from harvest to consumption. Virtually all postharvest diseases of fruit and vegetables are caused by fungi and bacteria. In some root crops and brassicas, viral infections present before harvest can sometimes develop more rapidly after harvest. However, in general, viruses are not a common cause of postharvest disease, but they can cause severe losses in terms of quantity and quality in bulb and flower production (Wang et al., 2010).

Postharvest diseases are often classified according to how the infection is initiated. The so-called quiescent or latent infections are those where the pathogen initiates the infection of the host at some point in time (usually before harvest) but then enters a period of inactivity or dormancy until the physiological status of the host tissue changes in such a way that the infection can proceed. The dramatic physiological changes that occur during fruit ripening are often the trigger for the reactivation of quiescent infections. Some examples of postharvest diseases that arise from quiescent infections include anthracnose in various tropical fruits caused by *Colletotrichum*

spp. (Santos et al., 2013) and grey mould in strawberries caused by *Botrytis cinerea* (Bristow et al., 2010).

The other major group of postharvest diseases are those that arise from infections initiated during and after harvest. Often these infections occur through surface wounds created by mechanical or insect injury. The wounds need not be large for an infection to take place and in many cases may be microscopic in size. Common postharvest diseases resulting from wound infections include blue and green mould (caused by *Penicillium* spp.) and transit rot (caused by *Rhizopus stolonifer*). Bacteria such as *Erwinia carotovora* (soft rot) are also common wound invaders. Many pathogens, such as banana crown rot fungi, also gain entry through the injury created by severing the crop from the plant.

Lanzhou Lily (*Lilium davidii var. unicolor Cotton*) is a native variety of China and is an important edible bulb that is mostly distributed in the middle region of Gansu Province in China (Zhang et al., 2018). Taxonomically, the *Lilium* genus comprises 115 species, approximately 55 of which originate from PR China (Chen et al., 2011). Lanzhou Lily is a specialty vegetable and profitable industry in Gansu Province. It is the only vegetable that has a combination of food value, medicinal value and ornamental value (Jin et al., 2008). It is not only famous in the China but also famous in the world. Lanzhou Lily is often cultivated as a perennial vegetable in industry. It contains nutrients such as sugars, proteins, vitamins, mineral elements and amino acids needed by the human body. It is known as "vegetable ginseng" (Ma et al., 2005).

Lanzhou Lily aids gastrointestinal function, decreases blood glucose and moderates lipemia, and it should be pointed out that the Lanzhou Lily transfers it health-related benefits to humans. Francis et al found that Lanzhou Lily contains kaempferol, kaempferol glycosides, quercetin glycosides, a regaloside, a chalcone and fatty acids (Francis et al., 2004). In addition, Lanzhou Lily scales, which are considered a health food due to their abundant nutritional components that include proteins, carbohydrates lipids and amino acids, are used in Chinese medicine in different forms, such as fresh bulbs, dried scales and powder to treat heart and lung ailments (Li et al., 2014).

Lanzhou Lily production has an acreage above 6,667 km^2 and an annual output of 30 million to 40 million kg. It is mainly sold to large and medium-sized cities in Guangzhou, Shanghai and Beijing. The Guangzhou market accounts for approximately 50% of the lily's total sales, the Shanghai market accounts for approximately 21% of the lily's total sales, and the Beijing market accounts for approximately 18% of the lily's total sales. The remaining scattered sales are in local markets, and some products are exported to the United States, Japan, Hong Kong, Taiwan and other places (She, 2012). The profits from planting lilies are much higher for the local people than those from other economic crops.

However, even though the Lanzhou Lily industry in Lanzhou is rapidly developing in terms of production, Lanzhou Lily bulbs face problems of rot deterioration, the degradation of certain varieties, and limited storage, transportation and preservation techniques. According to a survey the production areas, Lanzhou Lily bulbs are harvested only from mid-October to mid-November and from early February to early March. Lily bulbs collected after harvest are perishable during storage and transportation, which seriously affects the commercial value of the lilies. As a result, great economic losses have become an important factor in curbing lily production.

The diseases that affect Lanzhou Lily during storage are mainly fungal and bacterial diseases. The fungi that cause disease including bulbous base rot, bulbous rot disease, bulbous mildew rot, bulbous aspergillosis, bulb spot disease and bulb anthracnose from fungi and bacteria are found in a wide variety of environments such as soil, plants and water (Richard et al., 1980). In addition, bacterial diseases mainly include bacterial soft rot and bacterial blight (Mao & Li, 2007). The main pathogens are *Fusarium oxysporum* (Hjm et al., 1995), *Penicillium cyclopium* (Shang et al., 2005), *Penicillium corymbiforme* (Shi-sen et al., 2005), *R. stolonifer* (Tang et al., 1997), *Aspergillus niger* (Tang et al., 1997), *Fusar-ium solani* (Mart.) *Sacc*. (Wang et al., 1994), *Colletotrichumliliacear um* (West.) *Duck* (Tang et al., 1997), *E. carotovora var. carotovora* (Feng et al., 2007) and *Erwinia lilii* (Uyeda) *Magrow* (Tang et al., 1997).

The objectives of this study were to determine the species profiles associated with decayed Lanzhou Lily fruit using molecular phylogenetic and morphological approaches and to test the pathogenicity of the representative isolates in Lanzhou Lily fruit.

2 | MATERIALS AND METHODS

2.1 | Collection of isolates

In 2016 and 2017, harvested *L. davidii* fruit was obtained from commercial *L. davidii* fruit packing operations. The fruit was stored at 0°C for 5 weeks, and then, the decayed fruit was selected for the isolation of pathogens.

Lanzhou Lilies were separately surface sterilized with 70% ethanol for 3 min by gently swirling them and then washed two to three times with sterile distilled water (Lipa & Wiland, 1972). Then, the fruit was washed under tap water and surface disinfected with 1% sodium hypochlorite for 5 min, rinsed with sterilized distilled water three times and blot-dried using sterilized filter paper. The tissue segments containing symptomatic and asymptomatic areas were removed with a sterilized scalpel and separately transferred onto nutrient agar (NA), yeast extract peptone dextrose media (YPD), G1 synthetic medium and potato dextrose agar (PDA) with streptomycin.

The plates were incubated at 30°C for 2–3 days. At the end of the incubation period, bacterial, yeast and actinomycetes colonies were separated based on their colour and colony morphology. The separated bacterial yeast and actinomycetes colonies were transferred to another nutrient agar, yeast extract peptone dextrose media and G1 synthetic medium plate and incubated for 48–72 hr to grow. The purified bacterial, yeast and actinomycetes isolates were subcultured and stored in 20% glycerol at −80°C for further characterization studies. The isolated strains were identified by a number of morphological, biochemical, physiological and molecular techniques according to Bergey's Manual of Systematic Bacteriology, volumes 1

and 2 (Kandler, 1986; Krieg, 1984). All isolates identified in this study are publicly accessible.

2.2 | DNA extraction, PCR amplification and sequencing

For each isolate, genomic DNA was extracted by the method of Makimura and Russell (Makimura et al., 1994; Russell et al., 2001). The DNA pellets were dissolved in 50 μl Tris–EDTA buffer (10 mmol/L Tris–HCl, 1 mmol/L EDTA, pH 8.0). The isolated DNA was stored at −20°C until use.

The oligonucleotide primers 27F (5′-AGAGTTTGATCCTGG CTCAG-3′ as forward) and 1492L (5′-GGTTACCTTGTTACGACTT-3′ as reverse) (Macrogen) were used to amplify the 16S rRNA gene for each bacterial isolate (Demirci et al., 2013). The PCRs contained 5 μl 10× Taq DNA polymerase reaction buffer, 1.5 μl 10 mmol/L dNTP mix, 1.5 μl 10 pmol each of the opposing primers, 1 μl 5 U/μl Taq DNA polymerase (Fermentas), 3 μl $MgCl_2$, 2 μl genomic DNA and 34.5 μl dH_2O. PCR was performed under the following conditions: 2-min initial denaturation at 94°C; 35 cycles of denaturation (45 s at 94°C), annealing (60 s at 55°C) and extension (60 s at 72°C); and a final extension at 72°C for 10 min. Finally, the PCR products were analysed by electrophoresis on 1% agarose gels and then visualized under UV light by staining with ethidium bromide. The correct PCR products were sent to the Beijing Genomics Institute for sequencing. The primer pairs 518F (5′-CCAGCAGCCGCGGTAATACG-3′) and 800R (5′-TACCAGGGTATCTAATCC-3′) were used for sequencing. The obtained sequences were analysed by BLAST searches using the NCBI GenBank database (Altschul et al., 1990; Benson et al., 2013). Finally, the sequences were used to construct a phylogenetic tree to verify the isolate identification.

The D1/D2 region of the 26S rRNA gene was amplified by PCR with the primer pair NL-1 (5′-GCATATCAATAAGCGGAGGAAAG-3′) and NL-4 (5′-GGTCCGTGTTTCAAGACGG-3′) (Kurtzman & Robnett, 1997); the resulting PCR product was directly sequenced using the same primers (Sugita er al., 1998). The sequence data were analysed with the National Center for Biotechnology Information (NCBI; Bethesda, MD, USA) BLAST system (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE-TYPE=BlastSearch&LINK-LOC=blasthome). The strains with ≥99% D1/D2 26S rDNA sequence similarity were defined as conspecific. The DNA sequences were aligned using CLUSTAL W (Thompson et al., 1994) for the neighbour-joining analysis (Saitou & Nei, 1987); the distances between the sequences were calculated using Kimura's two-parameter model (Sugita et al., 1999).

2.3 | Phylogenetic analyses

All the obtained sequences of 16SrDNA and 26SrDNA were compared with the available sequence data, using a BLAST search against the NCBI GenBank database to identify the sequences. Multiple sequence alignment was manually performed with closely related reference sequences of other isolated pathogenic species available in the NCBI database using ClustalW2 software (http://www.ebi.ac.uk/Tools/phylogenecy/clustalw2-phylogeny/). A neighbour-joining tree was constructed with the combined data set of 16SrDNA and 26SrDNA according to the maximum-likelihood method using MEGA 6 software ver. 6.0. The reliability of the tree was evaluated with 1,000 bootstrap replicates for branch stability.

2.4 | Morphological observations

The colony morphology of the isolates was inspected on nutrient agar and yeast extract peptone dextrose media by direct observations or the use of a stereomicroscope (Vettraino et al., 2010). The shape of the cells was determined using a light microscope at ×1,000 magnification. Gram staining of the isolates was carried out based on the method of Claus (Claus, 1992). Capsule staining was carried out by negative staining. The motility of isolates was determined using a semisolid medium (Soutourina et al., 2001).

Conventional biochemical tests such as KIA, oxidase, catalase and indole tests were performed according to Bergey's Manual of Systematic Bacteriology, volumes 1 and 2 (Kandler, 1986; Krieg, 1984). Separately, the extracellular enzyme activity of the isolates such as amylase, proteinase, lipase, cellulase and chitinase activity was also determined. Protease activity was determined on agar plates including skim milk (Yu et al., 2009). Lipase activity was screened on rhodamine B (Sigma) agar plates including olive oil (Kouker & Jaeger, 1987). A starch hydrolysis test was used to detect amylase activity (Yu et al., 2009). Chitinase activity was determined according to the procedure of Sandalli (Sandalli et al., 2008). Cellulase activity was determined on nutrient agar plates that included carboxymethyl cellulose (0.5%). After 2 days of incubation, cellulase activity was determined with Congo red staining (Yu et al., 2009). The formation of clear zones around the colonies was determined as an indication of enzyme activity. Physiological tests such as those for temperature, pH and NaCl tolerance were also performed in LB and YPD broth to determine the physiological properties of the isolates. The physiological and biochemical characterization of the strain FJAT-14262 was analysed using API 20E and API 50 CH biochemical strips at 37°C for 48 hr.

2.5 | Pathogenicity tests

Koch's postulates that were filled by syringe inoculation with different concentrations of each microorganism (2.0×10^5–2.0×10^9 cfu/ml) were individually placed into disinfected (0.5% NaOCl) postharvest ripe Lanzhou Lily.

The bacterial isolates stocked at −80°C were initially streaked on nutrient agar plates to obtain single colony for each isolate. After, the single colonies were inoculated into nutrient broth medium using an inoculation loop and incubated at 30°C for 18 hr (48 hr for the slow-growing isolates). At the end of the incubation period, the bacterial density was measured at an absorbance of 600 nm and then adjusted to 1.89 (1.8×10^9 cfu/ml). Finally, 5 ml of these cultures was centrifuged at 1520 *g* for 15 min. The pellet was resuspended in 5 ml of sterilized

phosphate buffer solution (PBS) and used for the bioassay. Bacterial solutions that were prepared daily were used in the bioassays.

Freshly harvested organic Lanzhou Lily was obtained from a local blueberry farm and used for the pathogenicity tests. The fruits were organically produced avoiding potential interference from fungicide residues on/in the fruit. The fruits were surface-disinfested in 0.5% NaOCl (10% bleach) for 2 min, rinsed three times with sterile distilled water and then air-dried. The fruits were wounded with a sterile needle to a depth of 2 mm. Each fruit was inoculated by placing 1 ml of the bacterial or yeast suspensions of each isolate prepared as described above into the wound. Three Lanzhou Lily replicates were inoculated with each isolate at each temperature. For a control treatment, the fruits were wounded as described above and treated with 1 µl of sterile water instead of the conidial suspension. Fruit rot development was evaluated after incubation for 6 days at 20°C, 5 weeks at 4°C and 3 additional days at room temperature after being removed from storage at 0°C for 6 weeks. The inoculated fruit was placed on double-sided sticky tape in Petri dishes, and the dishes were kept in plastic boxes with a shallow layer of sterile water (approximately 0.5 cm) on the bottom to maintain high humidity. The plastic boxes were placed in incubators at the designated temperatures of 0, 4 or 20°C (commercial blueberries are commonly stored at 0–1°C). The leaves of the control group were treated with sterile PBS.

The incidence of decay was expressed as the percentage of the inoculated fruit that developed decay in each replicate. Reisolation of the isolates from the decayed fruit was attempted. The experiment was conducted twice.

2.6 | Chemotaxis

A capillary tube assay was used to quantify the chemotactic responses of the isolates. The bacteria were suspended in chemotaxis buffer PME (10 mM potassium phosphate, 1 mM MgCl2 and 0.1 mM EDTA) and placed in a 1.5 ml Eppendorf® tube (And & Ogawa, 1988). The PME buffer with the addition of 0.5% trehalose, fructose, fructosan or inositol was siphoned into disposable capillary tubes, which were then sealed with glue at one end. For the tomato leaf and root extract preparations, 0.05 g of fresh tissue samples was ground in 0.5 ml PME buffer, filtered through a 0.22 µm bacterial filter and siphoned into the capillary tubes. The bacteria were exposed to the attractant by inserting the open end of the capillary tube into the 1.5-ml Eppendorf® tube. At 1 and 2 days postinoculation, the bacterial cells per capillary tube were enumerated by plating samples on LB agar at appropriate dilutions. The results were expressed as the mean of three separate assays.

2.7 | Data analysis

The nucleotide sequences of the 16S rRNA genes belonging to the bacterial isolates were initially aligned with sequences of related bacterial species from the GenBank database using the multiple alignment program ClustalW packaged in BioEdit (Hall, 1999; Thompson et al., 1994). The phylogenetic relationships were determined by the neighbour-joining method with p-distance analysis (Saitou & Nei, 1987). The strength of the internal branches from the resulting tree was statistically tested by bootstrap analysis with 1,000 bootstrap replications (Felsenstein, 1985). The analyses were performed with MEGA 5.0 software (Tamura et al., 2011). The data were subjected to analysis of variance followed by post hoc LSD multiple comparison tests to compare the isolates with each other and the control in terms of mortality ($p < 0.05$). The statistical analyses were performed using SPSS 20.0 statistical software.

3 | RESULTS

3.1 | Phylogenetic analysis

Two different kinds of bacteria and two kinds of yeast were isolated from the nutrient agar, potato dextrose agar and yeast extract peptone dextrose media.

An approximately 1,500-bp fragment and 500-bp fragment of the 16S rRNA and 26S rRNA gene region, respectively, were also sequenced to further characterize the isolates and to construct a dendrogram using closely related species. According to the identification studies, the isolates were identified as *Metschnikowia* sp., Pb2-7; *Metschnikowia aff*., Pb4-1; *Bacillus safensis*., Pb1-5; and *Stenotrophomonas maltophilia*., Pb4-3 (Table 1). These identifications were also supported by the phylogenetic analysis (Figure 1).

The nucleotide sequence accession numbers and the GenBank database accession numbers for the 16S rRNA nucleotide sequences of the isolates are as follows: Pb1-5 and MG210673, Pb2-7 and MG210676, Pb4-1 and MG210677, Pb4-3 and MG210675.

3.2 | Morphological characteristics of the cultures and sporulation

In total, two different bacteria and two different yeasts were isolated from Lanzhou Lily and identified based on a variety of different techniques. The colonies of two isolates (Pb1-5 and Pb4-3) were cream-coloured on NA. The colonies of two isolates (Pb2-7 and

TABLE 1 Proposed identification of the isolates according to the BLAST search using the partial sequence of 16S rDNA and 26S rDNA gene

Isolate	Species	GenBank ID number	Query coverage (%)	Identity (%)
Pb1-5	*Bacillus safensis*	CP015611.1	100	100
Pb2-7	*Metschnikowia pulcherrima*	KT922394.1	92	99
Pb4-1	*Metschnikowia pulcherrima*	JX188179.1	91	99
Pb4-3	*Stenotrophomonas maltophilia*	AF100734.1	100	99

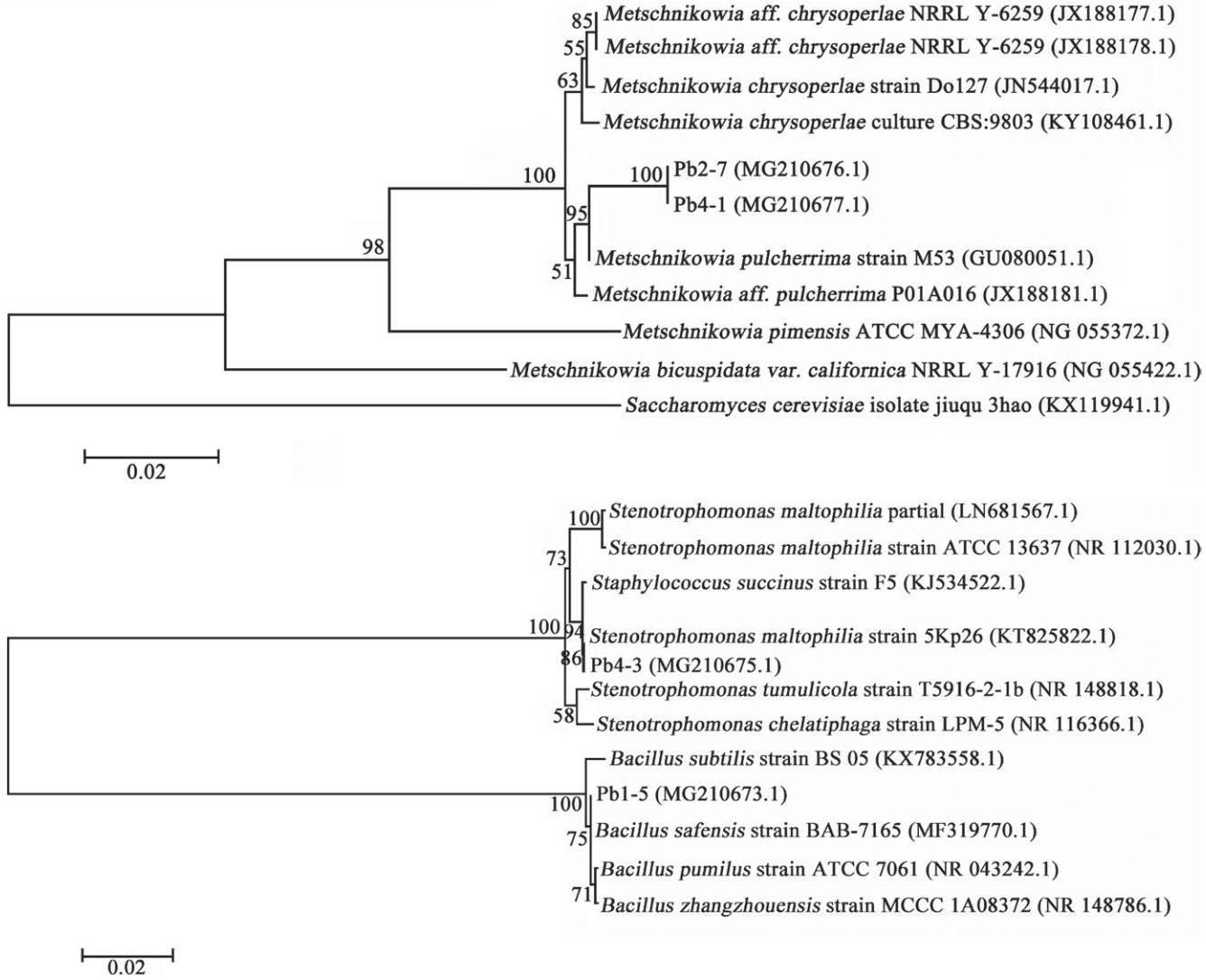

FIGURE 1 The neighbour-joining tree of the bacterial and yeast isolates and their closely related species. The approximately 1,500-bp sequence and 500-bp sequence of the 16S rDNA and 26S rDNA gene were used to construct the dendrogram. Bootstrap values based on 1,000 replicates were indicated above nodes. Bootstrap values C ≥ 70 are labelled. The scale on the bottom of the dendrogram indicates the degree of dissimilarity

TABLE 2 The morphological characteristics of the bacterial isolates

Isolate number	Pb1-5	Pb2-7	Pb4-1	Pb4-3
Colony colour	White	Yellow	Yellow	Light yellow
Colony shape	Smooth	Smooth	Smooth	Smooth
Shape of bacteria	Bacil	Coccus	Coccus	Coccus
Gram stain	+	+	+	+
Spore stain	−	−	+	+
Capsule	−	−	−	−
Motility	−	−	−	−
Turbidity when grown in LB	Turbid	Turbid	Turbid	Turbid
Source	Lanzhou Lily	Lanzhou Lily	Lanzhou Lily	Lanzhou Lily

Pb4-1) were coloured cream on YPD. Three isolates (Pb2-7, Pb4-1 and Pb4-3) were yellow, and one isolate (Pb1-5) was white. All isolates were smooth on NA and YPD. Three isolates (Pb2-7, Pb4-1 and Pb4-3) were bacillus, while one isolate was bacilli or coccobacilli. Four isolates (Pb1-5, Pb2-7, Pb4-1 and Pb4-3) were gram-positive. Two (Pb4-1 and Pb4-3) formed spores. None of them formed capsules. Four isolates (Pv2, Pv3, Pv5 and Pv9) were not motile. All isolates caused turbidity in LB broth (Table 2).

All isolates were catalase positive. Likewise, all isolates were positive in terms of gelatine hydrolysis. All of the isolates could hydrolyse starch. The carbon sources of the isolates were different from each other. Other biochemical properties of the different isolates are listed in Table 3. Their growth curve is shown below in Figure 2.

All isolates were able to grow in 3%, 5%, 7% and 10% NaCl concentrations; however, high concentrations of NaCl affected the growth of the strain (Figure 3). Only Pb4-3 and Pb1-5 grew in pH 3.0, pH 4.0 and pH 5.0, but they were variable with respect to growth at other pH values (Figure 4).

3.3 | Pathogenicity

All the inoculated fruit that were incubated developed an infection, except for the fruit treated with sterile water and a non-pathogenic bacterium (Table 4). The symptoms after inoculation were consistent with the typical rot symptoms that naturally occur with the disease. In the early stage of the disease, the outer scales of the bulbs produced brown depressed spots. The lesions had darker edges. The lesions were rotted in the centre and expanded to the surrounding areas gradually. The amount of rotten tissues increased continuously. Sometimes

TABLE 3 The carbon source utilization of the bacterial isolates

Isolate number	Pb1-5	Pb2-7	Pb4-1	Pb4-3
Mannitol	−	+	+	−
Maltose	−	+	+	−
D-Sorbitol	−	+	+	−
D- (+)-Trehalose dihydrate	+	+	+	−
Galactose	−	+	+	+
Raffinose	−	+	+	−
Fructose	+	+	+	−
Xylose	−	+	+	−
Cellobiose	+	+	+	−
Inositol	−	+	+	−
Melezitose	−	−	+	−
Arabinose	−	+	+	−
Lactose	−	+	+	−
Dulcitol	−	+	+	−
Glucose	+	+	+	+
Maltose	+	+	+	−
N-acetyl-D- (+)-glucosamine	−	+	+	−

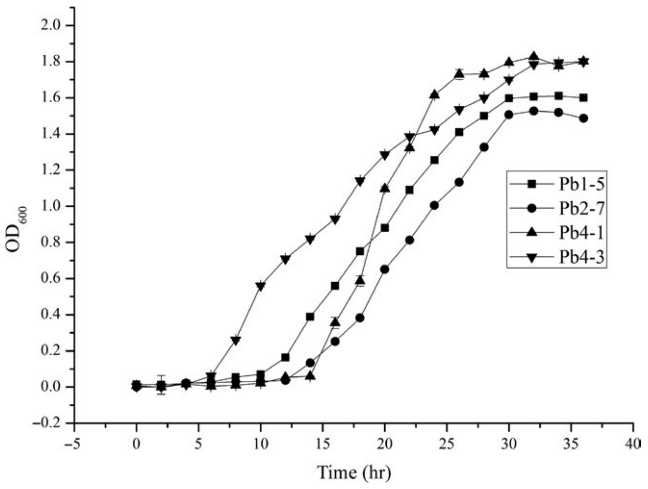

FIGURE 2 The growth curve of strain

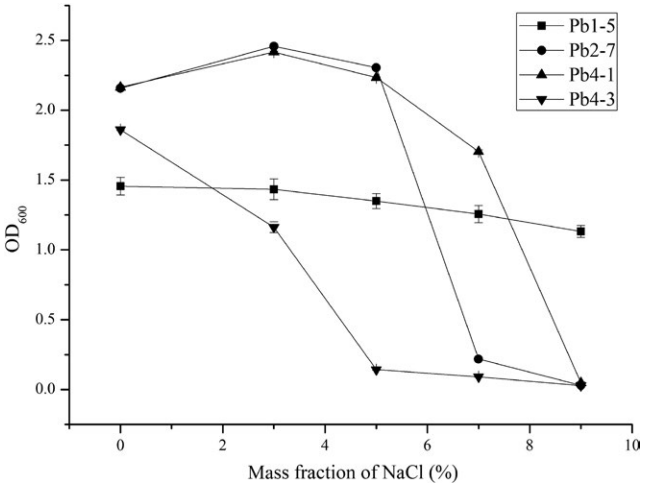

FIGURE 3 The effects of different mass fraction of NaCl on the growth

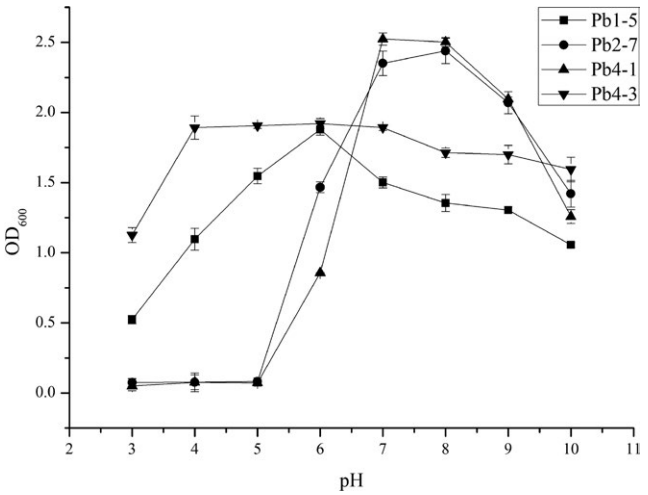

FIGURE 4 The effects of pH on the growth

TABLE 4 Incidence (%) and severity of rot on lily inoculated with different isolated strains

Species	Isolates	28°C for 7 days	
		Incidence	Severity
Bacillus safensis	Pb1-5	100 a	4.0 ± 0.1 ab
Metschnikowia sp. *pulcherrima*	Pb2-7	100 a	3.9 ± 0.2 bc
Metschnikowia pulcherrima	Pb4-1	100 a	3.9 ± 0.2 bc
Stenotrophomonas maltophilia	Pb4-3	100 a	4.2 ± 0.2 a
Sterile water	–	0 c	0 d
Nonpathogenic bacterium	–	0 c	0 d

Note. Severity was based on the lesion development on inoculated Lanzhou Lily using the following scale: 0, no decay; 1, lesion ≤12.5%; 2, 12.5% <lesion ≤25%; 3, 25% <lesion ≤50%; 4, 50% <lesion ≤75%; and 5, 75% <lesion ≤100%. Values followed by the same letter within the same column are not significantly different (p = 0.05).

the rotten parts produced a green or grey mould layer, the internal scales decayed slowly, and finally, the bulbs became dry and rotted. The diseased tissue was removed to isolate and culture the pathogens, and the result of microscopic observation was consistent with that of the inoculated bacteria. The experimental results showed that *B. safensis*, *Metschnikowia pulcherrima*, *M. pulcherrima* and *S. maltophilia* were the main pathogens of bulb decay of Lanzhou Lily during the storage period and that the main invasive route was through the bulb surface wound. The effect of the infection effect is shown in Figure 5.

3.4 | Chemotaxis

Chemotaxis was studied using a capillary tube assay (Sun et al., 2017). The yeast and bacterial cells in capillary tubes were enumerated at 2 days postinoculation. Different parts of the lily attracted the yeast and bacteria, and the bulb was the best energy source. The yeast and bacterial cell numbers reached over 10^5 cfu/ml and 10^7 cfu/ml at 2 days postinoculation, respectively. These results suggest that the plant bulb extract was the most suitable nutrient attractant for all isolates (Figure 6).

4 | DISCUSSION

Lily bulbs have medicinal and health functions such as moistening the lungs preventing coughing, lowering fevers and alleviating diuresis and are cultivated in many parts of China as traditional Chinese medicines for preventing and treating human tuberculosis and neurasthenia. With improvements in people's living standards, the ornamental value of the cut flowers of lilies is increasingly valued, and the cultivation area of lilies in China is also expanding. Lily bulb rot disease, which seriously affects the buds, is the main disease that occurs during storage of the lily. The plants grown from the diseased bulbs are dwarfed, do not flower and die prematurely. During the storage and transportation process, the damage to the bulb by rot can be devastating, causing greater losses from the production and cultivation of lilies.

In our study, we isolated two pathogenic bacteria and two pathogenic yeasts from Lanzhou Lily fruit. Notably, it is the first time in this area of research that these pathogenic bacteria species have been isolated from Lanzhou Lily.

Many of the fungi that cause postharvest disease belong to the phylum Ascomycota and closely related anamorphic fungi (Fungi Imperfecti). In the case of Ascomycota, the asexual stage of the fungus (the anamorph) is usually encountered more frequently than is the sexual stage of the fungus (the teleomorph) in postharvest diseases. Important genera of anamorphic postharvest pathogens include *Penicillium*, *Aspergillus*, *Geotrichum*, *Botrytis*, *Fusarium*,

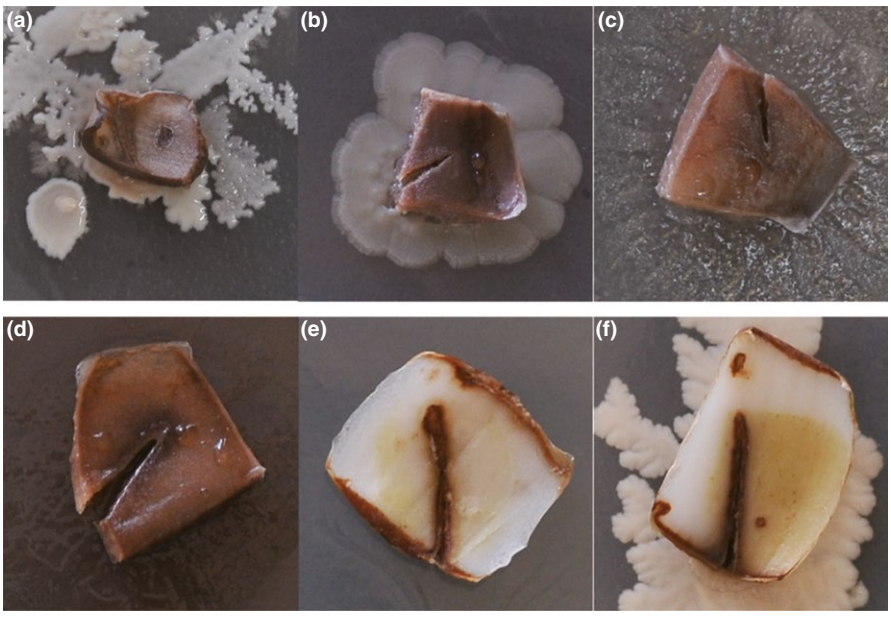

FIGURE 5 Pathogenicity of the bacterial and yeast isolates using the concentration of 1.67×10^7 cfu/ml against Lanzhou Lily within 7 days after application. (a) *Bacillus safensis*, (b) *Metschnikowia pulcherrima* sp., (c) *Metschnikowia pulcherrima* sp., (d) *Stenotrophomonas maltophilia*, (e) sterile water, (f) non-pathogenic bacterium [Colour figure can be viewed at wileyonlinelibrary.com]

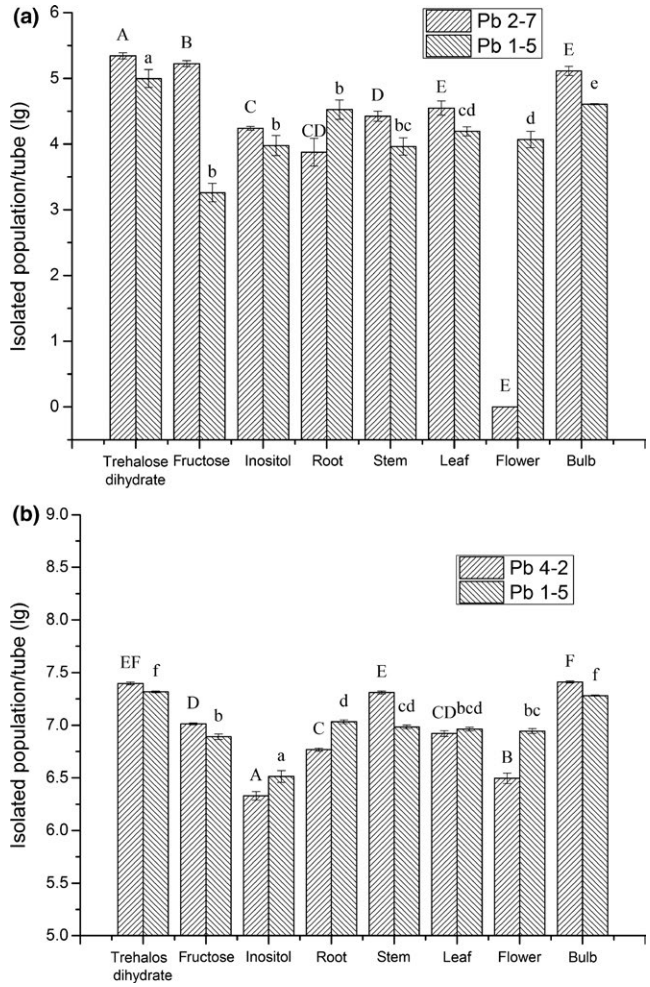

FIGURE 6 Chemotaxis of different isolates. (a) is yeasts' chemotaxis towards selected sugar resources, and tomato extract is shown. (b) is bacterial chemotaxis towards selected sugar resources, and tomato extract is shown. Bacterial concentration in capillary tubes was determined by plating samples at the appropriate dilutions on LB agar plates. The asterisks above the letter indicate significant differences by ANOVA (p = 0.05)

Alternaria, *Colletotrichum*, *Dothtorella*, *Diplodia* and *Phomopsis*. Some of these fungi also have ascomycete sexual stages.

Studies have shown that *F. oxysporum* is the main cause of soil disease in lilies (Wu et al., 2015). The major causal agents of bacterial soft rots are various species of *Erwinia*, *Pseudomonas*, *Bacittus*, *Lactobacillus* and *Xanthomonas*. Bacterial soft rots are common postharvest diseases in many vegetables, but they are generally of less importance in most fruit than it that of lilies. This is because most fruit has a low pH which inhibits the majority of bacterial plant pathogens (Coates & Johnson, 1997).

Shang et al showed that *Fusarium*, *Rhizoctonia*, *Verticillium*, *Penicillium* and *Ilyonectria* (*Neonectria*) in wilted samples may be pathogenetic root rot fungi (Shang et al., 2016). Und, Kotan, Sahin, and Ala, (2006) showed that *A. piechaudii*, *B. pumilus*, *Chromobacterium violaceum*, *E. intermedius*, *Erwinia rhapontici*, *Pantoea agglomerans*, *Pseudomonas aeruginosa*, *Pseudomonas huttiensis*, *Pseudomonas cichorii* and *Pseudomonas putida* are possible foliar pathogens in pome fruits. The results of a study based on biochemical testing, pathogenic testing and phylogenetic analyses showed that *Pseudomonas syringae pv. actinidiae* is the causal agent of "Hongyang" kiwifruit bacterial canker disease (Zhang et al., 2013). In addition to this bacterial pathogen, 14 endophytic fungi were also isolated and identified from kiwifruit leaves, including *Penicillium* sp. *Colletotrichum* sp. *Phomopsis* sp. *Alternaria* sp. and *Nigrospora* sp. Shang et al showed that *Fusarium tricinctum* was isolated from all inoculated plants (Shang et al., 2014). The species *A. tenuissima* has been reported to be the primary causal agent of Alternaria leaf spot and fruit rot in blueberries worldwide (Zhu & Xiao, 2015).

This research was the first to characterize the bacteria and yeast from Lanzhou Lily. Based on multiple DNA sequence comparisons and fungal morphological characteristics, we documented four species, including *B. safensis*, *M. pulcherrima*, *M. pulcherrima* and *S. maltophilia* that are responsible for fruit rot in *Lilium davidii var. unicolor* known as Lanzhou Lily. The experimental results showed that the incidence of disease from wound inoculation was 100%. The symptoms of the inoculated plants were consistent with the symptoms of natural lily bulb decay, and the original inoculum could be isolated from the site of inoculation. Therefore, *B. safensis*, *M. pulcherrima*, *M. pulcherrima* and *S. maltophilia* are the main pathogenic bacteria that cause decay in lily bulbs during storage. The main route of invasion is bulbous surface wounds.

The attraction of motile organisms to specific chemicals, defined as chemotaxis, is a well-documented phenomenon. Bacterial chemotaxis can be demonstrated by the accumulation of the bacteria at the tip of the capillary tube which contains the substance under study and is dipped into the bacterial suspension. How the bacteria are attracted and how the bacterial flagella are directed towards a source of stimulation is still unknown (Adler, 1969).

The possible role of chemotaxis in plant diseases caused by phycomycetes has been studied by Zentmyer, Royle and Hickman (Royle & Hickman, 2001; Zentmyer, 1961). Except for Zentmyer, these authors agree that the zoospore accumulation on roots of host and non-host plants is a non-specific phenomenon (Zentmyer, 1961).

For different isolates, chemotaxis could help them move to the root periphery, adhere to the root surface and aggregate near wound sites. The chemotaxis results from this study suggested that the plant bulb extract was the most suitable nutrient attractant for all isolates.

To reduce the use of pesticides, attention is currently focused on alternative control strategies based on improved cultural practices, biological control and plant defence promoters. Efforts are also being devoted to reducing postharvest losses of horticultural crops by using biological control and physical methods such as ultraviolet illumination, radiofrequency treatment, heat treatments (heat therapy) and storage technologies (Arvanitoyannis, 2007).

Among these methods, postharvest heat treatments such as hot water treatments, hot rinses in hot water along with brushing and hot air treatment provide quarantine security, reduce rot development, enhance fruit resistance to chilling injury in cold-sensitive

cultivars, retain fruit quality during cold storage and increase shelf life. The effect of heat therapy on horticultural crops has been thoroughly reviewed (Barkaigolan & Phillips, 1991; Mulas & Schirra, 2007). Despite the beneficial effects of heat treatments, the complete control of decay is rarely accomplished by heat therapy alone, especially when the fruit is subjected to cold storage prior to marketing. Thus, heat therapy should be combined with other treatments to enhance its efficiency.

However, these practices are usually not enough to protect the product from fungal infections. The use of synthetic fungicides has been the primary means of controlling postharvest diseases, although current trends focus on finding new practical and eco-friendly control alternatives (Youssef et al., 2012). Furthermore, pesticide use is regulated by EC Regulation 1107/2009 that replaced Directive 91/414/EEC, and it applies to any type of plant protection products on the market, including natural products such as plant extracts, semiochemicals and microorganisms (Alabouvette & Cordier, 2011).

Biological control using microbial antagonists has attracted much interest as an alternative to chemical products. The first study on postharvest biocontrol was published by Tronsmo and Dennis (Tronsmo & Dennis, 1977), and some years later, Wilson and Pusey (Wilson & Pusey, 1985) published the most well-known work on the potential of a *Bacillus subtilis* strain to control brown rot in peaches. Since the publication of those articles, many postharvest research laboratories have been searching for effective antagonists for controlling postharvest diseases. However, few of them have reached the marketing phase, mainly because there are steps in the research programme that still have to be developed such as large-scale production, formulation, packaging and shelf life studies (Ippolito & Sanzani, 2011). Additionally, most biocontrol products are being developed by private companies, and little information is available regarding the full use of microorganisms for commercial applications.

Postharvest fungal decays of fresh fruits and vegetables can result in serious losses (Ippolito & Nigro, 2000). Traditionally, these losses have been controlled by applications of fungicides after harvest or prior to shipping to markets. Alternative methods to control these losses have been investigated because fungicides are being removed from the market due to human health risk concerns (Scientific et al., 1987). In addition, fungicides frequently become ineffective due to their tendency to select for fungicide-tolerant strains of the pathogens (Bertrand & Saulie, 1978).

We suggest that an improved control strategy must be established to prevent disease during storage of Lanzhou Lily. For example, an extended study will be conducted to determine an optimal time interval for bioagent application, which may completely prevent the occurrence of rot disease. An alternative approach is to use one of the isolates from this study in addition to other biocontrol agents, such as *Bacillus and Streptomyces* spp. Several studies have demonstrated that a mixture of bioagents is more effective than a single bioagent for bacterial rot control (Kheirandish & Harighi, 2015).

In summary, in this study, we documented that Lanzhou Lily fruit rot in Lanzhou is caused by a combination of species that include *B. safensis*, *M. pulcherrima*, *M. pulcherrima* and *S. maltophilia*.

As different species of bacteria and yeasts may respond to fungicides differently, the findings in this study will provide the basis for the development of chemical control practices targeting specific pathogens and will provide a theoretical basis for how to systematically control lily disease.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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