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SHORT COMMUNICATION

# *Candida xinjiangensis* sp. nov., a new anamorphic yeast species isolated from *Scolytus scheryrewi* Semenov in China

**Xiao-feng Zhu[1,2] · Dian-peng Zhang[3] · Sen Yang[2] · Qing-wen Zhang[1]**

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**Abstract** Three yeast strains designated as S44, XF1 and XF2, respectively, were isolated from *Scolytus scheryrewi* Semenov of apricot tree in Shule County, Xinjiang, China, and were demonstrated to be a new member of the genus *Candida* by sequence comparisons of 26S rRNA gene D1/D2 domain and internal transcribed spacer (ITS) region. BLASTn alignments on NCBI showed that the similarity of 26S rRNA gene sequences of S44 (type strain) to all sequences of other *Candida* yeasts was very low (≦93 %). The phylogenetic tree based on the 26S rRNA gene D1/D2 domain and ITS region sequences revealed that the strain S44 is closely related to *C. blattae*, *C. dosseyi*, *C. pruni*, *C. asparagi*, *C. fructus* and *C. musae*. However, the strain S44 is distinguished from these *Candida* species by the physiological characteristics. Moreover, the strain S44 formed typical pseudohyphae when grown on cornmeal agar at 25 °C for 7 days, but did not form ascospores in sporulation medium for 3–4 weeks. Therefore, the name *Candida xinjiangensis* is proposed for the novel species, with S44 (=KCTC$^{T}$27747) as the type strain.

**Keywords** Apricot tree · *Candida xinjiangensis* sp. nov. · China · *Scolytus scheryrewi*

## Introduction

*Scolytus schevyrewi* Semenov (Coleoptera: Scolytidae) was recorded in 1902 in eastern Tianshan Mountain area of Xinjiang, China (Wood and Bright 1992). *S. schevyrewi* is a native pest in Asia that mainly distributed in China, Korea, Mongolia, Russia, Kazakhstan, Uzbekistan, Kyrgyzstan, Turkmenistan and Tajikistan (Douglas et al. 2013; Negrón et al. 2005; Yin et al. 1984). It was first reported in Colorado and Utah in 2003 (Negrón et al. 2005) and then entered Canada in 2006 (Langor et al. 2009). *S. schevyrewi* attacks a wide range of host plants of Ulmaceae, Fabaceae, Elaeagnaceae, Salicaceae and Rosaceae (Negrón et al. 2005). The pest not only can cause the death of a large number of elms, but also coexists with a variety of elm pathogens and spreads elm wilt disease (Jacobi et al. 2007, 2013). The ecology of this insect including its interactions with the hosts has been studied (Lee et al. 2011; Li et al. 2009); however, only a few symbiotic microorganisms of *S. scheryrewi* are well documented.

Yeasts occur naturally on fruits and vegetables and other niches such as soils, waters and insects (Droby et al. 1997; Miller and Phaff 1998). Some yeasts have been

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The GenBank/EMBL/DDBJ accession numbers for the 26S rRNA gene D1/D2 and ITS region sequences of strain *Candida* sp. S44 in this study are KU051541 and KU240035, respectively.

**Electronic supplementary material** The online version of this article (doi:10.1007/s00203-016-1294-5) contains supplementary material, which is available to authorized users.

✉ Sen Yang
yangsenxj@126.com

✉ Qing-wen Zhang
zhangqingwen@263.net

[1] Department of Entomology, College of Plant Protection, China Agricultural University, Beijing 100094, China

[2] Institute of Plant Protection, Xinjiang Academy of Agricultural Sciences, Urumqi 830091, China

[3] Institute of Plant and Environment Protection, Beijing Academy of Agriculture and Forestry Sciences, Beijing 100097, China

isolated from the gut or surfaces of insects that feed on a variety of materials, including basidiomycete fruiting bodies, woody substrates, ephemeral flowers and nectar exudates (Kurtzman 2001; Lachance and Bowles 2002a, b; Lachance et al. 2001a, b, 2005; Pimentel et al. 2005; Nguyen et al. 2006, 2007). In the investigation of symbiotic microorganisms of *S. scheryrewi*, a new *Candida* species was discovered. The objective of this study is to describe the new yeast species belonging to the genus *Candida*.

## Materials and methods

### Yeast strain and culture conditions

Several bark beetles were collected from different apricot trees (*Prunus aremeniaca* L. cv. Saimaity) in Shule County of Kashi Prefecture, Xinjiang, China, in the years of 2014 and 2015 and identified as *S. scheryrewi* Semenov (Fig. 1) by its morphological characteristics (Wood and Bright 1992; Yin et al. 1984). The bark beetles were suspended in a 300-ml conical flask containing 100 ml of sterile deionized water and then incubated at 27 °C for 20 min on rotary shake at 150 rpm. One hundred microliters of the suspension was aliquoted and transferred to YPDA plates (20 g $l^{-1}$ dextro-glucose, 20 g $l^{-1}$ peptone casein, 10 g $l^{-1}$ yeast extract, 20 g $l^{-1}$ agar, streptomycin sulfate 100 mg $l^{-1}$), and the plates were subsequently incubated at 30 °C for 48 h. The cultures were purified twice by streaking on YPDA plates. The three strains, S44, XF1 and XF2, were, respectively, isolated and maintained in PDA slants.

**Fig. 1** The bark beetle *Scolytus scheryrewi* of apricot tree

### DNA manipulation

The strain was identified by sequencing the internal transcribed spacer 1 (ITS1), 5.8S ribosomal RNA gene and internal transcribed spacer 2 (ITS2) according to White et al. (1990) and the D1/D2 domain at the 5′ end of the 26S rRNA gene according to Kurtzman and Robnett (1998). The DNA from antagonist cell suspensions grown in YPD for 48 h was extracted using NucleoMag 96 Plant Kit (Macherey–Nagel, Oensingen, Switzerland) and Kingfisher magnetic particle processor (Thermo Labsystems, Basingstoke, UK) following the manufacturers' protocols. The ITS regions were amplified using genomic DNA as a template and universal primers ITS1 and ITS4. The D1/D2 domains were amplified using the primers NL-1 and NL-4 on the genomic DNA. Each 20 µl PCR contained 1 µl of DNA template (50 ng), 200 mM of each deoxynucleotide triphosphate, 2 µl of 10X buffer (Taq DNA Polymerase, Qiagen, Chatsworth, CA, USA), 0.7 mM each primer and 1.0 U Taq DNA Polymerase (Qiagen). PCR program for ITS regions followed: 95 °C, 3 min; 34 cycles: 94 °C, 15 s; 55 °C, 45 s; 72 °C, 55 s; 72 °C, 7 min; and 4 °C. The program for D1/D2 domain was: 95 °C, 10 min; 30 cycles: 94 °C, 30 s; 55 °C 30 s; 72 °C, 45 s; 72 °C, 7 min; and 4 °C. A 10 µl aliquot of PCR products from each reaction was electrophoresed in 2.0 % agarose gel in TBE buffer and then stained with SYBR SAFE (Invitrogen, Eugene, OR, USA). Gel images were acquired with a Gel Doc 1000 System (Bio-Rad Laboratories, Hercules, CA, USA). PCR amplification products were cloned into the PCR4 TOPO vector (Invitrogen) using the TOPO TA cloning kit following the manufacturer's protocol and sequenced by Tianyihuayuan Sequencing company (Beijing, China) using an IIIumina HiSeq 2000 Sequencer (lllumina, USA).

### Phylogenetic analysis

The sequences were analyzed by using the software BLASTn (Basic Local Alignment Search Tool; Altschul et al. 1990) for similarity. Sequence data were first aligned with the program CLUSTAL_X (Thompson et al. 1997) and then subjected to the program MEGA 4.10. A phylogenetic tree was constructed from evolutionary distance data that were calculated with the neighbor-joining method (Saitou and Nei 1987) using Kimura's two-parameter distance measure (Kimura 1980). Confidence limits for phylogenetic trees were estimated from bootstrap analysis (1000 replications). Reference sequences were retrieved from GenBank under the accession numbers indicated in the tree.

### Morphology and physiology tests

To morphologically and physiologically characterize the new *Candida* species, three strains were tested according to the standard methods as described by Yarrow (1998).

## Results and discussion

### Culture isolation

Three strains were isolated from *S. scheryrewi*. The three strains were designated as S44, XF1 and XF2, respectively. The specific information on the strains is shown in Table 1.

### Phylogeny

BLASTn results showed that the similarity of 26S rRNA gene sequences of the type strain S44 to all sequences of other known yeasts was very low (≦93 %). Phylogenetic analysis of the 26S rRNA gene D1/D2 domain and ITS region sequences revealed that the strain S44 is closely related to *Candida blattae* ATCC MYA-4360$^T$, *C. dosseyi* NRRL Y-27950$^T$, *C. pruni* sp-Quan$^T$, *C. asparagi* SN 15-1$^T$, *C. fructus* CECT 11884$^T$ and *C. musae* CECT 11882$^T$. The strain S44 was placed into the same clade with *C. blattae*, *C. dosseyi*, *C. pruni*, *C. asparagi*, *C. fructus* and *C. musae* (Fig. 2). However, the sequence similarity of 26S rRNA gene D1/D2 domain and internal transcribed spacer (ITS) region sequences of S44 was very lower (≦90 %) to those of other six *Candida* yeasts. Regarding the sequences of ITS, compared with the type strain "S44," the strains "XF1" and "XF2," respectively, showed 100 % (372/372) and 99 % (371/372) similarity with the type strain "S44." Regarding the sequences of 26S rRNA gene, the strains "XF1" and "XF2" showed 99 % (544/547) and 99 % (543/547) similarity with the type strain "S44," respectively. In the phylogenetic tree, the three novel stains were placed into one single clade. When the phylogenetic tree was constructed by maximum likelihood method, the three novel strains were also placed into one single clade (Fig. 3), which confirmed the results obtained by neighbor-joining analysis. Based on the phylogenetic analysis, the strain "S44" together with strains "XF1" and "XF2" is described as a new species of *Candida* and named as *Candida xinjiangensis* sp. nov.

### Morphology and physiology

Morphological culture showed that the cells of *C. xinjiangensis* (strain S44) are (3.0 − 9.0) × (2.0 − 9.5) μm in size and are globose to ovoid, single, in pairs and in short chains when grown in yeast malt extract medium (YM) broth at 25 °C for 3 days, and the sediment is formed (Fig. 4). The colonies are cheese-like in shape and appear off-white in color when grown on YM agar plates at 25 °C for one month (Fig. 5a). When grown on cornmeal agar at 25 °C for 7 days, the yeast produced pseudohyphae (Fig. 5b). No sexual state was observed in cultures of the strain when grown on 5 % malt extract agar at 25 °C for 1–3 weeks or on cornmeal agar at 25 °C for 1–4 weeks or on YPD agar for 1–3 weeks or on PDA for 1–3 weeks.

Physiological tests (Table 2) indicated that *C. xinjiangensis* (strain S44) is distinguished from *C. blattae* by its sucrose fermentation and its growth at 37 °C, distinguished from *C. dosseyi* by its non-fermentation of raffinose and its growth at 37 °C and distinguished from *C. pruni* by its sucrose fermentation, assimilation of D-ribose and inassimilation of L-rhamnose and nitrate. *C. xinjiangensis* is distinguished from *C. asparagi* by its fermentation of galactose and sucrose, and its growth at 37 °C or in vitamin-free medium, and distinguished from *C. fructuse* and *C. musae* by its fermentation of galactose and sucrose, and assimilation of galactose and by its growth at 37 °C or in vitamin-free medium. Moreover, *C. xinjiangensis* formed typical pseudohyphae when grown on cornmeal agar at 25 °C for 7 days, but did not form ascospores in sporulation medium for 3–4 weeks.

### Description of *Candida xinjiangensis* sp. nov.

*Candida xinjiangensis* (xinjiang'ensis. L. gen. n. Geographic name, a province of China, from where the three strains were collected).

**Table 1** Strains of *Candida xinjiangensis* sp. nov., isolated from the beetles of different apricot trees

| Strains | Source of isolation | Coordinates/height above sea level | GenBank accession number D1/D2 | ITS |
|---|---|---|---|---|
| S44 | *Scolytus scheryrewi* collected from apricot tree in Shule County of Kashi Prefecture, Xinjiang, China, August 2015 | 76°4′6″E, 39°22′48″N 1250 m H | KU051541 | KU240035 |
| XF1 | *Scolytus scheryrewi* collected from apricot tree in Shule County of Kashi Prefecture, Xinjiang, China, August 2014 | 76°4′6″E, 39°22′48″N 1250 m H | KX644909 | KX644907 |
| XF2 | *Scolytus scheryrewi* collected from apricot tree in Shule County of Kashi Prefecture, Xinjiang, China, August 2014 | 76°4′6″E, 39°22′48″N 1250 m H | KX644910 | KX644908 |

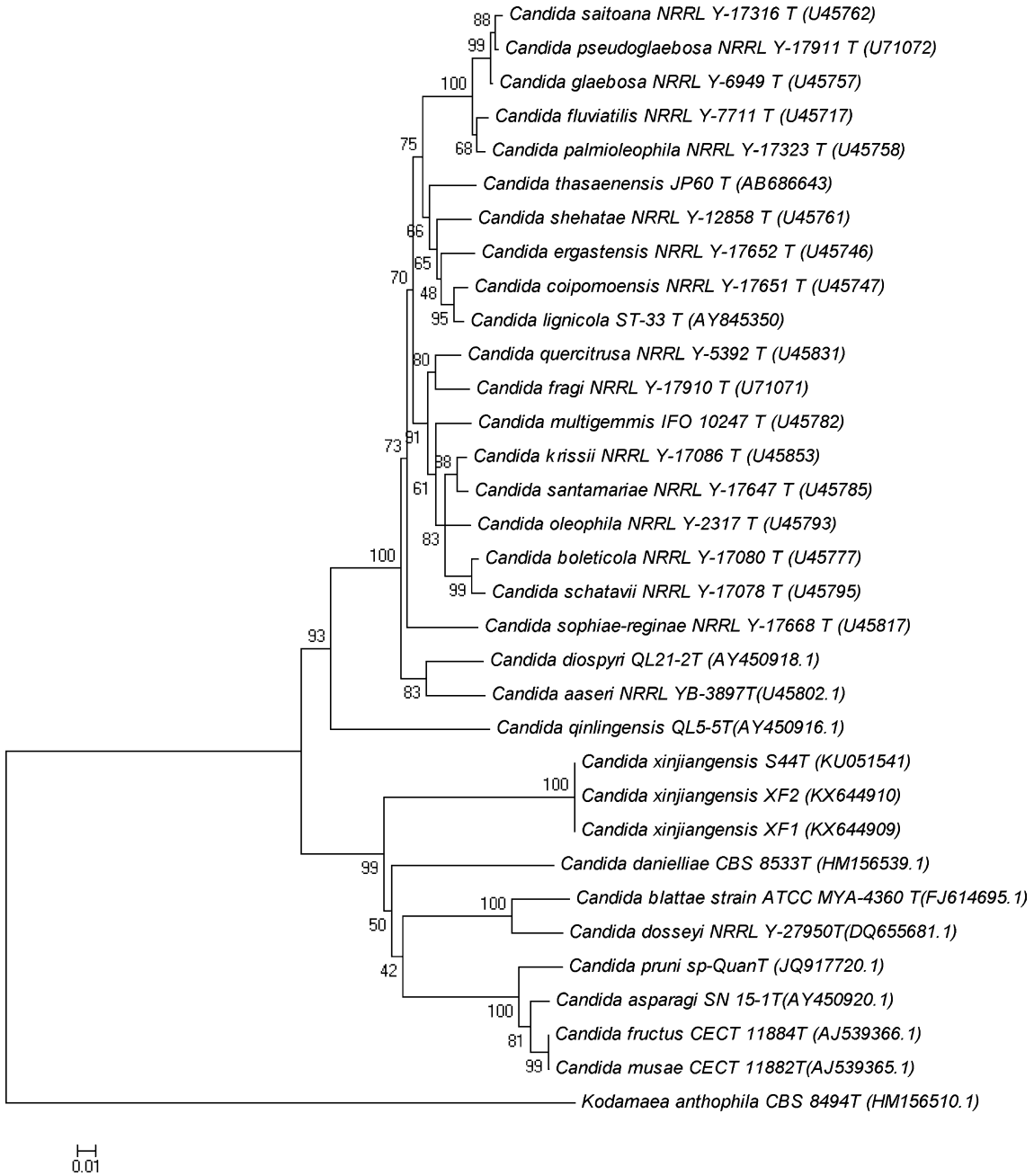


**Fig. 2** Phylogenetic relationships between *Candida xinjiangensis* sp. nov. S44$^T$ and members of the *Candida* clade inferred using neighbor-joining analysis (Kimura two-parameter mode) of combined sequences of the D1/D2 domains of the 26S rRNA gene and ITS regions. *Kodamaea anthophila* represents the outgroup. Scale represents 0.02 nucleotide substitutions per site. Bootstrap values (%) based on 1000 replications are given on each node. Bar means 1 % sequence divergence. Reference sequences were retrieved from GenBank or CBS under the accession numbers indicated in parentheses

Growth in YM broth: After incubation for 3 days at 25 °C, the cells are (3.0–9.0) × (2.0–9.5) μm in size and are globose to ovoid in shape and occur singly, in pairs or in short chains (Fig. 3). In addition, the sediment is formed and budding is multilateral. Growth on YM agar: After 7 days at 25 °C, the colonies of the streak culture

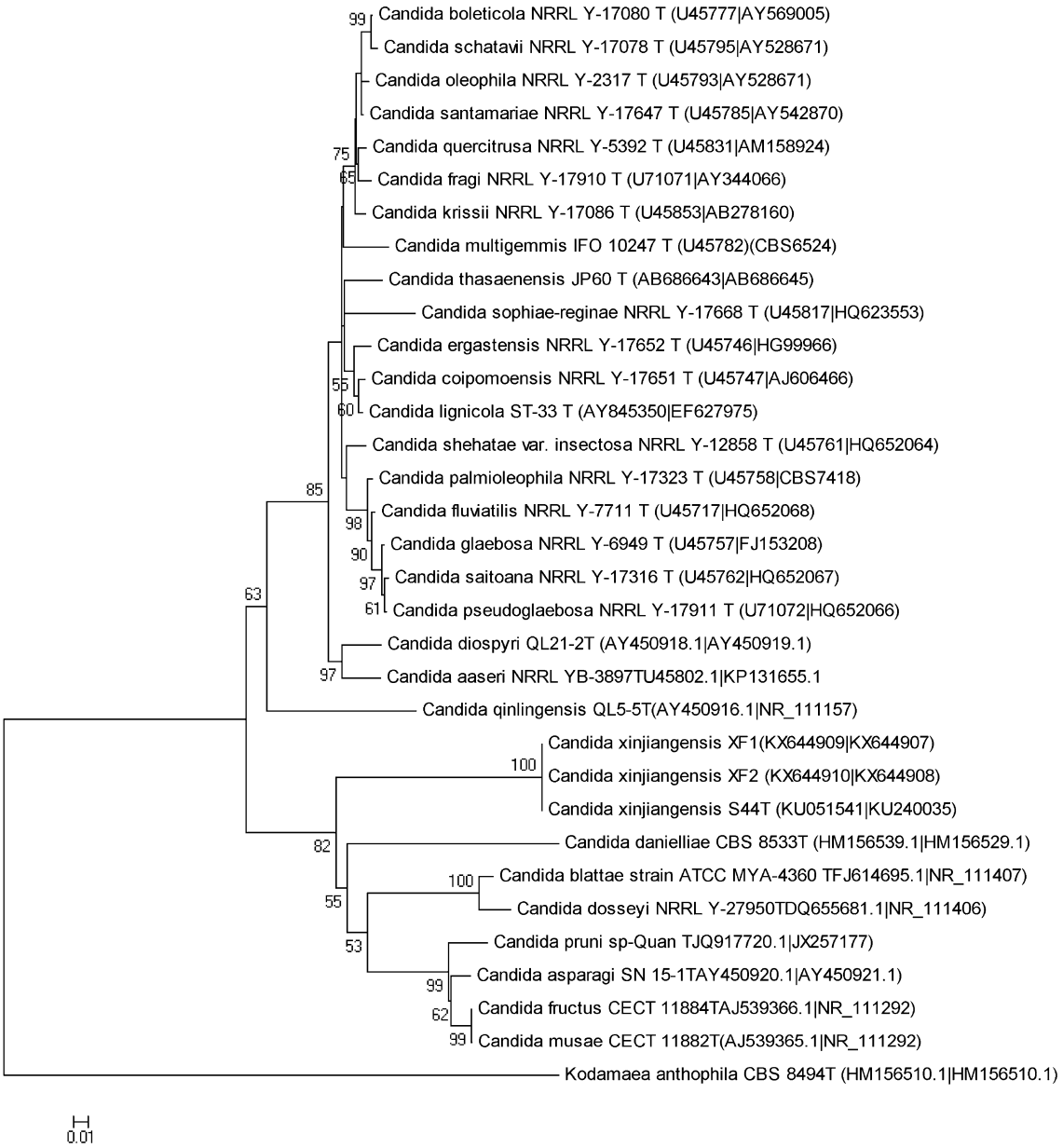


**Fig. 3** Phylogenetic relationships between *Candida xinjiangensis* sp. nov. S44$^T$ and members of the *Candida* clade inferred using maximum likelihood analysis of combined sequences of the D1/D2 domains of the 26S rRNA gene and ITS regions. *Kodamaea anthophila* represents the outgroup. Scale represents 0.02 nucleotide substitutions per site. Bootstrap values (%) based on 1000 replications are given on each node. Bar means 1 % sequence divergence. Reference sequences were retrieved from GenBank or CBS under the accession numbers indicated in *parentheses*

are cheese-like in shape, off-white in color, folded and procumbent, with a wavy border. Dalmau plate culture on cornmeal agar: After 7 days at 25 °C, true hyphae are not formed, but typical pseudohyphae are observed (Fig. 4).

Glucose, galactose and sucrose are fermented; maltose, lactose and raffinose are not. Glucose, galactose, sucrose, maltose, D-arabinose, cellobiose, trehalose, raffinose, D-xylose D-mannitol, ribitol, L-lysine, cadaverine, citrate and succinic acid are assimilated; L-arabinose, D-ribose, lactose, soluble starch, L-rhamnose, erythritol and inositol are not. Nitrates are not assimilated; growth in vitamin-free medium is positive. Growth at 37 °C is positive. The type

**Table 2** Comparison of the physiological characteristics of *Candida xinjiangensis* sp. nov. with six closely related species

| Characteristics | *C. xinjiangensis* | *C. blattae* | *C. dosseyi* | *C. pruni* | *C. asparagi* | *C. fructus* | *C. musae* | *C. danielliae* |
|---|---|---|---|---|---|---|---|---|
| Fermentation | | | | | | | | |
| Galactose | + | + | + | − | + | − | − | − |
| Sucrose | + | − | + | − | − | − | − | *w* |
| Maltose | − | − | + | − | − | − | − | − |
| Raffinose | − | − | + | − | − | − | − | − |
| Assimilation | | | | | | | | |
| Galactose | + | + | + | + | + | − | − | − |
| L-Arabinose | − | − | − | − | − | − | − | − |
| D-Arabinose | d | + | d | − | + | − | − | − |
| D-Ribose | − | − | d | + | *w* | + | − | − |
| Maltose | + | + | + | + | + | − | + | − |
| Cellobiose | + | + | + | + | + | − | − | − |
| Sucrose | + | + | + | + | + | − | + | + |
| Raffinose | + | + | + | − | − | − | − | − |
| L-rhamnose | − | + | + | − | − | − | − | − |
| Inositol | − | n | n | − | − | − | − | n |
| Other tests | | | | | | | | − |
| Nitrate | − | − | − | + | + | + | + | − |
| Succinic acid | + | n | n | + | + | + | + | n |
| Citric acid | + | n | n | + | *w* | + | + | n |
| Growth at 37 °C | + | − | − | + | − | − | − | + |
| Vitamin-free medium | + | + | + | + | − | − | − | − |

The lists of carbon sources and nitrates and other tests were prepared using standard methods described by Yarrow (1998). In response to fermentation, assimilation and other tests, the three strains of *C. xinjiangensis* sp. nov. were tested and their characteristics are the same

+ positive, − negative, *d* delayed positive, *w* weak and *n* no data

Data of reference species were taken from Zhang et al. (2014) and Nguyen et al. (2007)

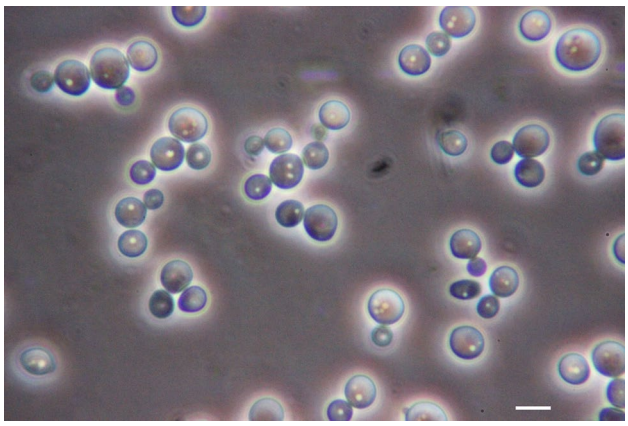

**Fig. 4** Cells of *Candida xinjiangensis* sp. nov. after 3 days of growth in YM broth at 25 °C, *bar* 10 µm

strain, S44, isolated from the bark beetles (*S. scheryrewi*) collected in Shule County of Kashi Prefecture, Xinjiang province of China, has been deposited in the Korean Collection for Type Cultures (KCTC), Seoul, Korea, as KCTC 27747$^{T}$.

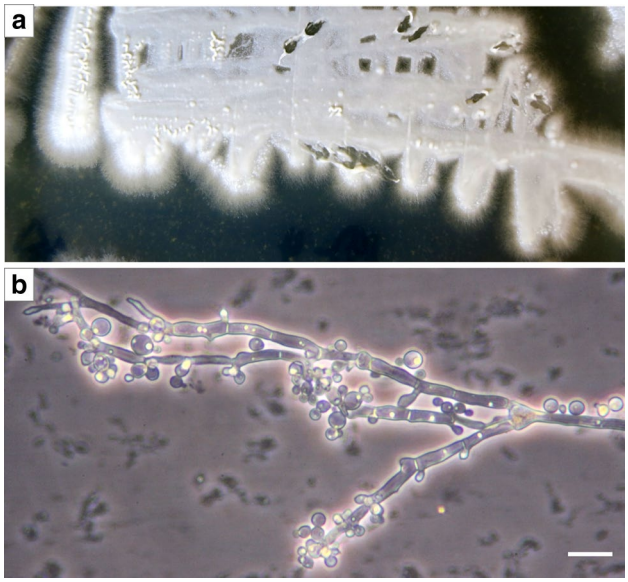


**Fig. 5** **a** Colony morphology and **b** pseudohyphae of *Candida xinjiangensis* sp. nov. after 7 days of growth on CM agar at 25 °C, *bar* 30 µm

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