Kazachstania taianensis sp. nov., a novel ascomycetous yeast species from orchard soil

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Three teleomorphic ascomycetous yeast isolates (TA11TR-1^T, TA11TR-4 and TA11TR-6) from orchard soil from Tai'an, Shandong province, China, were shown to represent a novel species within the genus *Kazachstania* based on phenotypic characterization and sequence analyses of the 18S rRNA gene, internal transcribed spacer (ITS) regions and 26S rDNA gene D1/D2 domain. The name *Kazachstania taianensis* sp. nov. (type strain TA11TR-1^T = AS 2.4160^T = CBS 11405^T) is proposed. *K. taianensis* sp. nov. clustered in a branch together with *Kazachstania sinensis*, *Kazachstania naganishii* and the *Kazachstania telluris* complex with moderate bootstrap support in the neighbour-joining tree reconstructed from combined 18S and D1/D2 sequences. The novel species possessed unusual ITS 1 (338 bp) and ITS 2 (488 bp) sequences. The total length of the ITS–5.8S rDNA gene region of the species was 983 bp, being much longer than those of other ascomycetous yeast species described so far.

Postharvest pathogens can cause major losses of apples during storage. Biological control of postharvest diseases of apple fruit with antagonists has emerged as the most effective alternative to fungicides in recent years (Droby, 2006). Many recognized antagonistic yeasts isolated from fruits, leaves and soils have been reported (Lima *et al.*, 2006; Janisiewicz, 1996; Janisiewicz & Korsten, 2002; Sansone *et al.*, 2005; Wilson *et al.*, 1993). Among the microbial antagonists used for the successful control of postharvest diseases of fruits and vegetables listed in a recent review by Sharma *et al.* (2009), 19 are yeast species, including 13 ascomycetes and six basidiomycetes. Of those antagonistic yeasts, nine species have been used for effective control of postharvest diseases of apple fruit, including *Candida oleophila*, *Cryptococcus laurentii*, *Metschnikowia pulcherrima* and *Rhodotorula glutinis* (Sharma *et al.*, 2009).

To obtain effective antagonistic yeasts to control Fuji apple (*Malus domestica* Borkh.) fruit decay, we investigated the yeast diversity on the surface of Fuji apple fruit and in the

soil below the apple trees. Approximately 315 yeast strains were isolated from the Tai'an area in Shandong province, China. These strains were identified based on the sequences of the 26S rDNA gene D1/D2 domain and internal transcribed spacer (ITS) region. A total of 28 species belonging to 12 genera were recognized. Among the yeast isolates, three were found to represent an undescribed species in the genus *Kazachstania*. The novel species is hereby described as *Kazachstania taianensis* sp. nov.

The yeast strains TA11TR-1^T, TA11TR-4 and TA11TR-6 were isolated from a soil sample collected from an apple orchard in Tai'an, Shandong Province, China, by an enrichment method using YM broth containing chloramphenicol (200 µg ml⁻¹) to inhibit the growth of bacteria. Morphological, physiological and biochemical characteristics were examined according to standard methods for yeast taxonomy (Yarrow, 1998). Nuclear DNA was extracted by using the method of Makimura *et al.* (1994). The ITS region sequence, including the 5.8S rDNA gene and 26S rDNA gene D1/D2 domain, was determined by the methods described previously (Bai *et al.*, 2002). 18S rDNA gene sequences were determined according to Wang *et al.* (2003). Sequences were aligned by using the CLUSTAL X program (Thompson *et al.*, 1997). Phylogenetic trees were reconstructed from the evolutionary distance data calculated from Kimura's two-parameter model (Kimura, 1980) by using the neighbour-joining method (Saitou & Nei,

Abbreviation: ITS, internal transcribed spacer.

The GenBank/EMBL/DDBJ accession number for the 18S rDNA gene, ITS region and 26S rDNA gene D1/D2 domain sequences of strain TA11TR-1^T is GQ181169.

Electrophoretic karyotypes of strains TA11TR-1^T, TA11TR-6 and TA11TR-4 are available with the online version of this paper.

1987). Bootstrap analyses (Felsenstein, 1985) were performed from 1000 random resamplings. Reference sequences were retrieved from the GenBank database under the accession numbers indicated on the tree (Fig. 1). Electrophoretic karyotyping was performed using the method described by Lu *et al.* (2004).

Morphology and physiology

Strains TA11TR-1^T, TA11TR-4 and TA11TR-6 reproduced asexually by multilateral budding. They produced ascospores on diluted V8 juice agar (1:19) after 7 days at 25 °C. Vegetative cells transformed directly into persistent asci, each containing one or two globose or ellipsoidal ascospores (Fig. 2). Glucose and galactose were fermented. Only glucose, galactose, D-ribose and ethanol were assimilated among the carbon compounds tested. Based on phenotypic characters, it was difficult to classify the two strains into a specific genus.

The physiological differences between *K. taianensis* sp. nov. and closely related species are shown in Table 1. Specifically, *K. taianensis* sp. nov. differed from the species in the *K. telluris* complex (Kurtzman *et al.*, 2005) by its ability to ferment and assimilate galactose and its inability

to grow at 40 °C, and from *K. sinensis* and *K. naganishii* by its inability to ferment sucrose and to assimilate sucrose, L-lysine and cadaverine (Table 1).

Phylogenetic analysis and electrophoretic karyotyping

The D1/D2 and ITS sequences of strains TA11TR-1^T, TA11TR-4 and TA11TR-6 were identical. A BLAST search of the GenBank database showed that the closest matches to the D1/D2 sequence of strain TA11TR-1^T were *Kazachstania servazzii*, *Kazachstania aquatica* and an undescribed strain ST-394 from Thailand. The novel isolates differed from the type strains of the two described species and the Thailand strain by 22–25 (3.8–4.2 %) mismatches (15–17 substitutions and 7–8 indels) in the approximately 590 bp D1/D2 domain analysed. The ITS sequence of the three novel strains was quite unique. The lengths of the ITS 1 and ITS 2 regions were 338 and 488 bp, respectively, making the total length of the ITS–5.8S rDNA gene region (983 bp) much longer than those of other ascomycetous yeast species recognized so far. A BLAST search of GenBank using either the ITS 1 or ITS 2 region sequences revealed no sequences with significant similarity.

Fig. 1. Phylogenetic tree reconstructed from neighbour-joining analysis of the combined sequences of the 18S rDNA gene and 26S rDNA gene D1/D2 domain, depicting the relationships of strain TA11TR-1^T with closely related taxa. Bootstrap percentages over 50 % from 1000 bootstrap replicates are shown. Reference sequences were from the type strains of the species retrieved from GenBank under the accession numbers indicated. Bar, 0.01 substitutions per nucleotide position.

Fig. 2. Vegetative cells of strain TA11TR-1^T grown in YM broth for 3 days at 25 °C (a), and asci and ascospores formed on diluted V8 juice agar (1 : 19) after 7 days at 25 °C (b). Bars, 10 µm.

Since it was not possible to align the ITS sequence of strain TA11TR-1^T with those of other related ascomycetous yeast species, the 18S rDNA gene and D1/D2 domain sequences were used in the phylogenetic analysis. In the neighbour-joining tree reconstructed from combined 18S and D1/D2 sequences, strain TA11TR-1^T was located in a clade containing several *Kazachstania* species with strong bootstrap support (Fig. 1). The strain was clustered in a branch together with *Kazachstania sinensis*, *Kazachstania naganishii* and the *Kazachstania telluris* complex (Kurtzman *et al.*, 2005) with only moderate or weak support. Strain TA11TR-1^T differed from these described species by 40–50 (6.8–8.5 %) mismatches in the D1/D2 domains. ITS sequence comparison of strain TA11TR-1^T with its phylogenetic neighbours provided no evidence that the extra length in both the ITS 1 and ITS 2 regions of the novel strain was caused by insertion events. The sequence analyses clearly indicate that strains TA11TR-1^T, TA11TR-4 and TA11TR-6 represent a novel species of the genus *Kazachstania*, for which the name *Kazachstania taianensis* sp. nov. is proposed.

Since the three strains studied were isolated from the same soil sample, showed the same phenotypic characteristics and possessed identical ITS and D1/D2 sequences, their chromosomal DNA banding patterns were compared to examine whether or not these strains were independent. Ten chromosomal bands representing at least 14 chromosomes (wider bands or bands with stronger relative intensity may correspond to doublets or triplets) were resolved for each strain (Supplementary Fig. S1 available in IJSEM Online). The banding patterns of strains TA11TR-1^T, TA11TR-4 and TA11TR-6 were almost identical, suggesting that they were clones of the same strain. Although only a single strain representing the novel species is available at present, the clear isolation of its phylogenetic position and its unusual ITS sequence suggest that the proposal of the new species is beyond question.

Latin diagnosis of *Kazachstania taianensis* R. Chen, S.-C. Wei, Q.-M. Wang & F.-Y. Bai sp. nov.

In medio liquido YM post dies 3 ad 25 °C, cellulae ellipsoideae, ovoidae, 2.8–5.2 × 4.8–6.0 µm, singulae et binae. Post 1 mensem sedimentum formatur. In agar farinae Zea maydis confecto pseudomycelium non observatae. Asci inconjugatio fiunt. Ascosporae globosae vel ellipsoideae, 1 vel 2 in quoqueasco.

Glucosum et galactosum fermentantur at non sucrosum, maltosum, lactose nec raffinose. Glucosum, galactosum, D-ribosum (lente et exigue) et ethanolum (lente et exigue) assimilantur at non L-sorbose, sucrosum, maltosum, cellobiosum, trehalosum, lactosum, melibiosum, melezitolum, raffinose, inulinum, amyllum solubile, D-xylosum, L-arabiosum, D-arabiosum, L-rhamnosum, D-glucosaminum, methanolum, glycerolum, erythritolum, ribitolum, galactitolum, D-mannitolum, α-methyl-D-glucoside, salicinum, DL-lacticum, acidum succinicum, acidum citricum, inositolum net hexadecane. Ammonium sulfatum at non natrum nitrosum, kalium nitricum, L-lysinum, ethylaminum assimilantur net cadaverinum. Ad crescentiam vitamina externa necessaria sunt. Maxima temperatura crescentiae:

Table 1. Physiological characteristics that differentiate the novel strains from closely related species

Species: 1, *Kazachstania taianensis* sp. nov.; 2, *K. bovina*; 3, *K. heterogenica*; 4, *K. pintolopesii*; 5, *K. slooffiae*; 6, *K. telluris*; 7, *K. naganishii*; 8, *K. sinensis*. +, Positive; w, weakly positive; –, negative.

Characteristic	1	2	3	4	5	6	7	8
Fermentation of:								
Galactose	+	–	–	–	–	–	+	–
Sucrose	–	–	–	–	–	–	+	+
Assimilation of:								
Galactose	+	–	–	–	–	–	+	+
Sucrose	–	–	–	–	–	–	+	+
L-Lysine	–	–	–	–	–	–	+	+,w
Cadaverine	–	–	–	–	–	–	+	+,w
Growth at 40 °C	–	+	+	+	+	+	–	–

34 °C. *Materia amyloidea iodophila non formantur. Diazonium caeruleum B non respondens. Ureum non hydrolysatur. Typus: isolatus ex terra, TA11TR-1^T, depositus in collectione China General Microbiological Culture Collection Center, Academia Sinica (AS 2.4160^T).*

Description of *Kazachstania taianensis* R. Chen, S.-C. Wei, Q.-M. Wang & F.-Y. Bai sp. nov.

Kazachstania taianensis (tai.an.en'sis. N.L. fem. adj. *taianensis* pertaining to Tai'an, the geographical origin of the type strain).

In YM broth, after 3 days at 25 °C, cells are ellipsoid or ovoid, 2.8–5.2 × 4.8–6.0 µm and occur singly or in pairs. Budding occurs singly. After 1 month at 25 °C, sediment is present. Pseudohyphae are not observed in cultures grown on cornmeal agar. Sporulation is observed on V8 juice agar (1:19) after 7 days at 25 °C; vegetative cells transform directly into persistent asci, each containing one or two globose or ellipsoid ascospores. Glucose and galactose are fermented; sucrose, maltose, lactose and raffinose are not fermented. Glucose, galactose, D-ribose (weak and delayed) and ethanol (weak and delayed) are assimilated; sucrose, L-sorbose, maltose, cellobiose, trehalose, lactose, melibiose, melezitose, raffinose, inulin, soluble starch, D-xylose, L-arabinose, D-arabinose, L-rhamnose, D-glucosamine, methanol, glycerol, erythritol, ribitol, galactitol, D-mannitol, methyl α-D-glucoside, salicin, DL-lactic acid, succinic acid, citric acid, inositol and hexadecane are not assimilated. Ammonium sulfate is assimilated; potassium nitrate, sodium nitrite, L-lysine, ethylamine hydrochloride and cadaverine hydrochloride are not assimilated. Growth in vitamin-free medium is negative. Maximum growth temperature is 34 °C. Starch-like compounds are not produced. Diazonium blue B reaction is negative. Urease activity is negative.

The type strain, TA11TR-1^T (=AS 2.4160^T =CBS 11405^T), was isolated from apple orchard soil in Tai'an, Shandong province, in August 2008.

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