Description of *Scheffersomyces henanensis* sp. nov., a New D-Xylose-Fermenting Yeast Species Isolated from Rotten Wood

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**Abstract**

Two strains of a D-xylose-fermenting yeast species were isolated from rotten wood samples collected from the Baotianman Nature Reserve in Henan Province, central China. These strains formed hat-shaped ascospores in conjugated and deliquescent ascii. Multilocus phylogenetic analysis that included the nearly complete small subunit (SSU), the internal transcribed spacer (ITS) region and the D1/D2 domain of the large subunit (LSU) rRNA genes, as well as RNA polymerase II largest subunit (RPB1) gene demonstrated that the two strains represent a novel yeast species closely related to *Scheffersomyces segobiensis*. A sequence comparison of xylose reductase (*XYL1*) gene, which was recently recommended for rapid identification of cryptic species in the *Scheffersomyces* clade, revealed a significant sequence divergence of 25 nucleotides between the novel strains and their closest relative *S. segobiensis*, supporting their classification as a distinct species. Furthermore, these new strains can be clearly distinguished from *S. segobiensis* by a number of morphological and physiological characteristics. Therefore, a novel yeast species, *Scheffersomyces henanensis* sp. nov., is proposed to accommodate these strains. The type strain is BY-41\(^T\) (≡ CICC 1974\(^T\) = CBS 12475\(^T\)).

**Introduction**

The genus *Scheffersomyces* was proposed by Kurtzman and Suzuki based on phylogenetic analysis from the combined sequences of the D1/D2 domain of the large subunit (LSU) and the nearly complete small subunit (SSU) rRNA genes [1]. At the time of description, the genus contained three species, *Scheffersomyces stipitis*, *S. segobiensis* and *S. spartinae*, which were transferred from the genus *Pichia* [1,2]. The genus *Scheffersomyces* was later expanded by the inclusion of seven related *Candida* species as new combinations, as well as three novel species, *S. illinoensis*, *S. quercinus* and *S. virginianus*, which were isolated from rotten wood [3]. Thus, 13 species were included in this genus, which clustered in an independent clade based on a multilocus phylogenetic analysis that included the traditional SSU and LSU markers, the orthologous RPB1, and the recently proposed ITS barcoding region for fungi [3,4]. More recently, several new species of the genus *Scheffersomyces* including *S. cryptocercus* [5], *S. parashehatae* and *S. xylosfermentans* [6] have been recovered from wood-ingesting insects.

Yeasts of the genus *Scheffersomyces* have been found to occupy habitats rich in xylose, including decaying wood [3,7–9], wood-feeding insects [3,5,6,10] and their resulting frass [10,11]. Many of these yeast species, such as *S. cryptocercus*, *S. illinoensis*, *S. insectosa*, *S. lignosus*, *S. quercinus*, *S. segobiensis*, *S. shehatae*, *S. stipitis* and *S. virginianus*, possess the rare ability to produce ethanol by fermentation of D-xylose, which gives them economic potential for the production of bioethanol from plant waste residues [12–14]. *S. shehatae* and *S. stipitis* are considered the best ethanol producers among these naturally D-xylose-fermenting yeasts [13,15]. Despite the existence of these microorganisms, obtaining high ethanol yields from pentose sugars on a large scale remain a challenge [16], as microorganisms that robustly convert pentose sugars into ethanol at high yields while withstanding fermentation inhibitors have not yet been identified [17]. Therefore, there is a need for identifying new yeasts capable of efficient xylose fermentation for bioethanol production. Identification of yeast strains that ferment hemicellulosic sugars will lead to improved prospects for lignocellulosic ethanol production [18]. Such strains can be obtained by isolation from the environment, strain mutation and selection in the laboratory [8,19] or by engineering strains of *Saccharomyces cerevisiae* capable of fermenting D-xylose [20].

During an investigation of the yeast community associated with rotten wood obtained from the Baotianman Nature Reserve of Henan Province, central China, we isolated two D-xylose-fermenting yeasts whose physiology and ascospore morphology typically resembled those of the genus *Scheffersomyces*. Multilocus phylogenetic analysis and nucleotide sequence comparison of the single copy xylose reductase (*XYL1*) gene indicated that these strains represent a novel yeast species closely related to *S. segobiensis*. In this paper, we describe this new species as *Scheffersomyces henanensis* sp. nov.
other strain BY-58 was found in a sample from a deciduous
forest in August 2009, whereas the maintained at
suspended in YM broth supplemented with 10% glycerol and
streaking technique on YM agar plates. Purified yeast strains were
[21]. Representative colonies were purified by the conventional
with 0.025% sodium propionate and 200 mg/L chloramphenicol
1% glucose; adjusted to pH 4.0–4.5 with 1 M HCl) supplemented
out by the enrichment technique using yeast extract-malt extract
for the described field studies. Isolation of the strains was carried
Chinese diversity rules, and all necessary permits were obtained
forest in June 2010. The field collections were made according to
Materials and Methods
Yeast Isolation and Culture
One hundred and five yeast strains were isolated from 23
samples of rotten wood collected from the Baotianman National
Nature Reserve in Henan Province, central China (33°27’47’’N
and 111°48’32’’E). Strain BY-41T was isolated from a sample
collected in a mixed deciduous forest in August 2009, whereas the
other strain BY-58 was found in a sample from a deciduous Quercus
forest in June 2010. The field collections were made according to
Chinese diversity rules, and all necessary permits were obtained
for the described field studies. Isolation of the strains was carried
out by the enrichment technique using yeast extract-malt extract
(YM) broth (0.3% yeast extract, 0.3% malt extract, 0.5% peptone,
1% glucose; adjusted to pH 4.0–4.5 with 1 M HCl) supplemented
with 0.025% sodium propionate and 200 mg/L chloramphenicol
[21]. Representative colonies were purified by the conventional
streaking technique on YM agar plates. Purified yeast strains were
suspending in YM broth supplemented with 10% glycerol and
maintained at −80°C.

Morphological, Physiological and Biochemical Characteristics
The morphological, physiological and biochemical characteris-
tics were examined according to standard methods that are
employed in yeast taxonomy [2,22,23]. All assimilation tests were
performed three times, and the results were read after 5 and 21
days of incubation. For the examination of ascospores, the strains
were incubated on YM agar, McClary’s acetate agar, cornmeal
agar and 5% malt extract agar [23], either individually or as
pairwise mixtures on the sporulation medium. Ubiquinones were
extracted and purified by the method of Yamada and Kondo with
slight modifications and determined by HPLC as described
previously [24,25].

Amplification and Sequencing of DNA
Genomic DNA was extracted with a Dr. GenTLE (from Yeast)
High Recovery (Takara Bio, Shiga, Japan). The concentration,
integrity and purity of total extracted DNA were confirmed by gel
electrophoresis in 0.8% agarose in 0.5
– Tris-Borate-EDTA (TBE). The nuclear rRNA genes for SSU, ITS and D1/D2
LSU were amplified and sequenced as described previously [26–
28]. Two protein-coding genes, RPBI and XYL1, were amplified
using the following degenerate primer pairs: RPBI-Af (5’-
GARTGYCCDGGDCAYTTYGG-3’) and RPBI-Cr (5’-CCNGFCDAINTCRCTRTCCATTRA-3’) for
RPBI [29,30]; XYL1-forward (5’-GGTYYTGGMGMTGYTGGAAARSTC-3’) and XYL1-
reverse (5’-AAGWATTGWWGCRCRAWGWGA-3’) for
XYL1 [3,5]. The PCR conditions recommended in the references
for each primer pair were employed. The purified PCR products
were sequenced using a Dye Terminator cycle sequencing kit
(Applied Biosystems, Warrington).

Table 1. GenBank accession numbers of the nucleotide sequences used in this study*.

| Species                  | Codes            | SSU   | ITS   | LSU   | RPBI  | XYL1  |
|-------------------------|------------------|-------|-------|-------|-------|-------|
| C. bolitotheri          | NRRL Y-27587T    | AY242142 | FJ623599 | AY242249 | JN804828 | –     |
| C. terraborum           | NRRL Y-27573T    | AY426956 | FJ623596 | AY309810 | JN804831 | –     |
| C. panamericana         | NRRL Y-27567T    | AY242164 | FJ623601 | AY242273 | JN804835 | –     |
| S. coipomonaensis       | NRRL Y-17651T    | HQ651931 | HQ652070 | HQ651966 | KC507420 | –     |
| S. lignicola            | CBS 10610T       | AY845351 | HQ652074 | AY845350 | –       | –     |
| S. ergatensis           | NRRL Y-17652T    | AB013524 | EU343826 | U54764  | EU440998 | JQ436926 |
| S. insectosa            | NRRL Y-12854T    | AB013583 | HQ652064 | U54773  | JN804842 | JQ235697 |
| S. lignous              | NRRL Y-12856T    | HQ651941 | JN943262 | U54772  | JN804837 | JQ235693 |
| S. segobiensis          | NRRL Y-11571T    | AB054288 | DQ409166 | U54742  | EF599429 | JQ436925 |
| L. elongisporus         | NRRL YB-4239T    | HQ876033 | HQ876042 | HQ876050 | AY653537 | –     |
| C. tropicalis           | NRRL Y-12968T    | EU438785 | AB437068 | U54749  | –       | –     |
| S. quercinina           | NRRL Y-48722T    | –      | HM566445 | HM566445 | –       | –     |
| S. gosingicus           | CBS 11433T       | HQ876040 | HQ999978 | HQ999955 | –       | –     |
| S. spinatina            | NRRL Y-7322T     | FJ153139 | HQ876044 | U54764  | –       | –     |
| S. stipitis             | NRRL Y-7124T     | AB054280 | JN943257 | U54741  | JN804841 | JQ235696 |
| Scheffersomyces sp.     | NRRL Y-48762T    | JF826438 | JF826438 | –       | –       | –     |
| S. shehatae             | NRRL Y-12858T    | AB013582 | JN943264 | JQ25409 | JQ36927 | JQ235691 |
| S. quercicus            | NRRL Y-48825T    | JN940981 | JN943260 | JN70957 | JN804838 | JQ00829 |
| S. virginianus          | NRRL Y-48822T    | JN940969 | JN943259 | JN70958 | JN804839 | JQ235695 |
| S. illinoensis          | NRRL Y-48827T    | JN940968 | JN943261 | JN70959 | JN804840 | JQ235694 |
| S. cryptocercus         | NRRL Y-48824T    | JQ714001 | JQ713977 | JQ714021 | JQ713989 | JQ714031 |
| S. parashetatae         | CBS 12535T       | HQ651936 | HQ652051 | HQ651972 | JQ23138 | KC479716 |
| S. xylasafermentans     | CBS 12540T       | HQ876038 | HQ652061 | HQ652020 | JQ23142 | KC479722 |
| S. henanensis           | CBS 12475T       | JF896577 | HQ127627 | HQ127626 | KF690371 | KF690374 |

*Sequences generated in this work shown in bold. T = type strain.
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Materials and Methods

Yeast Isolation and Culture

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Phylogenetic Analyses

Comparisons with sequences from the international GenBank database (http://www.ncbi.nlm.nih.gov/) were done using BLASTN search. Sequences were aligned using the multiple sequence alignment program CLUSTAL X 1.83 [31]. Phylogenetic trees were constructed using the neighbour-joining and maximum parsimony programs in MEGA software version 5.0 [32]. The evolutionary distance data was calculated from Kimura’s two-parameter model [33] in the neighbour-joining analyses [34]. The heuristic search (close-neighbour-interchange) was used in the maximum parsimony analyses. The sites containing gaps in the alignments of a single gene or combined sequences were excluded. Bootstrap analyses [35] were performed from 1000 random resamplings.

Figure 1. Phylogenetic tree constructed from neighbour-joining analysis of the combined sequences of SSU, ITS, D1/D2 LUS and RPB1, depicting the relationships of Scheffersomyces henanensis sp. nov. with closely related taxa in the Scheffersomyces clade. Candida tropicalis was used as an outgroup taxon (in gray). Bootstrap percentages over 50% from 1000 bootstrap replicates are shown. Bar, 0.01 substitutions per nucleotide position.

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Table 2. Nucleotide differences and percentages of homology between Scheffersomyces henanensis sp. nov. and the type cultures of closest relatives, S. segobiensis, S. stipitis and S. illinoinensis.

| Species                 | SSU | ITS | D1/D2 LUS | RPB1  | XLY1  |
|-------------------------|-----|-----|-----------|-------|-------|
| S. segobiensis T        | 99% | 99% | 99%       | 99%   | 99%   |
| S. stipitis T           | 99% | 99% | 99%       | 99%   | 99%   |
| S. illinoinensis T      | 99% | 99% | 99%       | 99%   | 99%   |

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Yeast Isolation and Diversity

Results and Discussion

Yeast Isolation and Diversity

A total of 105 yeast strains were isolated from rotten wood samples obtained from Baotianman Nature Reserve, central China. Initial biochemical characterization of fermentation abilities was performed on all the isolates, which showed that only 17 yeast strains had the ability to ferment D-xylose. Based on the rapid identification of the D1/D2 domain of the LSU rRNA gene, the majority of these D-xylose-fermenting yeast strains were identified as known species that included S. stipitis and their nearest phylogenetic neighbour, by 3.8% sequence divergence (25 substitutions, 0 gaps) in 525 nt (Table 2 and Fig. 2). Phylogenetic analysis based on the nucleotide sequence of XYL1 alone supported the separation of these strains as a unique species, as also determined by the multilocus phylogenetic construction (Table 1, Fig. 1 and 2). These results clearly indicated that the new strains were representatives of a novel species closely related to S. segobiensis.

The sequence analysis of the easily amplified XYL1 was recently recommended for rapid identification of cryptic species in the Scheffersomyces clade [3,5]. Therefore, XYL1 was amplified from the two strains of the proposed new species and sequenced. The XYL1 sequences of these strains were identical with each other, but differed significantly from those of S. segobiensis, their nearest phylogenetic neighbour, by 3.8% sequence divergence (25 substitutions, 0 gaps) in 525 nt (Table 2 and Fig. 2). Phylogenetic analysis based on the nucleotide sequence of XYL1 alone supported the separation of these strains as a unique species, as also determined by the multilocus phylogenetic construction (Table 1, Fig. 1 and 2). These results described above further confirm our provisional characterization of these strains as a new species of the genus Scheffersomyces.

Cells of two isolates were spherical to ellipsoidal (Fig. 3a), reproduced by multilateral budding, formed one to two hat-shaped ascospores (Fig. 3b), produced pseudoasci but not true ascospores. The sequence analysis of the easily amplified XYL1 was recently recommended for rapid identification of cryptic species in the Scheffersomyces clade [3,5]. Therefore, XYL1 was amplified from the two strains of the proposed new species and sequenced. The XYL1 sequences of these strains were identical with each other, but differed significantly from those of S. segobiensis, their nearest phylogenetic neighbour, by 3.8% sequence divergence (25 substitutions, 0 gaps) in 525 nt (Table 2 and Fig. 2). Phylogenetic analysis based on the nucleotide sequence of XYL1 alone supported the separation of these strains as a unique species, as also determined by the multilocus phylogenetic construction (Table 1, Fig. 1 and 2). These results described above further confirm our provisional characterization of these strains as a new species of the genus Scheffersomyces.

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Nomenclature

The electronic version of this article in Portable Document Format (PDF) in a work with an ISSN or ISBN will represent a published work according to the International Code of Nomenclature for algae, fungi, and plants, and hence the new names contained in the electronic publication of a PLOS ONE article are effectively published under that Code from the electronic edition alone, so there is no longer any need to provide printed copies.

In addition, new names contained in this work have been submitted to MycoBank from where they will be made available to the Global Names Index. The unique MycoBank number can be resolved and the associated information viewed through any standard web browser by appending the MycoBank number contained in this publication to the prefix http://www.mycobank.org/MB. The online version of this work is archived and available from the following digital repositories: PubMed Central; LOCKSS.

Proposal of New Yeast Species

Two strains BY-41T and BY-58 were found to share identical nuclear rRNA genes (SSU, ITS and LSU) and RPB1, indicating their conspecificity. In order to obtain a clearer phylogenetic circumscription of the novel strains and their closely related species in the Scheffersomyces clade, we used a multilocus phylogenetic analysis that included the traditional rRNA genes (SSU, ITS and LSU) and the orthologous RPB1 as defined by Urbina and Blackwell [3,5]. A phylogenetic tree constructed by the neighbour-joining method based on the multilocus sequence analysis showed that our isolates connected to S. segobiensis with 100% bootstrap support and constituted a cluster with S. stipitis and S. illinoisensis in the Scheffersomyces clade (Table 1 and Fig. 1). The same tree topology was derived from the maximum parsimony analysis (results not shown). The nucleotide differences between the new strains and their closest relatives, S. segobiensis, S. stipitis and S. illinoisensis are given in Table 2. These results clearly indicated that the new strains were representatives of a novel species closely related to S. segobiensis.
hyphae, fermented D-xylose, gave negative diazonium blue B reaction and contained Q-9 as the major ubiquinone. These characteristics fit well with those of species of the genus *Scheffersomyces*. However, these two strains also exhibited a number of distinct physiological characteristics that clearly differentiated them from *S. segobiensis* and other closely related species of the genus *Scheffersomyces* (Table 3). For instance, they ferment melezitose, whereas both *S. stipitis* and *S. segobiensis* ferment trehalose. In addition, the novel strains are able to assimilate inulin, galactitol and D-galacturonic acid unlike the other *Scheffersomyces* species described to date.

On the basis of the multilocus sequence analyses of the nuclear rRNA genes and two protein-coding genes, as well as other taxonomic characteristics reported above, we conclude that the two strains represent a single novel species belonging to the genus *Scheffersomyces*. The novel species is described as *Scheffersomyces henanensis* sp. nov., with type strain BY-41T (= CICC 1974T = CBS 12475T).

**Description of *Scheffersomyces henanensis* Hui, Ren, Chen & Niu sp. nov.**

Hui et al. 2014, sp. nov. [urn:lsid:imycobank.org:names:MB 805938.

In YM broth after 3 days at 25°C, cells are spherical or ovoid (2–6.5 × 2–7 μm) and occur singly or in pairs (Fig. 3a). Budding is multilateral. On YM agar after 3 days at 25°C, the streak culture is butyrous, white, raised with a smooth surface and has an entire margin. In Dalmau plates after 7 days on cornmeal agar at 25°C, pseudohyphae are formed, but true hyphae are not formed. On cornmeal agar and 5% malt extract agar after 6 days at 25°C, conjugated asci are formed and each ascus contains one to two hat-shaped ascospores. Asci are deliquescent (Fig. 3b). The major ubiquinone is Q-9. A summary of the physiological and other growth characteristics of *S. henanensis* is given in Table 4.

**Type strain.** CICC 1974T (= CBS 12475; BY-41) is preserved as a lyophilized preparation in China Center of Industrial Culture Collection (CICC), Beijing, China, and the Yeast Collection of the Centraalbureau voor Schimmelcultures (CBS), Utrecht, the Netherlands. The strain was isolated from rotten wood collected in August 2009 from Baotianman Nature Reserve in Henan Province, central China, the coordinates for which are 33°27′47″N and 111°48′32″E.

**Etymology.** The species name *henanensis* (he.nan.en’sis. L. nom. masc. adj.) refers to Henan Province, central China, the geographical origin of the species.

**Author Contributions**

Conceived and designed the experiments: FH QN. Performed the experiments: YR LC. Analyzed the data: YR LC. Contributed reagents/materials/analysis tools: YR LC. Wrote the paper: FH QN.

![Figure 3. Morphological characterization of *Scheffersomyces henanensis* sp. nov. BY-41T.](a) Budding cells grown on YM broth for 3 days at 25°C. (b) Asci formed on cornmeal agar after 6 days at 25°C. Bar, 10 μm. doi:10.1371/journal.pone.0092315.g003

*Table 3. Physiological characteristics that differentiate *Scheffersomyces henanensis* sp. nov. from related species*.  

| Characteristic | *S. henanensis* | *S. stipitis* | *S. segobiensis* |
|---------------|-----------------|---------------|-----------------|
| **Fermentation** | | | |
| Maltose | + | +, D | – |
| Trehalose | – | –, +, D | D |
| Cellobiose | – | D, – | – |
| Melezitose | D, W | – | – |
| Starch | D, W | D, – | – |
| **Assimilation** | | | |
| L-Sorbose | – | D, – | D |
| D-Ribose | – | +, D | + |
| Melezitose | D | +, D | – |
| Inulin | + | – | – |
| Soluble starch | + | + | – |
| Erythritol | D, W | + | – |
| Galactitol | + | – | – |
| D-Glucuronate | + | D, – | – |
| D-Galacturonic acid | + | – | – |

*Data for reference species were taken from Barnett et al. (2000). Symbols: +, Positive; –, negative; D, delayed positive; and W, weakly positive.

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Table 4. Physiological characteristics of *Scheffersomyces henanensis* sp. nov.*

| Fermentation | D-Glucose | + | Inulin | – |
|--------------|-----------|---|--------|---|
| D-Galactose  | +         | Cellobose | – |
| Sucrose      | –         | Methyl-a-D-glucoside | – |
| Maltose      | +         | Melibiose | – |
| Lactose      | –         | Melilizose | D, W |
| Raffinose    | –         | Starch | D, W |
| a,α-Trehalose| –         | D-Xylose | D |
| Carbon assimilation | D-Glucose | + | Raffinose | – |
| D-Galactose  | +         | Melezitose | D |
| L-Sorbose    | –         | Inulin | + |
| D-Glucosamine| –         | Soluble starch | + |
| D-Ribose     | –         | Glycerol | + |
| D-Xylose     | +         | Erythritol | D, W |
| L-Arabinose  | –         | Ribitol | + |
| D-Arabinose  | +         | Xyritol | + |
| L-Rhamnose   | +         | L-Arabinitol | – |
| Sucrose      | +         | D-Gluconolate | + |
| Maltose      | +         | D-Mannitol | + |
| Trehalose    | +         | Galactitol | + |
| Methyl-a-D-glucoside | D | myo-Inositol | – |
| Cellobose    | +         | DL-Lactate | – |
| Salicin      | +         | Succinate | + |
| Arbutin      | +         | Citrate | + |
| Melibiose    | –         | Methanol | – |
| Lactose      | –         | Ethanol | W |
| D-Gluconate  | +         | D-Galacturonic acid | + |
| Nitrogen assimilation | Nitrate | – | Creatine | – |
| Nitrite      | –         | Creatinine | – |
| Ethylamine   | +         | Glucosamine | – |
| L-Lysine     | +         | Imidazole | – |
| Cadaverine   | +         | D-Tryptophan | – |

Growth tests

| 10%NaCl/5% glucose | – | 0.1% Cycloheximide | + |
| 50% Glucose | – | Vitamin-free medium | – |
| Starch formation | – | Growth at 35°C | + |
| 1% Acetic acid | – | Growth at 37°C | – |

Additional tests

| Starch formation | – | Urea hydrolysis | – |
| Acetic acid production | – | Diazonium blue B reaction | – |

*Symbols: +, Positive; –, negative; D, delayed positive; and W, weakly positive.
doi:10.1371/journal.pone.0092315.t004

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