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FULL PAPER

Reclassification of citrus pseudo greasy spot causal yeasts, and a proposal of two new species, *Sporobolomyces productus* sp. nov. and *S. corallinus* sp. nov.

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Abstract Three basidiomycetous yeast strains, No. 3 (=MAFF 654001), No. 4 (=MAFF 654002), and No. 6 (=MAFF 654003), isolated from diseased leaves of *Citrus unshiu* and formerly identified as *Sporobolomyces roseus*, were reported to cause citrus pseudo greasy spot (PGS). In the course of investigating the causal agent of citrus PGS, we confirmed, using conventional and molecular phylogenetic methods, that these yeasts caused citrus PGS but did not belong to *S. roseus*. No. 3 (=MAFF 654001) and No. 4 (=MAFF 654002) were closely related to *S. subbrunneus*, and No. 6 (=MAFF 654003) to *S. dimmenae*, but each of them was distinctive from these species. We have proposed two new species, *S. productus* and *S. corallinus*, as the causal pathogens of citrus PGS.

Keywords Citrus pseudo greasy spot · Citrus greasy spot · Plant pathogenic yeast · *Sporobolomyces* · *Subbrunneus* lineage

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Introduction

Pseudo greasy spot (PGS) of citrus has been prevalent since the 1960s, exclusively in Japan. The disease first develops as tiny black spots on the surface of mature leaves, and then the spots gradually merge with each other, creating a halo around them. The disease sometimes causes defoliation and results in yield loss. PGS occurs in the open fields of southwestern Japan, and the damage is more serious in greenhouses all over the country (Ieki 2006). However, to date, there are no reports of its occurrence in other areas of the world.

Another serious disease affecting citrus leaves, that is, citrus greasy spot (CGS), is caused by two fungal species, namely, *Mycosphaerella citri* Whiteside and *M. horii* Hara. CGS is known as a great threat worldwide (Timmer and Gottwald 2000). PGS differs from CGS in that the black spots are more numerous and smaller in size in PGS, and PGS occurs primarily on vigorous trees (Koizumi and Kuhara 1984). However, because the symptoms of PGS in affected plants are similar to those of CGS, the diseases remain difficult to distinguish from one another. Frequent confusion and misidentification are the result in part of a paucity of information about PGS pathogens. This confusion causes problems because the isolation methods and the fungicidal controls differ for PGS and CGS. Therefore, it is very important to develop methods that clearly and correctly distinguish PGS from CGS.

During a long-term survey of the PGS pathogens, Koizumi and Kuhara (1984) found pestle-shaped bodies in the intercellular spaces of diseased leaves. Yeast-like microorganisms were isolated by the leaf-section-suspension method. Among them, three strains with elongated cells (6–9 × 2–3 μm) and salmon-colored colonies were

identified as the principal agents of PGS. This report was recognized as the first case of yeast-type microorganisms causing disease in plant leaves (Koizumi and Kuhara 1984). Koizumi (1986) identified these yeasts as *Sporobolomyces roseus* Kluyver & van Niel, an anamorph of *Sporidiobolus metaroseus* Sampaio & Valério, on the basis of the ability to utilize nitrate, inositol, and some other carbon sources and the ability to form pseudo- or true mycelia, according to the taxonomic system available at that time (Fell and Statzell 1984).

The yeasts of the genus *Sporobolomyces* are widely distributed in nature and are regarded as saprophytic inhabitants of the phyllosphere, especially on cereal leaves (Boekhout 1991; Last and Price 1969). New species are continually being found and described. Recently, 53 species were listed by Hamamoto et al. (2011). The authors mentioned that this genus was polyphyletic and was divided into four lineages. Knowing the correct taxonomic position of this PGS pathogen is necessary for development of an accurate method for discriminating the causative agent of PGS from that of CGS.

In this study, we reclassified the PGS pathogen, formerly identified as *S. roseus*. After phylogenetic and taxonomic studies of the causal agents of PGS, we found that these agents did not belong to *S. roseus* and thus they were classified as two novel species: *Sporobolomyces productus* and *S. corallinus*. We propose that these novel species are the primary PGS pathogens.

Materials and methods

Yeast isolates and culture conditions

Freeze-dried cultures of three basidiomycetous yeasts (No. 3, MAFF 654001; No. 4, MAFF 654002; No. 6, MAFF 654003) kept at the Ministry of Agricultural, Forestry, and Fisheries (MAFF) gene bank were used in this study. These strains were isolated from Satsuma mandarin (*Citrus unshiu* Marc.) leaves showing PGS symptoms. The strains were established after multiplication in diseased tissue suspensions in 1973 (Koizumi and Kuhara 1984).

All the strains were grown in yeast extract-malt extract (YM) medium (3 g yeast extract, 3 g malt extract, 5 g peptone, 10 g glucose, 1 l distilled water) or on yeast extract-malt extract agar (YMA) medium (20 g agar added to 1 l YM) at 24°C for a few days.

Injection and spray inoculation tests

Yeast cells grown on YMA plates were suspended in distilled water and adjusted to concentrations of approximately 10^6 cells/ml for inoculation tests. Mature leaves of Kawano Natsudaidai (*Citrus natsudaidai* Hay.) were injected with yeast cell suspensions in a syringe, and then the petioles were inserted into a sponge filled with water. The inoculated leaves were incubated in a plastic container at 24°C. Immature but fully expanded leaves on potted plants of Satsuma mandarin cv. Juman (*C. unshiu* Marc.) were used for spray inoculation following machine oil treatment to prevent damage by mites. As negative control, distilled water was used for both inoculation tests. Ten to 15 inoculated leaves were covered with plastic bags for 4 days, and the plants were incubated in a glasshouse controlled at 25°C/day and at 23°C/night. The plants were monitored for the development of disease symptoms for up to 6 months after inoculation.

Morphological, physiological, and biochemical properties

Morphological, physiological, and biochemical tests were performed according to the standard methods developed by Yarrow (1998). Maximum growth temperature was determined using yeast peptone glucose agar (YPGA) medium (10 g yeast extract, 5 g peptone, 4 g glucose, 20 g agar per liter of distilled water) in air incubators.

Sequence analysis of the internal transcribed spacer (ITS) region and the D1/D2 domain of the large subunit (LSU) rRNA gene

Yeast cells were picked from YMA cultures with a pipette tip and directly added to 20 µl of the polymerase chain reaction (PCR) reaction mix of the *Ex Taq* polymerase Hot Start version (Takara, Japan). Approximately 1 kbp DNA fragments, including ITS (ITS1 and ITS2, including 5.8S gene) and the D1/D2 domain of LSU rRNA gene, were amplified with primers ITS1 (5′-GTC GTA ACA AGG TTT CCG TAG GTG-3′) (White et al. 1990) and NL4 (5′-GGT CCG TGT TTC AAG ACG G-3′) (O'Donnel 1993). PCR was performed in a thermal cycler (Gene Amp PCR System 9700; Applied Biosystems, Foster City, CA, USA) as follows: initial denaturation of 94°C for 5 min, 30 cycles of 94°C for 1 min, 52°C for 1 min, and 72°C for 1.5 min. The amplified products were separated on a 1.5% agarose gel and visualized using ethidium bromide. Target bands were cut out from the gel, extracted, and purified using a QIAquick Gel Extraction kit (Qiagen, Germany), for sequencing. Cycle sequencing was performed using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). The forward primers were ITS1, NL1 (5′-GCA TAT CAA TAA GCG GAG GAA A-3′) (O'Donnel 1993), ITS-wf (5′-GAA CGC AGC GAA ACG CGA A-3′) (this study), and D1/D2-wf (5′-ATA GCG AAC AAG TAC

CGT G-3′) (this study). The reverse primers were ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′) (White et al. 1990), NL4, ITS-wr (5′-TTC GCG TTT CGC TGC GTT TC-3′) (this study), and D1/D2-wr (5′-CAC GGT ACT TGT TCG CTA T-3′) (this study). The nucleotide sequences were determined by direct sequencing using an Applied Biosystems 310 Genetic Analyzer (Applied Biosystems). Fragment sequences were analyzed using Geneious Pro 5.1 (Drummond et al. 2009) (Biomatters, New Zealand) and Clustal W (Thompson et al. 1994).

Molecular phylogenetic analyses

The three D1/D2 domain sequences of LSU rRNA gene that we determined were aligned by Geneious Pro 5.1 with the sequences of 43 other basidiomycetes (retrieved from the GenBank and DDBJ libraries) and adjusted manually. *Bulleromyces albus* was designated as an outgroup. Phylogenetic analysis was conducted by neighbor-joining (NJ) using PAUP* ver. 4.0 (Swofford 1998). A distance matrix was obtained using Kimura's two-parameter correction for multiple hits (Kimura 1980). Bootstrap analysis for 1,000 replicates was performed to provide confidence estimates for tree topologies.

The nucleotide sequence data reported in this article were deposited in the DDBJ, EMBL, and GenBank nucleotide sequence databases with the following accession numbers: AB638334, AB638335, and AB644404.

Results and discussion

Pathogenicity

All three strains, namely, No. 3 (=MAFF 654001), No. 4 (=MAFF 654002), and No. 6 (=MAFF 654003), caused chlorosis around the injection spots on Kawano Natsudaidai leaves 20 days after injection. Yeast cells were observed in the intercellular spaces under a microscope. In case of spray inoculation, chlorosis was observed 1 month later, and subsequently many tiny black spots appeared on the lower side of the leaves (Fig. 1a, b). These symptoms were commonly observed on leaves of all plants among the three strains. Negative controls did not show any symptoms (Fig. 1c). The symptoms developed on the upper side of the leaves 3 months after spray inoculation, and we observed dehiscence and suberization on the vein in some cases.

Koizumi and Kuhara (1984) showed the isolation, pathogenicity, and re-isolation of the diseased plants in their study; that is, Koch's postulates were fulfilled. This study also used the same strains as Koizumi and Kuhara (1984) and reproduced the same symptoms as those reported by Koizumi and Kuhara (1984). Additionally, by sequencing following PCR amplification, we confirmed that the DNA fragment amplified from the inoculated affected leaf was completely identical with that of the inoculum (data not shown).

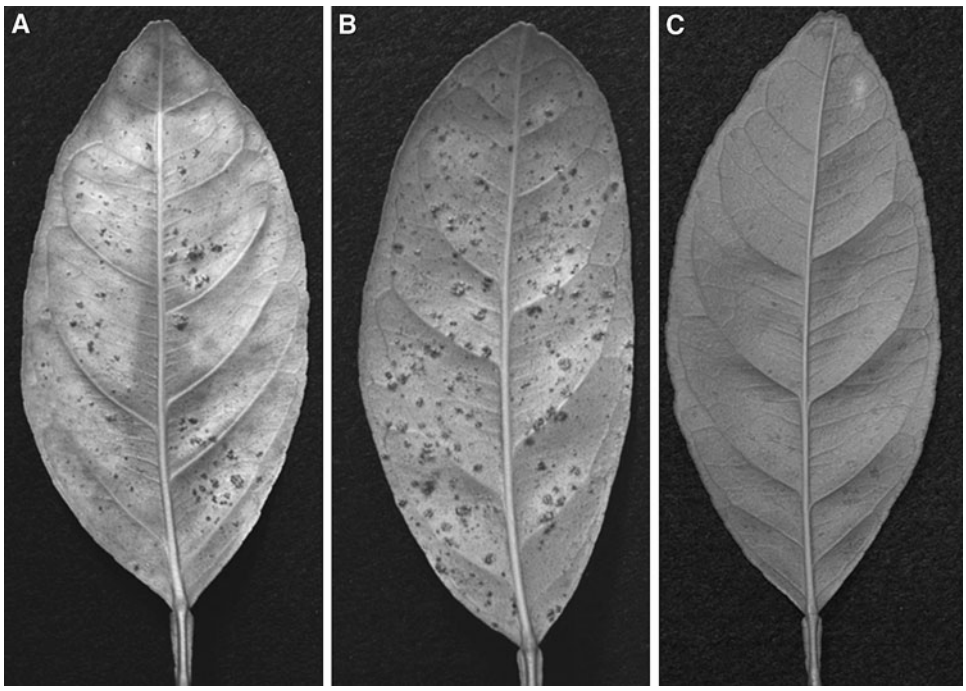

Fig. 1 Leaf symptoms of pseudo greasy spot on Kawano Natsudaidai (*Citrus natsudaidai* Hay.) 6 months after spray inoculation. **a** *Sporobolomyces productus* No. 3^T; **b** *Sporobolomyces corallinus* No. 6^T; **c** negative control

PGS is a unique disease in which yeast infects the plant leaves and causes symptoms in them. The results of this study were in agreement with the results of previous studies (Koizumi and Kuhara 1984; Koizumi 1986).

Morphology and physiology

The three strains apparently differed from *S. roseus* in the carbon compound assimilation reactions of D-arabinose, maltose, α,α-trehalose, cellobiose, raffinose, melezitose, soluble starch, glycerol, xylitol, D-glucitol, and DL-lactate (Table 1).

All three strains had similar colony morphologies, with soft and smooth surfaces, and also had similar maximum growth temperatures of 28°–29°C (Table 1). They neither fermented nor formed pseudomycelia in the Dalmau plate method. The shapes of ballistoconidia were bilaterally asymmetrical. All strains required *p*-aminobenzoic acid and thiamine in the vitamin requirement test. However, strains No. 3 (=MAFF 654001) and No. 4 (=MAFF 654002) formed pale brown to grayish-orange colonies, whereas strain No. 6 (=MAFF 654003) initially formed pale pink colonies. Strain No. 3 (=MAFF 654001) had smaller ballistoconidia (5.0–7.0) × (1.5–3.0) μm than strain No. 6 (=MAFF 654003) (6.0–9.0) × (2.0–3.5) μm (Figs. 3, 4). Strains No. 3 (=MAFF 654001) and No. 4 (=MAFF 654002) were similar to *S. subbrunneus* and No. 6 (=MAFF 654003) to *S. dimmenae*, which are members of the *Subbrunneus* lineage in genus *Sporobolomyces*. However, No. 3 (=MAFF 654001) and No. 4 (=MAFF 654002) were different from *S. subbrunneus* in the assimilation of D-arabinose, xylitol, D-gluconate, DL-lactate (weak), and citrate; and No. 6 (=MAFF 654003) was different from *S. dimmenae* in the assimilation of raffinose, glycerol, ribitol, and DL-lactate (Table 1). Additionally, mating was not observed between them.

Molecular properties

In the phylogenetic tree based on D1/D2 domain sequences, all of No. 3 (=MAFF 654001), No. 4 (=MAFF 654002), and No. 6 (=MAFF 654003) were included in the

Table 1 Differential characteristics among *Sporobolomyces productus*, *Sporobolomyces corallinus*, and the related species

Assimilation of	*S. productus* No. 3 (=MAFF 654001^T)	*S. productus* No. 4 (=MAFF 654002)	*S. subbrunneus*[a] JCM 5278^T	*S. corallinus* No. 6 (=MAFF 654003^T)	*S. dimmenae*[b] JCM 8762^T	*S. roseus*[c] CBS 486^T
D-Arabinose	−	nt	d	−	−	+
Maltose	−	−	−	−	−	+
α,α-Trehalose	+	+	+	+	+	−
Cellobiose	−	−	−	−	−	d
Raffinose	+	+	+	−	w	+
Melezitose	−	−	−	−	−	+
Soluble starch	−	nt	−	−	−	+
Glycerol	+	+	+	+	−	−
Ribitol	+	nt	+	+	−	−
Xylitol	−	nt	d	−	−	−
D-Mannitol	+	+	+	+	+	d
D-Glucitol	+	+	+	+	+	−
D-Gluconate	−	−	+	−	−	−
DL-Lactate	w	w	+	+	−	−
Succinate	+	nt	+	+	+	d
Citrate	–	nt	+	−	−	−
Maximum growth temperature (°C)	28–29	28–29	(30–31)[d] 29–30	28–29	(24–25)[d] 21–23	(nt)[d] 30

+ positive, − negative, *d* delay, *w* weak, *nt* not tested

[a] Nakase and Suzuki (1985)

[b] Hamamoto and Nakase (1995)

[c] Boekhout (1991)

[d] This study

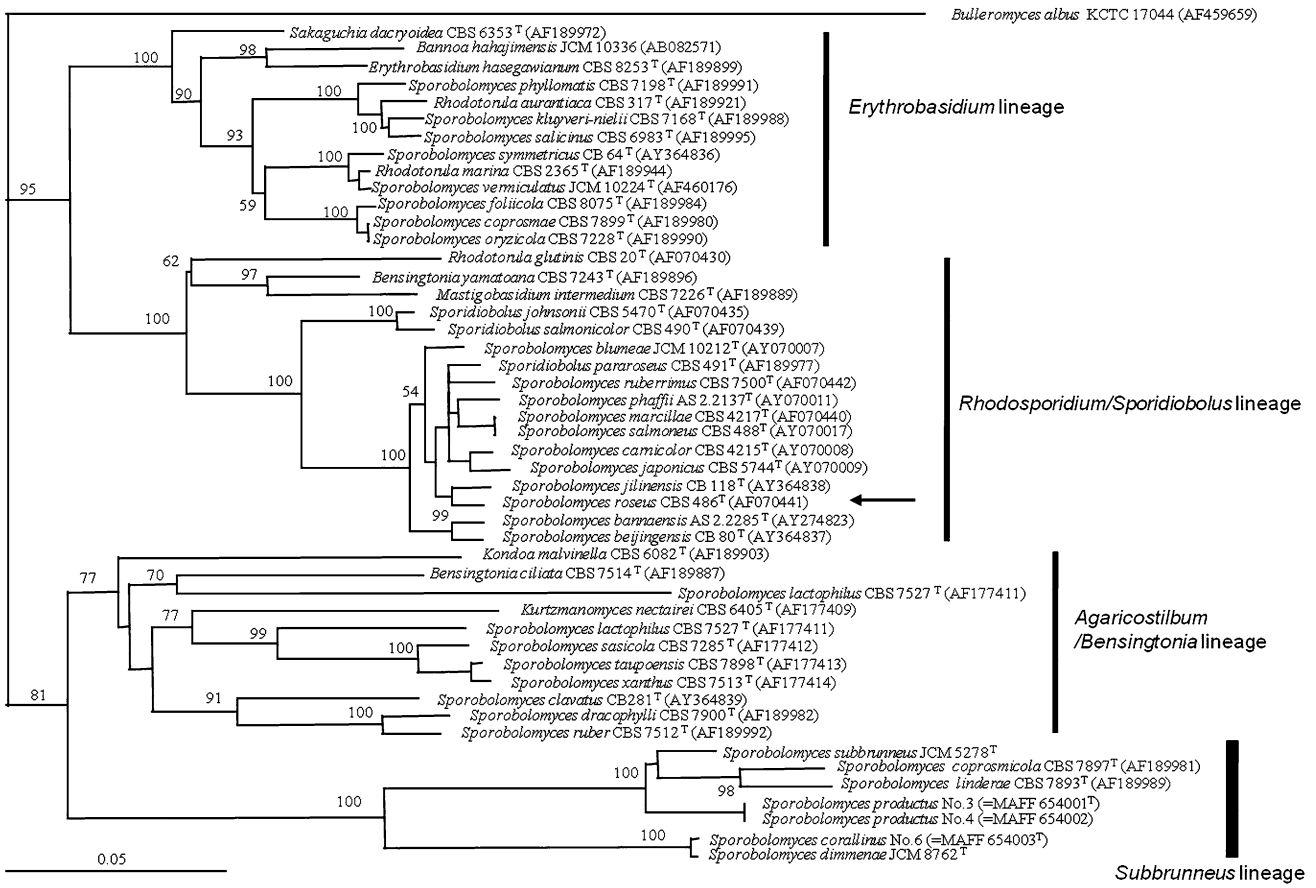

Fig. 2 Phylogenetic trees of the species of *Sporobolomyces* and related species based on the neighbor-joining method of the D1/D2 domain sequences of the LSU rRNA gene. These trees depict the relationship of the two novel *Sporobolomyces* species in the *Subbrunneus* lineage. *Bulleromyces albus* KCTC 17044 (AF459659) was the outgroup species. *Figures* represent percentages from bootstrap sampling with 1,000 replicates. Reference sequences retrieved from GenBank are indicated. *Arrow* indicates *S. roseus*

Subbrunneus lineage and separated from *S. roseus*, which was a member of the *Rhodosporidium/Sporidiobolus* lineage in genus *Sporobolomyces* (Fig. 2).

Although the nucleotide sequences of No. 3 (=MAFF 654001) and No. 4 (=MAFF 654002) were completely identical, sequence identity between No. 3 (=MAFF 654001) and No. 6 (=MAFF 654003) showed only 63.0% in the ITS1 and ITS2 regions (Table 2). The phylogenetic tree also demonstrated that No. 3 (=MAFF 654001) and No. 4 (=MAFF 654002), and No. 6 (=MAFF 654003), fell into two groups with *S. subbrunneus* and *S. dimmenae*, respectively (Fig. 2).

In general, species identification for basidiomycetous yeasts is assumed by less than 99% identity in the ITS sequence and the differences of more than three nucleotides in the D1/D2 domain sequences of LSU rRNA. However, some species, which could be separated by ITS analysis, such as the biologically distinct species *Cryptococcus ater* and *Filobasidium elegans*, could not be differentiated on the basis of the D1/D2 domain sequences (Scorzetti et al. 2002).

The sequence identities of No. 3 (=MAFF 654001) and No. 4 (=MAFF 654002) to the D1/D2 region of LSU rRNA gene (20 nt, transition:transversion = 6:14) and to the ITS region (96.2%; Table 2) of *S. subbrunneus* showed clearly that they were separate species.

In the case of No. 6 (=MAFF 654003) and *S. dimmenae*, although only one base difference was detected in the D1/D2 region, 98.1% identity of the ITS region indicated the distinctiveness of the former from the latter (Table 2). The higher growth temperature of this species also supported this separation. These results, in combination with assimilation patterns and maximum growth temperature differences, led us to conclude that No. 6 (=MAFF 654003) is distinct from *S. dimmenae*.

These results indicated that the three strains of the causal agents of PGS identified by Koizumi and Kuhara (1984) consisted of two species. Furthermore, we posit that strain No. 3 (=MAFF 654001) and strain No. 6 (=MAFF 654003) each represent a new species.

As already described, the genus *Sporobolomyces* is polyphyletic. The *Subbrunneus* lineage was genetically

Table 2 Sequence identities (%) among *Sporobolomyces productus*, *Sporobolomyces corallinus*, and the related species in ITS1 and ITS2

Species	Strain	Region of origin	Source	No. 4	No. 6	CBS 486	JCM 5278	JCM 8762
Sporobolomyces productus	No. 3 (=MAFF 654001^{T})	Kagoshima, Japan	Diseased leaf of *Citrus unshiu* infected with pseudo greasy spot	100	63.0	<20.0	96.2	62.5
Sporobolomyces productus	No. 4 (=MAFF 654002)	Kagoshima, Japan	Diseased leaf of *Citrus unshiu* infected with pseudo greasy spot	–	63.0	<20.0	96.2	62.5
Sporobolomyces corallinus	No. 6 (=MAFF 654003^{T})	Miyazaki, Japan	Diseased leaf of *Citrus unshiu* infected with pseudo greasy spot		–	<20.0	60.3	98.1
Sporobolomyces roseus	CBS 486^{T} (=JCM 5353)	–	–			–	<20.0	<20.0
Sporobolomyces subbrunneus	JCM 5278^{T}	Japan	Dead leaf of *Oryza sativa*				–	59.9
Sporobolomyces dimmenae	JCM 8762^{T}	New Zealand	Dead leaf of *Pseudowintera colonata*					–

distant from *S. roseus*, the type species of the genus. These results indicate that the *Subbrunneus* lineage may be reclassified into another genus in the future. In this article, we tentatively place these two species in the genus *Sporobolomyces*. We propose the names of these two species as *Sporobolomyces productus* N. Furuya et M. Takash sp. nov. and *S. corallinus* N. Furuya et M. Takash sp. nov., respectively. The appropriateness of proposing new species on the basis of only one strain may be debatable. However, considering the importance of correct identification of the PGS-causing agent for appropriate fungicidal control, the necessity of this proposal is high.

Description

Sporobolomyces productus N. Furuya et M. Takash sp. nov.

MycoBank No.: MB 561816

In liquido "YM," post tres dies ad 24°C, cellulae ellipsoidales, elongatae vel longi-ellipsoidales, (6.0–8.5) × (1.5–3.0) μm, singulae aut binae (Fig. 3a). Pellicula non formatur. In agaro "YM," post unum mensem ad 24°C, cultura cinereo-aurantia vel pallide brunnea, glabra aut rugulosa, mollis, margine integra. Mycelium et pseudohyphae non formantur. Ballistoconidia reniformes, (5.0–7.0) × (1.5–3.0) μm (Fig. 3b). Fermentatio nulla. Glucosum, saccharosum, trehalosum, raffinosum, D-xylosum, glycerolum, D-mannitolum, ribitolum, D-glucitolum, alpha-methyl-D-glucosidum, acidum DL-lacticum (exiguum), et acidum succinicum assimilantur, at non galactosum, L-sorbosum, maltosum, cellobiosum, lactosum, melibiosum, melezitosum, amylum solubile, L-arabinosum, D-arabinosum, D-ribosum, L-rhamnosum, ethanolum, erythritolum, salicinum, acidum D-glucuronicum, acidum citricum, inositolum, xylitolum, L-arabinitolum, D-glucitolum nec galactitolum.

Assimilatio potassii nitrati et sodii nitriti positiva. Temperature crescentiae maximae: 28–29°C. Ad crescentiam acidum *p*-aminobenzonicum et thiaminum necessarii sunt. Holotypus: Isolatus ex folio "*Citrus unshiu*," Kagoshima, Japonia, ii. 1974, M. Koizumi, MAFF 654001 (originaliter ut No. 3); in collection 'National Institute of Agrobiological Sciences (NIAS) Genebank,' Tsukuba, Japonia.

Growth in YM broth: after 3 days growth at 24°C, ellipsoidal, elongated, or long-ellipsoidal (6.0–8.5) × (1.5–3.0) μm cells occurring singly or in pairs were observed (Fig. 3a).

Growth on YM agar: after 1 month of growth at 24°C, grayish-orange to pale brown, smooth, dull, and soft colonies with entire margins were observed.

Dalmau plate culture on cornmeal agar: true and pseudohyphae were not formed.

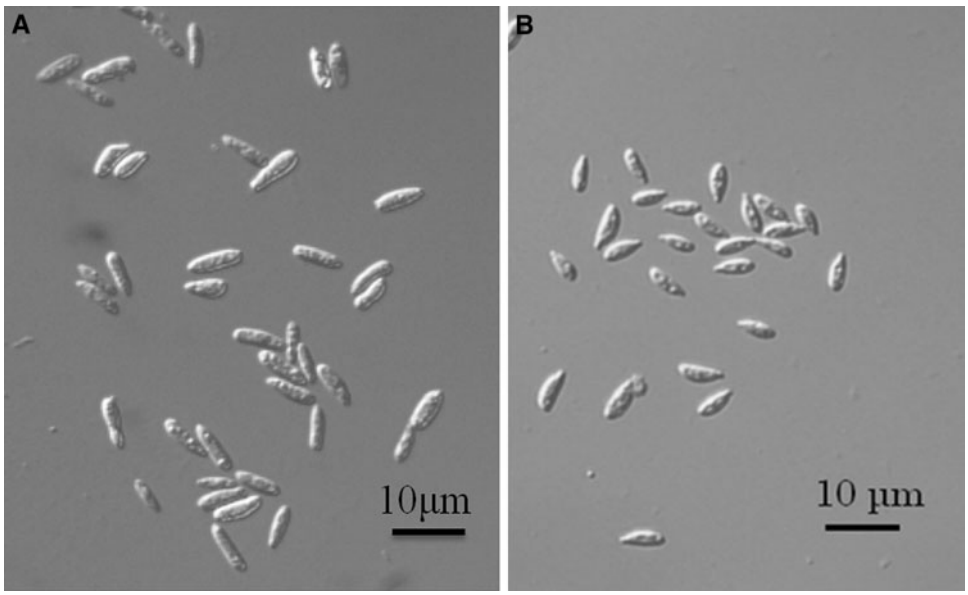

Fig. 3 Morphology of *Sporobolomyces productus* sp. nov. No. 3[T]. **a** Vegetative cells grown in yeast extract-malt extract (YM) broth for 3 days at 24°C; **b** ballistoconidia produced on cornmeal agar after 3 days at 24°C

Formation of ballistoconidia: ballistoconidia are formed on cornmeal agar (after 3 days at 24°C). They are bilaterally asymmetrical, reniform, and of the size (5.0–7.0) × (1.5–3.0) μm (Fig. 3b).

Fermentation: not observed

Assimilation of carbon compounds:

Glucose, sucrose, trehalose, raffinose, D-xylose, glycerol, D-mannitol, ribitol, D-glucitol, methyl-α-D-glucoside, DL-lactate (weak), and succinate are assimilated.

D-Galactose, L-sorbose, maltose, cellobiose, lactose, melibiose, melezitose, soluble starch, L-arabinose, D-arabinose, D-ribose, L-rhamnose, D-glucosamine, ethanol, meso-erythritol, salicin, citrate, myo-inositol, xylitol, L-arabinitol, D-glucuronic acid or D-galacturonic acid are not assimilated.

Assimilation of nitrogen compounds: nitrate and nitrite

Maximum growth temperature: 28–29°C.

Vitamins required: *p*-aminobenzoic acid and thiamine

Type: the type species is strain No. 3 (=MAFF 654001) isolated from citrus leaf in Kagoshima. This strain was deposited as the holotype in Genebank, National Institute of Agrobiological Sciences, Tsukuba, Ibaraki, Japan, as MAFF 654001. The isotype was deposited in the Japan Collection of Microorganisms, Saitama, Japan, as "JCM 1789."

Etymology: *productus* means that this species abundantly produces ballistoconidia.

Colonies of this yeast on YMA after 1 month at 24°C showed pale brown to grayish orange with a smooth, dull, and soft surface similar to *S. subbrunneus*. In addition, these yeasts abundantly formed asymmetrical ballistoconidia, and the production of ballistoconidia continues more than 1 month on cornmeal agar (CMA) plates as well as *S. subbrunneus*. However, there is a clear difference in the sequence similarity of ITS1 and ITS2 (96.2%).

Sporobolomyces corallinus N. Furuya et M. Takash sp. nov.

MycoBank No.: MB 561817

In liquido "YM," post tres dies ad 24°C, cellulae vegetativae, ovoideae, ellipsoideae vel elongatae, (5.0–7.0) × (2.0–3.5) μm, singulae aut binae. In agaro "YM," post unum mensem ad 24°C, cultura primo corallina demum laete aurantia, glabra aut rugulosa, mollis, margine integra (Fig. 4a). Ballistoconidia reniformes aut falcatae, (6.0–9.0) × (2.0–3.5) μm (Fig. 4b). Mycelium et pseudohyphae non formantur. Fermentatio nulla. Glucosum, saccharosum, trehalosum, D-xylosum, glycerolum, ribitolum, D-mannitolum, D-glucitolum, acidum DL-lacticum, alpha-methyl-D-glucosidum, et acidum succinicum assimilantur, et non L-sorbosum, galactosum, maltosum, cellobiosum, lactosum, melibiosum, melezitosum, raffinosum, amylum solubile, L-arabinosum, D-arabinosum, D-ribosum, L-rhamnosum, ethanolum, erythritolum, salicinum, acidum D-glucuronicum, acidum citricum, xylitolum, L-arabinitolum, galactitolum nec inositolum. Assimilatio potassii nitrati et sodii nitriti positiva. Temperatura crescentiae maximae: 28–29°C. Ad crescentiam acidum *p*-aminobenzonicum et thiaminum necessarii sunt. Holotypus: Isolatus ex folio "*Citrus unshiu*," Miyazaki, Japonia, ii. 1974, M. Koizumi, MAFF 654003 (originaliter ut No. 6); in collection 'National Institute of Agrobiological Sciences (NIAS) Genebank,' Tsukuba, Japonia.

Growth in YM broth: after 3 days of growth at 24°C, ovoid, ellipsoidal to elongate vegetative cells of (5.0–7.0) × (2.0–3.5) μm size occurring singly or in pairs (Fig. 4a).

Growth on YM agar: after 1 month of growth at 24°C, the streak culture is initially pale pink, which then turns to

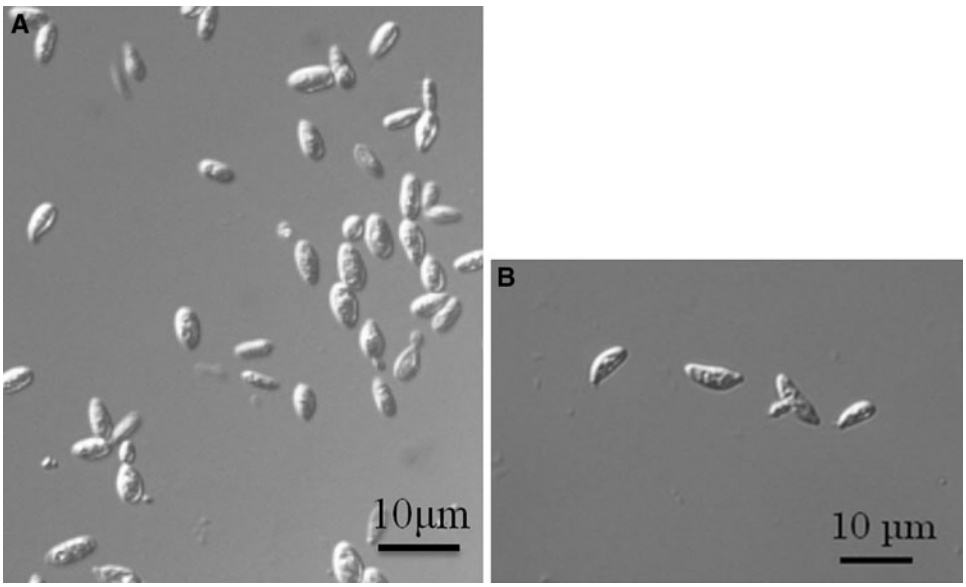

Fig. 4 Morphology of *Sporobolomyces corallinus* sp. nov. No. 6^T. **a** Vegetative cells grown in YM broth for 3 days at 24°C; **b** ballistoconidia produced on cornmeal agar after 3 days at 24°C

light orange, and then forms smooth, dull, and soft colonies with an entire margin.

Dalmau plate culture on cornmeal agar: true and pseudohyphae are not formed.

Formation of ballistoconidia: ballistoconidia are produced on cornmeal agar (after 3 days at 24°C). They are bilaterally asymmetrical, reniform, and of the size (6.0–9.0) × (2.0–3.5) μm (Fig. 4b).

Fermentation: negative

Assimilation of carbon compounds:

Glucose, sucrose, trehalose, D-xylose, glycerol, ribitol, D-mannitol, D-glucitol, DL-lactate, methyl-α-D-glucoside, and succinate are assimilated.

L-Sorbose, D-galactose, maltose, cellobiose, lactose, melibiose, melezitose, raffinose, soluble starch, L-arabinose, D-arabinose, D-ribose, L-rhamnose, ethanol, meso-erythritol, salicin, D-gluconate, citrate, xylitol, L-arabinitol, D-galacturonic acid, or myo-inositol are not assimilated.

Assimilation of nitrogen compounds: nitrate and nitrite

Maximum growth temperature: 28–29°C

Vitamins required: *p*-aminobenzoic acid and thiamine.

Type: strain No. 6 (=MAFF 654003) isolated from citrus leaf in Miyazaki is the type species. This strain was deposited as the holotype in Genebank, National Institute of Agrobiological Sciences, Tsukuba, Ibaraki, Japan, as MAFF 654003. The isotype was deposited in Japan Collection of Microorganisms, Saitama, Japan, as "JCM 17889."

Etymology: the specific epithet *corallinus* means that the initial color of the colony of this species is similar to the pink color of coral.

The color of the colony of this yeast on YMA was initially pale pink, and then it became light orange after 2 weeks of incubation. Its surface was smooth, whereas colonies of *S. dimmenae* were light orange, and wrinkled. The maximum growth temperature of *S. dimmenae* is 21–23°C in the original description (Hamamoto and Nakase 1995), and our results obtained using an air incubator showed a higher maximum growth temperature (24–25°C). However, the maximum growth temperature for this yeast is 28–29°C, which is 4°C higher than that for *S. dimmenae*.

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