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Metschnikowia sirindhorniae f.a., sp. nov. and *Moniliella princeps* sp. nov., Two Novel Yeasts Species Isolated from Toddy Palm (*Borassus flabellifer* L.) Inflorescence Sap in Thailand

SASITORN JINDAMORAKOT[1*], SOMJIT AM-IN[1], RUNGLUK KAEWWICHIAN[2],
PANNIDA KHUNNAMWONG[3] AND SAVITREE LIMTONG[3,4]

[1]*National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), Pathum Thani 12120, THAILAND*
[2]*Microbiology Program, Department of Science, Faculty of Science and Technology, Bansomdejchaopraya Rajabhat University, Bangkok 10600, THAILAND*
[3]*Department of Microbiology, Faculty of Science, Kasetsart University, Bangkok 10900, THAILAND*
[4]*The Academy of Science, The Royal Society of Thailand, Bangkok 10300, THAILAND*
*Corresponding author. Sasitorn Jindamorakot (sasitorn@biotec.or.th)
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ABSTRACT.– Two strains, YSP-519 and YSP-478, respectively representing novel ascomycetous and basidiomycetous yeast species, were obtained from toddy palm inflorescence sap collected in Thailand. The ascomycetous yeast strain YSP-519 was closely related to *Metschnikowia* agaves CBS 7744^T. There were 1.6% nucleotide substitutions (9 nucleotide substitutions and 2 gaps out of 555 nt) in the D1/D2 domains of the LSU rRNA gene and 0.7% nucleotide substitutions (3 nucleotide substitutions out of 454 nt) in the ITS regions. The basidiomycetous yeast strain YSP-478 was closely related to *Moniliella pollinis* CBS 461.67^T. It had 0.5 % nucleotide substitutions (3 nucleotide substitutions out of 554 nt) in the D1/D2 domains of the LSU rRNA gene and 2.0% nucleotide substitutions (9 nucleotide substitutions out of 473 nt) in the ITS regions. Phylogenetic analysis based on the concatenated sequences of the ITS regions and the D1/D2 domains confirmed that the two strains represent two new species. The name *Metschnikowia sirindhorniae* f.a., sp. nov. is proposed for YSP-519^T), and *Moniliella princeps* sp. nov. is proposed for YSP-478^T. The holotypes of *M. sirindhorniae* and *Mo. princeps* are TBRC 10642^T and TBRC 10641^T, respectively and the ex-type are CGMCC 2.6079^T and CGMCC 2.6078^T, respectively. The MycoBank numbers of *M. sirindhorniae* and *Mo. princeps* are MB858878 and MB858879, respectively.

KEYWORDS: ***Metschnikowia sirindhorniae* f.s., sp. nov., *Moniliella princeps* sp. nov., novel yeast species, Thailand**

INTRODUCTION

The genus *Metschnikowia* in the family Metschnikowiaceae was first proposed by Kamienski in 1899. In The Yeasts: A Taxonomic Study, 5th Edition, 39 species have been accepted into this genus (Lachance, 2011). In 2016, based on the short DNA sequences such as the D1/D2 domains of the LSU or concatenated sequences of a few genes, Lachance (2016) reported that the family Metschnikowiaceae comprises 83 *Metschnikowia* species with the species of the genus *Clavispora*, sister species of the genus *Metschnikowia*, and related *Candida* species. In the same article, the genus *Metschnikowia* is defined as a clade that consists of 81 species in which the sexual stage forms elongated asci containing only two ascospores, which are usually needle-shaped. However, in a small number of species, the sexual stage could not be detected. Furthermore, eleven novel species have been described. They include *Metschnikowia persimmonesis*, which was described based on a strain isolated from a fruit stalk and calyx of *Diospyros kaki* obtained in South Korea (Kang et al., 2017), *Metschnikowia maroccana* that was described from strains isolated from floral nectar in Morocco (de Vega et al., 2018), *Metschnikowia anglica* isolated from an unknown source, *Metschnikowia leonuri* from flowers, a mushroom and the gummy exudate of a wild cherry tree in the USA, *Metschnikowia peoriensis* from flowers and wild blackberries from the USA and *Metschnikowia rubicola* from thimbleberries and the flowers of red clover from the USA (Kurtzman et al., 2018), *Metschnikowia citriensis* isolated from the surface of citrus leaves collected in Chongqing Province, China (Liu et al., 2018), *Metschnikowia baotianmanensis* isolated from the gut of *Allomyrina dichotoma* (Coleoptera: Scarabeidae) collected from Henan Province, Central PR China (Chai et al., 2019), *Metschnikowia miensis* isolated from flowers growing in fields of mustard and broad beans in Japan (Shibayama et al., 2020) and *Metschnikowia ahupensis* isolated from the gut of the wood-feeding termite *Nasutitermes* sp. in Maharashtra, India (Tiwari et al., 2023). More recently, *Metschnikowia ratanii* was isolated from the nectar of *Lantana camara* flowers in Pune, India (Avchar et al., 2025). Six *Candida* species have been transfered to *Metschnikowia* as new combinations: *Metschnikowia chrysomelidarum*, *Metschnikowia gelsemii*, *Metschnikowia kofuensis*, *Metschnikowia picachoensis*, *Metschnikowia pimensis* and *Metschnikowia rancensis* (Kurtzman et al., 2018).

The genus *Moniliella* was introduced to accommodate two novel species, *Moniliella acetoabutens* and *Moniliella tomentosa* (Stolk & Dakin, 1966). Nine species of the genus *Moniliella* are listed in The

Yeasts, A Taxonomic Study 5th edition in 2011, *Moniliella acetoabutens*, *Moniliella fonsecae, Moniliella megachiliensis*, *Moniliella mellis*, *Moniliella nigrescens*, *Moniliella oedocephalis*, *Moniliella pollinis*, *Moniliella spathulata*, and *Moniliella suaveolens* (de Hoog et al., 2011). Later, two novel yeast species associated with fatty substances that contaminated meat processing tools, *Moniliella carnis*, *Moniliella dehoogii*, were proposed (Thanh et al., 2012). Then, twelve strains of yeast were described as *Moniliella byzovii*, they were isolated from flowers of *Ipomoea pes-caprae* and *Calotropis gigantea* by using an enrichment medium containing 50 % glucose (Thanh et al, 2013). Thanh et al. (2018) did an enumeration and identification of the yeasts that occurred at different stages of Vietnamese soy paste (tuong) production. They described 4 novel species comprising new *Moniliella* species, described as *Moniliella sojae*, the species of *Moniliella suaveolens* was reassigned to two novel species and named as *Moniliella pyrgileucina* and *Moniliella casei,* and an emended novel species combination are provided as *Moniliella macrospora* emend. comb. nov. In 2019, a novel species of black yeast isolated from flowers in Vietnam was described as *Moniliella foricola* (Thanh and Hien, 2019). Recently, *Moniliella aeria* was isolated from air in Yibin, Sichuan Province, PR China (Lei et al., 2022). *Moniliella zaluziensis* was isolated from blossoms of a peach tree (*Prunus persica*) and soil adjacent to a pear tree (*Pyrus communis*) in the Malé Záružie locality in the south-west of Slovakia (Pipiková et al., 2023). Currently, there are 19 *Moniliella* species. Members of this genus can be recognized by the formation of greyish to olivaceous black colonies and asexual reproduction by multilateral budding and the formation of hyphae and arthroconidia (de Hoog et al., 2011).

Toddy palm inflorescence sap is a product of the toddy palm (*Borassus flabellifer* L.). It is obtained by tapping the tip of the young inflorescence, as is done with the inflorescence of coconut (*Cocos nucifera* L.). The toddy palm inflorescence sap has a high nutritive value. It is rich in carbohydrates, minerals, and vitamins (Okafor 1978; Barh and Mazumdar, 2008; Sarkar et al., 2023). Of the total sugars present in palm sap, sucrose is the most predominant followed by glucose, fructose, inositol, and raffinose in minor quantities. Sugar obtained from *Borassus flabeliffer* and *Phoenix sylvestris* sap has higher calcium and protein contents, 1.32 mg/100 ml and 3.63%, respectively (Sama et al., 2022). There are numerous products obtained from toddy palm infloresence sap including palm sugar, palm syrup, palm wine (toddy), and vinegar (Sharma and Meghwal, 2021). The microbial flora is richer in toddy palm infloresence sap compared to coconut infloresence sap (Chrystopher et al., 1988a; 1988b). The microflora commonly found in toddy palm infloresence sap has been reported as *Saccharomyces cerevisiae*, *Kloeckera apiculata*, *Schizosaccharomyces pombe*, *Bacillus cereus*, *Lysinibacillus sphaericus* (*Bacillus sphaericus*), and *Cytobacillus firmus (Bacillus firmus)*. Among these, *S. cerevisiae* is the predominant microorganism and the best ethanol fermenting yeast species (Theivendirarajah and Chrystopher, 1987).

In the present study, we examined the yeasts found in toddy palm inflorescence sap in Thailand and found two strains, YSP-519 and YSP-478, representing two novel species. Based on phylogenetics, genetic and phenotypic properties, the strain YSP-519 strain represents a novel species of the genus *Metschinokowia*. The strain YSP-487 strain is proposed as a novel species of the genus *Moniliella* (a black yeast). Since 2018, we have worked to isolate a second strain of these two novel species with no success. This paper describes two novel yeast species based on only these two strains as *Metschnikowia sirindhorniae* sp. nov. and *Moniliella princeps* sp. nov.

MATERIALS AND METHODS

Yeast isolation

Strains YSP-519^T and YSP-478^T were obtained from toddy palm inflorescence sap collected from San Buri district, Chai Nat province, Thailand on 9 May 2018 and Ban Lat district, Phetchaburi province, Thailand on 4 April 2018, respectively. A dilution plate technique was used for yeast isolation. A sample was serially diluted, and a 0.1 ml aliquot was spread on yeast extract-malt extract (YM) agar (0.3% yeast extract, 0.3% malt extract, 0.5% peptone, 1% glucose and 1.5% agar) supplemented with 0.02% chloramphenicol and 0.025% sodium propionate and incubated at 25 ºC for 3 days. Different yeast colony morphologies were picked and purified by repeated cross-streaking on YM agar. Purified yeast strains were suspended in YM broth supplemented with 10% (v/v) glycerol and maintained at -80 ºC.

DNA sequencing and phylogenetic analysis

Nuclear DNA of YSP-478 and YSP-519 were isolated and purified according to the procedure reported by Limtong et al. (2007). The nucleotide sequences of the D1/D2 domains of the large subunit (LSU) rRNA gene and the internal transcribed spacer (ITS) regions were determined from PCR products and amplified from genomic DNA extracted from cells. Amplification of the D1/D2 domains of the LSU rRNA gene was conducted using PCR with the forward primer NL1 (59-GCATATCAATAAGCGGAGGAA

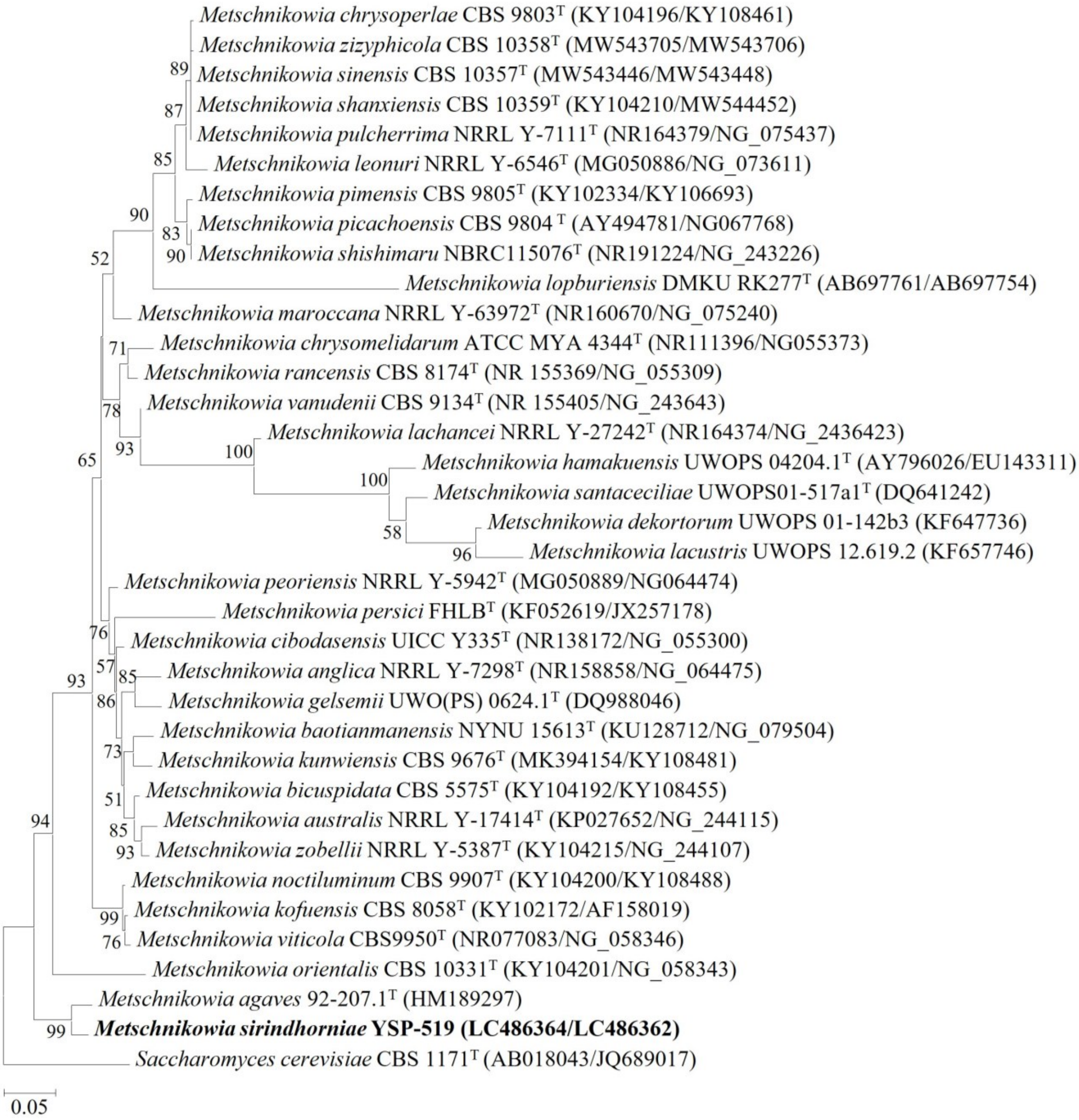

FIGURE 1. Phylogenetic tree based on the concatenated sequence of the ITS and the D1/D2 domains of the LSU rRNA gene, indicating the positions of *Metschnikowia sirindhorniae* f.a. sp. nov. with respect to closely related species. The phylogenetic tree was reconstructed using the maximum-likelihood method with MEGA software version 12. Numbers at nodes indicate percentages of bootstrap support, derived from 1000 samples. Numbers in parentheses are GenBank accession numbers in the order of ITS/D1/D2. *Saccharomyces cerevisiae* CBS 1171^T (AB018043/JQ689017) was used as the outgroup in these analyses. *Bar,* patristic distance of 0.05.

A AG-39) and the reverse primer NL4 (59-GGTCCG TGTTTCAAGACGG-39) (Kurtzman and Robnett, 1998). The ITS regions were amplified with the forward primer ITS5 (59-GGAAGTAAAAGTCGTAA CAAGG-39) and the reverse primer ITS4 (59-TCCTC CGCTTATTGATATGC-39) (White et al., 1990).

The PCR conditions were: 94 ºC for 5 min; 30 cycles of 94 ºC for 1 min, 52 ºC for 1.30 min, and 72 ºC for 2 min 30 s; 72 ºC for 10 min for D1/D2 domains and 94 ºC for 3 min; 30 cycles of 94 ºC for 30 s, 52 ºC for 30 s and 72 ºC for 45 s; 72 ºC for 10 min for D1/D2 and ITS regions. The PCR products were purified using a GenepHlow^{TM} Gel/PCR Kit (Geneaid Biotech Ltd., Taiwan) according to the manufacturer's instructions. Sanger sequencing was performed by Macrogen, Inc. (Korea). The sequence divergence of the D1/D2 domains of the LSU rRNA gene and the ITS regions of the yeast strains were compared with similar sequences

TABLE 1. Phenotypic characteristics that differentiate *Metschnikowia sirindthorniae* f.a. sp. nov. and *Moniliella princeps* sp. nov. from its closest species, *Metschnikowia agaves* and *Moniliella pollinis*, respectively.

Characteristics	*M. sirindhorniae* f.a. sp. nov.	*M. agaves*	*Mo. princeps* sp. nov.	*Mo. pollinis*
Fermentation:				
Glucose	-	s	+	+
Galactose	-	-	-	-/w
Maltose	-	-	-	+
Assimilation of carbon compounds:				
Galactose			s	-
Inulin	-	-	w	-
Soluble Starch	w	-	-	v
D-Xylose	+	s	-	v
Glycerol	+	-	+	+
Erythritol	-	-	1	+
D-Mannitol	+	+	s	+
Methyl α-D-Glucoside	+	-	-	-
D-Glucono-1,5-lactone	1	-	+	nd
Succinic acid	+	s	1	+
D-Gluconic acid	1	-	-	nd
D-Glucosamine	1	-	-	nd
Hexadecane	-	w	-	nd
Other characteristics:				
Growth in Vitamin-free medium	w	-	+	v
Growth at 37 °C	+	+	-	+

Growth reactions: -, no growth; w, weak growth; s, slow growth; +, strong growth; v, variable; nd, not determined.

Data for *Metschnikowia sirindhorniae* f.a. sp. nov. YSP-519T and *Moniliella princeps* sp. nov. YSP-478T are from the present study, for *M. agaves* and *Mo. pollinis* are from Lachance, M.A. (2011).

in the GenBank database using a nucleotide BLAST analysis tool (Altschul et al.,1997). The phylogenetic placement of the proposed novel species along with neighbouring taxa was analysed using the concatenated sequences of the ITS and D1/D2 domains of the LSU rRNA gene. The sequences of the ITS regions and D1/D2 domains were aligned with those of the type strains of related species (retrieved from the NCBI GenBank database) using MUSCLE (Edgar, 2004). The phylogenetic trees were constructed using the maximum-likelihood method based on the Tamura-Nei model (Tamura and Nei, 1993). Evolutionary analyses were conducted in MEGA12 (Kuma, et al., 2024). Confidence levels of the clades were estimated from bootstrap analysis with 1000 replicates (Felsenstein, 1985).

Phenotypic characterization

The strains, YSP-519^{T} and YSP-478^{T}, were characterized morphologically, biochemically and physiologically according to the standard methods described by Kurtzman et al. (2011). Formation of pseudohyphae and true hyphae was investigated by cultivation on potato dextrose agar (PDA; 20% potato infusion, 2% glucose and 1.5% agar) and cornmeal agar (CMA; 2% cornmeal infusion and 1.5% agar) in slide culture at 25 °C for up to 2 weeks. Ascospore formation was investigated on YM agar, 5% malt extract agar (5% malt extract and 1.5% agar), Fowell's acetate agar (0.5% sodium acetate trihydrate and 2% agar), corn meal agar (2% corn meal infusion and 1.5% agar), Gorodkowa agar (0.1% glucose, 0.5% sodium chloride, 1% peptone and 2% agar) at 15 and 25 °C for up to 4 weeks. Growth at various temperatures was determined by cultivation in Yeast Malt (YM) broth (0.3% yeast extract, 0.3% malt extract, and 1% dextrose). Carbon and nitrogen source assimilation tests were conducted in liquid media. Starved inoculums were used in nitrogen assimilation tests (Kurtzman et al., 2011).

RESULTS

Novel species delineation and phylogeny

Analysis of the D1/D2 domains of the LSU rRNA gene sequence revealed that YSP-519 showed 1.6% substitutions (9 nucleotide substitutions and 2 gaps out of 555 nt) from the type strain of *M. agaves* CBS 7744^{T}, the closest related species. The nucleotide sequence of the ITS region differed by 0.7% substitutions (3 nucleotide substitutions and 6 gaps out of 484 nt) from *M. agaves*. In the Maximum Likelihood phylogenetic tree constructed based on the ITS and D1/D2 sequences further demonstrated that the strain YSP-519 was in the *Metschnikowia* clade, clustered with *M. agaves* CBS

7744^T by 99% bootstrap (Fig. 1). Strain YSP-519 also differed in several phenotypic characteristics from *M. agaves*, the closest species, as shown in Table 1. Ascospore formation was not observed on any of the sporulation media tested at 15 and 25 °C for up to 4 weeks. The strain YSP-519 represented a novel species of the genus *Metschnikowia* based on the sequences divergence of the D1/D2 domains and the ITS regions, the phylogeny, and phenotypic properties. The name *Metschnikowia sirindhorniae* f.a., sp. nov. is proposed. According to the new rules of the Melburne Code, the suffix f.a. (forma asexualis) indicates that the sexual cycle is not known (McNeill et al., 2012).

The D1/D2 domains of the LSU rRNA sequence of the YSP-478 strain showed 0.5% substitutions (3 nucleotides substitutions) and 5 gaps out of 554 and 559 nt from the closest related species, *Moniliella pollinis* CBS 461.67^T and *Moniliella megachiliensis* CBS 190.92^T, respectively. The nucleotide sequence of the ITS regions differed by 2% substitutions (9 nucleotide substitutions) and 14 gaps out of 473 nt from the closest related species *Mo. pollinis* CBS 461.67^{T,} and 4.9% substitutions (22 nucleotides substitutions) and 11 gaps out of 464 nt from *Mo. megachiliensis* CBS 190.92^T. In the Maximum Likelihood phylogenetic tree constructed based on the ITS and D1/D2 sequences, the YSP-478 strain formed a new lineage, distant from all known taxa (Fig. 2). *Mo. pollinis* and *Mo. megachiliensis* were the least divergent known species in terms of pairwise sequence divergence and were supported by 97 bootstrap confidences. In the phylogenetic trees based on the ITS and the D1/D2 sequences clearly show that *Mo. byzovii* and *Mo. fonsecae* forming a well-supported, distinct clade that is separate from the main cluster of *Moniliella* because they showed a high level of sequence divergence. *Tilletiaria anomala* was used as an outgroup in phylogenetic analysis because molecular evidence has confirmed its distantly related taxon distinct. Strain YSP-478 also differed from *Mo. pollinis*, the closest related species by its ability to assimilate galactose and inulin and its growth at 37 ºC (Table 1). The strain showed the morphological characteristics of black colonies typical of *Moniliella* species. Since the sequences of the ITS and D1/D2 domains, and the phenotypic properties are distinguished from the closest related species and include typical morphological characteristics, this strain represents as a novel species of basidiomycetous yeasts of the genus *Moniliella* and the name *Moniliella princeps* sp. nov. is proposed.

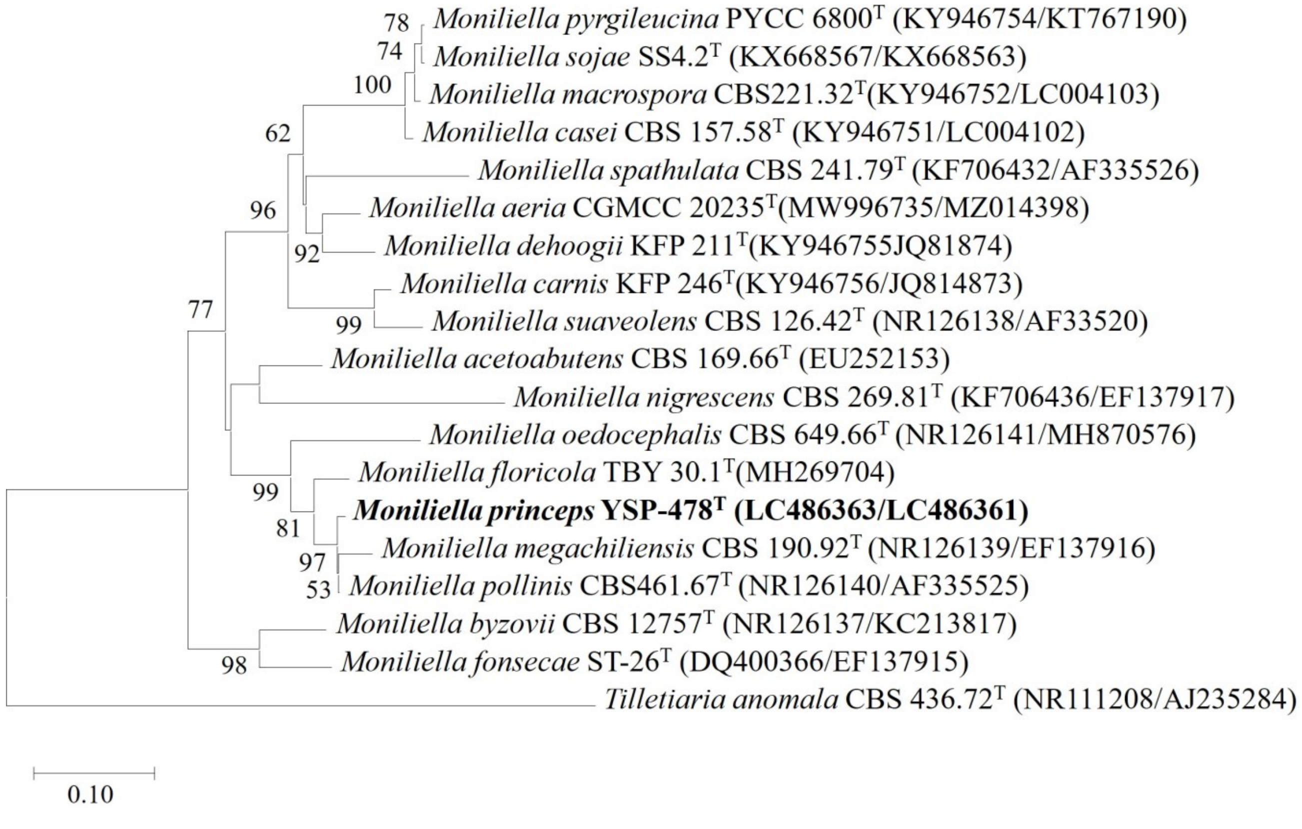

FIGURE 2. Phylogenetic tree based on the concatenated sequence of the ITS and the D1/D2 domains of the LSU rRNA gene, indicating the positions of *Moniliella princeps* sp. nov. with respect to closely related species. The phylogenetic tree was reconstructed using the maximum-likelihood method with MEGA software version 12. Numbers at nodes indicate percentages of bootstrap support, derived from 1000 samples. Numbers in parentheses are GenBank accession numbers in the order of ITS/D1/D2. *Tilletiaria anomala* CBS 436.72^T (NR111208/AJ235284) was used as the outgroup in these analyses. *Bar,* patristic distance of 0.10.

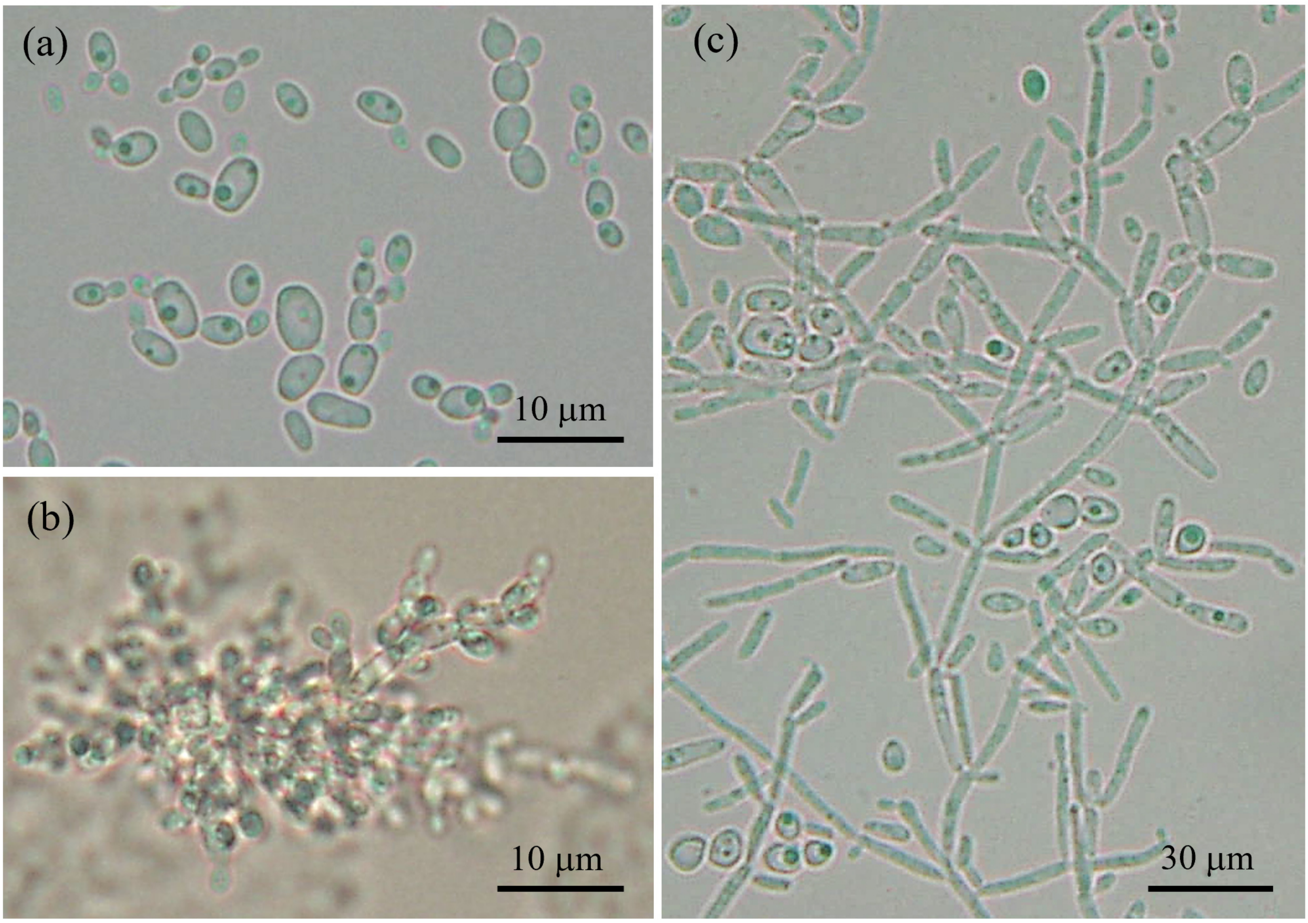

FIGURE 3. *Metschnikowia sirindhorniae* sp. nov. YSP-519^T. **A.** budding cells in YM broth after 3 days at 25 °C (*bar*, 10 µm); **B.** pseudomycelium formed on PDA after 7 days at 25 °C (*bar*, 10 µm); **C.** pseudomycelium in YM broth after 14 days at 25 °C (*bar*, 10 µm).

Taxonomy and Systematic Descriptions

Metschnikowia sirindhorniae sp. nov.

MycoBank No: 858878

(Fig. 3)

Etymology.– The species name "*sirindhorniae*" is named to honour Her Royal Highness Princess Maha Chakri Sirindhorn, who passionately plays an important role in promoting the conservation of biodiversity and the environment of Thailand.

Type strain.– YSP-519^T (TBRC 10642^T) is the holotype of *Metschnikowia sirindhorniae* sp. nov. It was obtained from toddy palm inflorescence sap, in Sankhaburi district, Chai Nat province, Thailand. It is permanently preserved in a metabolically inactive state in the culture collection of the Thailand Bioresource Research Center (TBRC), National Center for Genetic Engineering and Biotechnology, Pathum Thani province, Thailand. The isotype culture has been deposited in the China General Microbiological Culture Collection Center (CGMCC), China, as strain CGMCC 2.6079^T. The MycoBank number for *Metschnikowia sirindhorniae* is MB858878.

Description.–

Growth in YM broth: After 3 days at 25 °C, cells are spherical to ellipsoidal (1.0–4.8 x 1.4–5.7 µm) and occur singly or in pairs. Short chains of pseudohyphae and sediment are present. Budding is multilateral (Fig. 3A). Growth on YM agar: After 3 days at 25 °C, the streak culture is butyrous, cream-coloured, circular, raised, with a smooth surface and an entire margin. Pseudohyphae are produced on a slide culture on CMA and PDA after 7 days at 25 °C (Fig. 3B) and were found in YM broth after 14 days (Fig. 3C). Ascospores are not produced on common sporulation media including YM agar, 5% malt extract agar, Fowell's acetate agar, corn meal agar and Gorodkowa agar after 4 weeks at 25 °C. Fermentation of glucose, galactose, sucrose, maltose, lactose, melibiose, raffinose, xylose and fructose are negative. Glucose, galactose, L-sorbose, su-

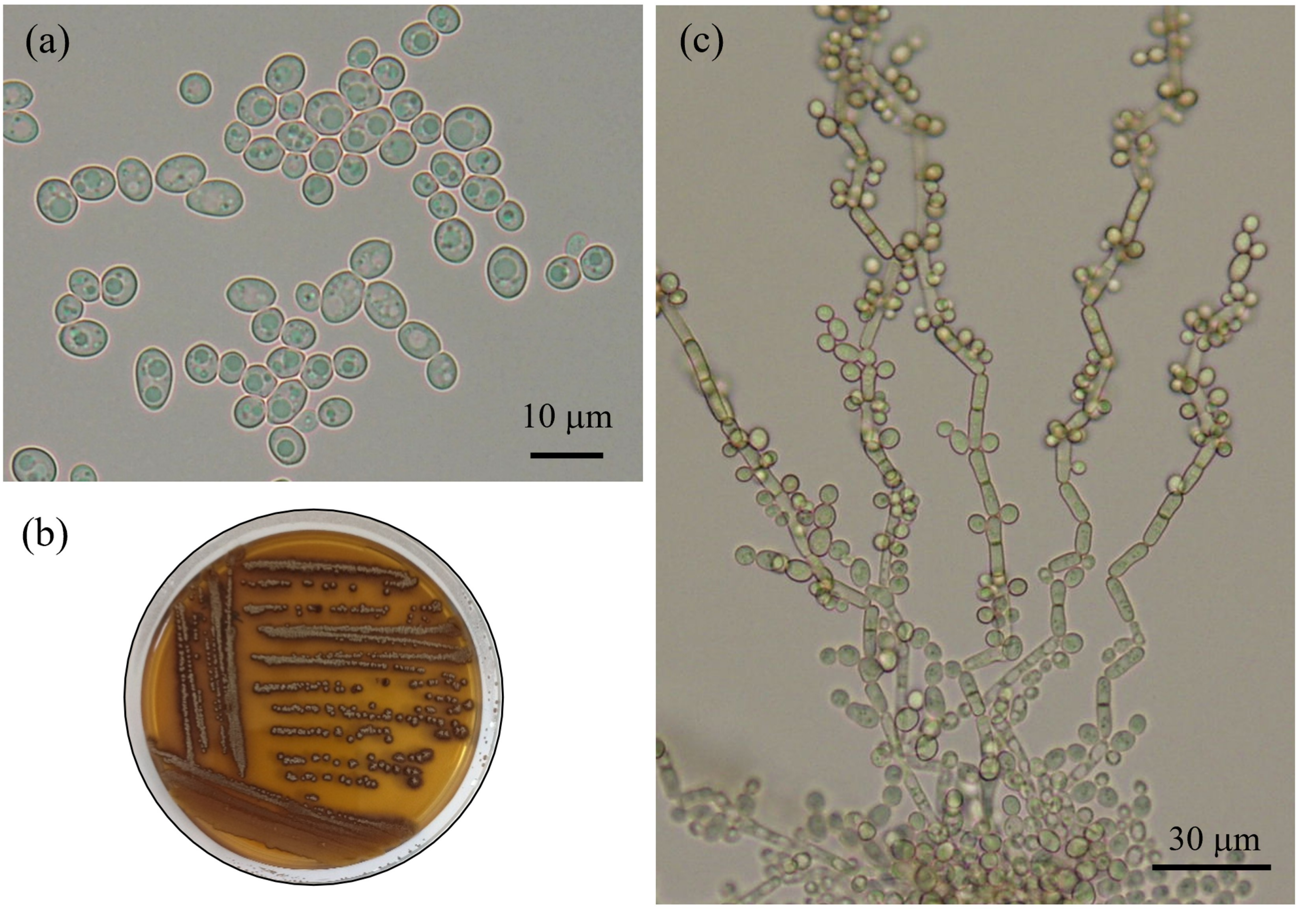

FIGURE 4. *Moniliella princeps* sp. nov. YSP-478^{T}. **A.** budding cells in YM agar after 3 days at 25 °C (*bar*, 10 µm); **B.** growth on YM agar after 7 days at 25 °C. **C.** pseudomycelium and arthospore formed on YM agar after 14 days at 25 °C (*bar*, 30 µm).

crose, maltose, cellobiose, trehalose, melezitose, soluble starch (weak), D-xylose, ethanol, glycerol, ribitol, D-mannitol, D-glucitol, α-methyl-D-glucoside, salicin, D-glucono-1,5-lactone, 2-keto-D-gluconic acid, succinic acid, citric acid, xylitol, D-gluconic acid, D-glucosamine, and *N*-acetyl-D-glucosamine are assimilated. Lactose, melibiose, raffinose, inulin, L-arabinose, D-arabinose, D-ribose, L-rhamnose, erythritol, galactitol, 5-keto-D-gluconic acid, DL-lactic acid, inositol, D-glucuronic acid, D-galacturonic acid, L-arabinitol, propane1, 2 diol, butane 2, 3 diol, methanol, and hexadecane are not assimilated. Ammonium sulfate, ethylamine, L-lysine, and cadaverine are assimilated, but potassium nitrate and sodium nitrite are not. Growth in vitamin free medium is weakly positive. Growth at 37 °C is positive. Growth in the presence of 10% (w/v) sodium chloride plus 5% (w/v) glucose is positive but weakly positive in 15% (w/v) sodium chloride plus 5% (w/v) glucose. Growth on media containing 50 and 60% (w/v) glucose and growth in the presence of 0.01% and 0.1% cycloheximide are absent. Acid formation, starch formation, and liquefaction of gelatin are negative. Urease activity and the diazonium blue B color reaction are negative.

Moniliella princeps sp. nov.

MycoBank No: 858879

(Fig. 4)

Etymology.– The specific epithet derivation is from the Latin '*princeps*' for 'the first, the foremost, the most eminent', in relation to Her Royal Highness Princess Maha Chakri Sirindhorn, who passionately plays an important role in promoting the conservation of biodiversity and the environment of Thailand.

Type strain.– YSP-478^{T} (TBRC 10641^{T}) is the holotype of *Moniliella princeps* sp. nov. It was obtained from toddy palm inflorescence sap, Ban Lat, Petchaburi province, Thailand. It is permanently preserved in a metabolically inactive state in the culture collection of the Thailand Bioresource Research Center (TBRC), National Center for Genetic Engineering and Biotechnology, Pathum Thani province, Thailand. The isotype culture has been deposited in the China General Microbiological Culture Collection Center (CGMCC), China, as strain CGMCC 2.6078^T. The MycoBank number for *Moniliella princeps* is MB858879.

Description.–

Growth in YM broth: After 3 days at 25 °C, cells are oval, subglobose, ellipsoidal to cylindrical (1.4–8.1 x 2.4–11.9 µm) and occur singly or in pairs. Short chains of pseudohyphae, pellicles, and sediment are formed. Asexual reproduction is by multilateral budding (Fig. 4A). Growth on YM agar: After 3 days at 25 °C, the streak culture has a rough surface, butyrous with a slightly lobed margin. Its colour gradually changed from brownish to olivaceous brown (Fig. 4B). Pseudohyphae and true hyphae are produced that broke into arthroconidia (Fig. 4C) on a slide culture on YM agar, CMA, and PDA after 14 days at 25 °C. Fermentation of glucose, sucrose, fructose (slow) are positive, but galactose, maltose, lactose, melibiose, raffinose and xylose are negative. Glucose, galactose (slow), sucrose, maltose, cellobiose, inulin (weak), ethanol, glycerol, erythritol (latent), D-mannitol (slow), D-glucitol, D-glucono-1,5-lactone, succinic acid (latent), citric acid, and xylitol (weak) are assimilated, but L-sorbose, trehalose, lactose, melibiose, raffinose, melezitose, soluble starch (weak), D-xylose, L-arabinose, D-arabinose, D-ribose, L-rhamnose, ribitol, galactitol, α-methyl-D-glucoside, salicin, 2-keto-D-gluconic acid, 5-keto-D-gluconic acid, DL-lactose, inositol, D-glucuronic acid, D-galacturonic acid, L-arabinitol, D-gluconic acid, D-glucosamine, *N*-acetyl-D-glucosamine, propane1, 2 diol, butane 2,3 diol, methanol, and hexadecane are not assimilated. Ammonium sulfate, potassium nitrate, sodium nitrite, ethylamine, L-lysine, and cadaverine are assimilated. Growth in vitamin free medium is positive. Growth at 36 °C is positive, but negative at 37 °C. Growth in the presence of 10% (w/v) sodium chloride plus 5% (w/v) glucose and in 15% (w/v) sodium chloride plus 5% (w/v) glucose is latent positive. Growth on media containing 50 and 60% (w/v) glucose is positive. Growth in the presence of 0.01 and 0.1% cycloheximide is negative. Acid formation and liquefaction of gelatin are positive. Starch formation is negative. Urease activity and the diazonium blue B color reaction are positive.

DISCUSSION

Strains YSP-519 and YSP-478, which are proposed to be *M. sirindhorniae* sp. nov. and *Mo. princeps* sp. nov., were isolated from toddy palm inflorescence sap. However, this sap may not be the source of these yeasts. Inflorescence sap may be contaminated during its collection by tapping. Tapping toddy palm inflorescence for their sap is the process in which the inflorescence tip is cut and sap flows out (Nguyen and Saka, 2016). During this process, insects, especially bees, consume the sap. These insects may bring yeasts to the sap. We confirmed this idea by using aseptic technique to collect sap and did not find any yeast or other microorganisms in the sap (data not shown). In Thailand, only three *Metschnikowia* species were reported. They are *Metschnikowia koreensis* found in the phylloplane of bitter bush and sugarcane (Limtong and Koowadjanakul, 2012; Limtong et al., 2014). *Metschnikowia saccharicola* and *Metschnikowia lopburiensis* were isolated from sugarcane phylloplane (Kaewwichian et al., 2012). Recently, *Metschnikowia lannaensis* was isolated from the flowers of tea, *Camellia sinensis* var. *assamica*, collected from the Suthep subdistrict, Muang district, Chiang Mai province, Thailand (Kanpiengjai et al., 2023). The habitat of *Mo. princeps* cannot be determined due to limited strain availability. Among the members of *Moniliella*, most are related to man-made products (e.g., cheese, fermented meats, and other foods), flowers, and insects. In 2009, strains of *Moniliella fonsecae* were isolated from flowers in Thailand, Cuba, and Brazil (Rosa et al., 2009). It is a ubiquitous microorganism adapted to flowers of tropical forest ecosystems and to the insects that visit these flowers. In terms of its evolutionary relationship, *Mo. princeps* is closely related to *Mo. pollinis*, which was found in pollen in honeycombs. *Mo. megachiliensis* seems to be restricted to the larval gut and frass of the alfalfa leafcutter bee (de Hoog et al., 2011), and *Mo. floricola*, which was found in flowers (Thanh and Hien, 2019). It may be assumed that *Mo. princeps* is a member of the flower and insect-associated yeasts.

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