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Moniliella sojae sp. nov., a species of black yeasts isolated from Vietnamese soy paste (*tuong*), and reassignment of *Moniliella suaveolens* strains to *Moniliella pyrgileucina* sp. nov., *Moniliella casei* sp. nov. and *Moniliella macrospora* emend. comb. nov.

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Abstract

The presence of yeasts at different steps of Vietnamese soy paste production was studied. Yeast growth occurred during primary soybean fermentation, with the cell density reaching 4.10^6 c.f.u. ml^{-1}, and terminated during brine fermentation. The dominant species were *Pichia kudriavzevii* and *Millerozyma farinosa*. Over the span of 14 years, nine strains of *Moniliella* were isolated. The strains had identical PCR fingerprints generated with primer $(GAC)_5$ and identical D1/D2 and internal transcribed spacer (ITS) sequences. A D1/D2-based phylogeny indicated that the strains were closest to a group of four previously assigned as *Moniliella suaveolens* strains. Together they form a new lineage that is well separated from all known species, including *M. suaveolens* (over 12.7 % divergence). ITS sequences indicated the presence of four species differing from each other by 9–57 nt. The name *Moniliella sojae* sp. nov. is proposed to accommodate the strains isolated from Vietnamese soy paste, *Moniliella pyrgileucina* sp. nov. is proposed for PYCC 6800 and *Moniliella casei* sp. nov. is proposed for CBS 157.58. An emended combination *Moniliella macrospora* is proposed for CBS 221.32 and CBS 223.32. The type strains and MycoBank numbers are: *M. sojae* sp. nov., SS 4.2^T=CBS 126448^T=NRRL Y-48680^T and MB 822871; *M. pyrgileucina* sp. nov., PYCC 6800^T=CBS 15203^T and MB 823030; *M. casei* sp. nov., CBS 157.58^T=IFM 60348^T and MB 822872; *M. macrospora* emend. comb. nov., CBS 221.32^T (=MUCL 11527^T) and MB 822874.

Condiments made from fermented soybean are popular in Asian cuisine. *Tuong* (soy paste) is a Vietnamese variety. *Tuong* is produced from soybean and glutinous rice in a multi-step fermentation process. For the production of *tuong*, after removal of the seed coat, soybeans are roasted, ground and soaked in water. Spontaneous fermentation of ground soybeans is carried out for about a week at ambient temperature. In a separate process, glutinous rice is cooked, spread on bamboo trays in a 3–5 cm layer and subjected to the growth of environmental fungi. After 2–3 days of solid-state fermentation, rice granules are covered with greenish-yellow fungal mycelia, mainly of *Aspergillus oryzae*. The mash is then transferred to a closed container and saccharification is carried out for 1 day by the action of fungal enzymes. The process releases heat and the temperature of the hydrolysing mash may reach 50–55 °C. Partially hydrolysed rice is then mixed with the fermented soybean suspension and sea salt (10–12 %). Brine fermentation is carried out for about 3 months with periodical mixing and sunning. The final product is amber-coloured, salty sweet and has a typical aroma of fermented soybean [1]. Based on the method of production, *tuong* is most similar to soy sauce but differs from the latter by the relatively low salt concentration, the use of rice instead of wheat as an ingredient and the primary fermentation of roasted soybeans. Until now, the production of *tuong* was largely an artisan process and relied on micro-organisms introduced from the environment. The microbiology of *tuong* is poorly documented except for the presence of *A. oryzae* as the main hydrolytic enzyme producer [2] and the sporadic isolation of *Bacillus amyloliquefaciens* [3] and *Enterobacter mori* [2]. In this study, we conducted enumeration and identification of the yeasts that occurred at different stages of *tuong* production. The description of three new *Moniliella* species and an emended novel species combination are provided.

YEAST ISOLATION

A small survey of 24 samples was conducted. Samples (two) at six different stages of fermentation were collected from

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Abbreviation: ITS, internal transcribed spacer.

two *tuong* workshops at Ban Yen Nhan, a locality famous for *tuong* production in the northern part of Vietnam. Samples were stored in sterile 50 ml plastic tubes and analysed the same day. In order to avoid possible osmotic shock, serial dilutions were made using physiological solutions supplemented with 0, 5 or 10 % NaCl and spread on malt–glucose agar plates (1 % malt, 1 % glucose, 0.01 % chloramphenicol, 2 % agar) supplemented with NaCl at the same concentrations as for the sample dilutions. Yeast enumeration and isolation were conducted after a week of incubation at 28 °C. Yeast growth occurred mainly during spontaneous fermentation of roasted soybean and the cell density reached 4.10^6 c.f.u. ml^{-1}. Upon addition of salt and partially hydrolysed rice, the yeast count decreased by two orders of magnitude within 1 day of brine fermentation. Yeasts were not detectable (fewer than 500 c.f.u. ml^{-1}) after 2 weeks of brine fermentation or in the final product. The yeast counts using the media supplemented with 0 or 5 % NaCl were similar and approximately 100 times higher than in the media with 10 % NaCl. On the isolation plates, the vast majority of yeast colonies were whitish cream in colour. Singular greenish-black colonies typical of *Moniliella* species were detected sporadically. Amongst 23 whitish-cream isolates randomly taken from eight samples, 19 were identified by D1/D2 sequencing as *Pichia kudriavzevii* and four as *Millerozyma farinosa*. Both *P. kudriavzevii* and *M. farinosa* are known to occur in traditional Vietnamese fermentation processes. *P. kudriavzevii*, formerly known as *Issatchenkia orientalis*, is frequently detected in alcoholic fermentation starter (*banh men*) [4] and during alcoholic fermentation of rice [5]. Similarly, *M. farinosa* (=*Pichia farinosa*) was isolated from traditional Vietnamese fermented meat (*nem chua*) [6]. Although all yeasts detected were halotolerant and could grow in the presence of 10 % NaCl, they did not grow during brine fermentation. Most probably, the combination of high salt concentrations and excessive heat during sunning has a detrimental effect on the yeasts. In this regard, *tuong* production is different from soy sauce fermentation, where yeast growth occurs during brine fermentation and the species *Candida famata* (*Debaryomyces hansenii*), *Candida versatilis* and *Zygosaccharomyces rouxii* are common. For soy sauce production, the temperature of the brine fermentation is kept under 30 °C [7]. Although they do not play an active role in substrate hydrolysis, yeasts are believed to contribute to the aroma and organoleptic quality of the final product. In industrial production of soy sauce, selected yeast cultures are used [7].

Since the greenish-black yeast occurred at a low density and a low frequency, its role in *tuong* production remained unclear. This rare yeast was a focus for subsequent study. Over the span of 14 years, nine strains of the greenish-black yeast were isolated (SS 4.2 in 2001; TBY 1065.1, TBY 1065.2 and TBY 1065.3 in 2012; TBY 3832.1, TBY 3832.2, TBY 3838.1, TBY 3838.2 and TBY 3838.3 in 2014). D1/D2 sequencing indicated that the yeast represents a new species of *Moniliella*. The yeast will be described hereafter as *Moniliella sojae*.

GENETIC CHARACTERIZATION

The nine *Moniliella* isolates were compared by PCR fingerprinting using the microsatellite primer $(GAC)_5$. For DNA extraction, a fresh culture grown in YMA (0.5 % peptone, 0.3 % yeast extract, 0.3 % malt extract, 1 % glucose, 2 % agar) was used (black pigments formed in old cultures of *Moniliella* may inhibit the PCR reaction). One loop-full of cells was transferred to a microtube containing 1 ml 2×SSC (15 mM sodium citrate, 150 mM NaCl, pH 7.0). The tubes were heated at 99 °C for 10 min using a dry heater block (Grant bio). Cells were collected by centrifugation at 10 000 ***g*** for 1 min. To the cell pellet, approximately 100 µl glass beads (0.2–0.5 mm in diameter; Roth), 100 µl phenol/chloroform and 150 µl water were added. The cells were disrupted using a Mini-Beadbeater-8 (Biospec) for 45 s. The tubes were centrifuged at 14 000 ***g*** for 10 min and the upper layer was transferred to a new microtube. The DNA solutions were further purified using a Silica Bead DNA Gel Extraction kit (Thermo Scientific) according to the manufacturer's instructions. PCR was carried out in a C1000 Touch Thermal Cycler (BioRad) using the following thermal program: 94 °C for 3 min; 30 cycles of 94 °C for 40 s, 52 °C for 40 s and 72 °C for 1 min 30 s; 72 °C for 10 min. PCR products were separated in 1 % agarose gel in 0.5× TAE (20 mM Tris, 10 mM acetic acid, 0.5 mM EDTA). PCR fingerprints generated by the $(GAC)_5$ primer were identical for all nine strains (Fig. 1). The primer was used widely for studying genetic diversity at sub-species level [8–10]. The uniformity of *Moniliella* strains isolated from fermented soybean over the span of 14 years was quite surprising. Previously, when isolating *Moniliella* species from other substrates such as the meat processing environment or flowers, we often obtained much higher diversities. From the meat processing environment, we have detected four different species of *Moniliella* [11]. Similarly, a flower sample may

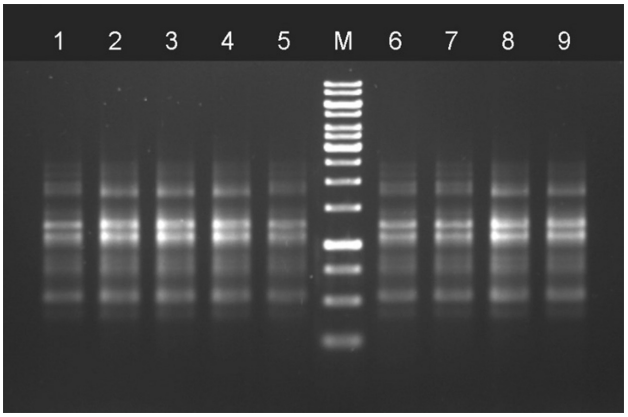

Fig. 1. Microsatellite PCR profiles of strains of *Moniliella sojae* sp. nov. generated with primer $(GAC)_5$. M, GeneRuler 1 kb DNA Ladder (Fermentas); 1–9, strains SS 4.2, TBY 1065.1, TBY 1065.2, TBY 1065.3, TBY 3832.1, TBY 3832.2, TBY 3838.1, TBY 3838.2 and TBY 3838.3, respectively.

harbour four distinct genetic groups of *Moniliella* [12]. The narrow diversity yeasts associated with *tuong* production might reflect the selective pressure that the production process exercises on the yeasts.

The *Moniliella* strains were compared and identified by sequencing of the D1/D2 LSU rRNA gene and internal transcribed spacer (ITS) regions. For amplification and sequencing of the D1/D2, primer pair NL1 and NL4 [13] was used. Similarly, primer pair ITS1 and ITS4 [14] was used for the ITS region. The PCR conditions were as follows: 94 °C for 3 min; 30 cycles of 94 °C for 40 s, 52 °C for 40 s and 72 °C for 60 s; 72 °C for 10 min. PCR products were sequenced using the service provided by First BASE Laboratories Sdn Bhd (Selangor, Malaysia). Sequences were aligned by the fast Fourier transform algorithm using the online version of MAFFT [15] (www.ebi.ac.uk/Tools/msa/mafft/) hosted at the European Bioinformatics Institute (Hinxton, Cambridgeshire, UK). Phylogenetic trees were reconstructed by using the neighbour-joining method [16] in the MEGA version 7.0 software package [17]. Bootstrap analyses were performed from 1000 random re-samplings [18]. The ITS and D1/D2 sequences of *Tilletiaria anomala* CBS 436.72^T (NR 111208, AJ235284) were used as outgroups.

ITS and D1/D2 sequences were obtained for four strains: SS4.2, TBY 1065.1, TBY 3832.1 and TBY 3838.1. In both the ITS and the D1/D2 regions, all four strains were identical, which agreed well with the microsatellite fingerprinting data (Fig. 1). In a neighbour-joining tree reconstructed based on ITS–D1/D2 sequences, the new isolates together with strains CBS 157.58, CBS 221.32, CBS 223.32 and PYCC 6800 formed a new lineage, distant from all known taxa (Fig. 2). *Moniliella dehoogii* was the least divergent known species in terms of pairwise sequence divergence but differed from strains of the lineage by 66–77 nt (or 12.7–14.8 % divergence) in the D1/D2 region. In the CBS database, CBS 157.58, CBS 221.32 and CBS 223.32 had been assigned to *Moniliella suaveolens*. According to the GenBank database, PYCC 6800 (accession number KT767190) was also placed under *M. suaveolens*. The D1/D2 sequences indicated that the group is unrelated to

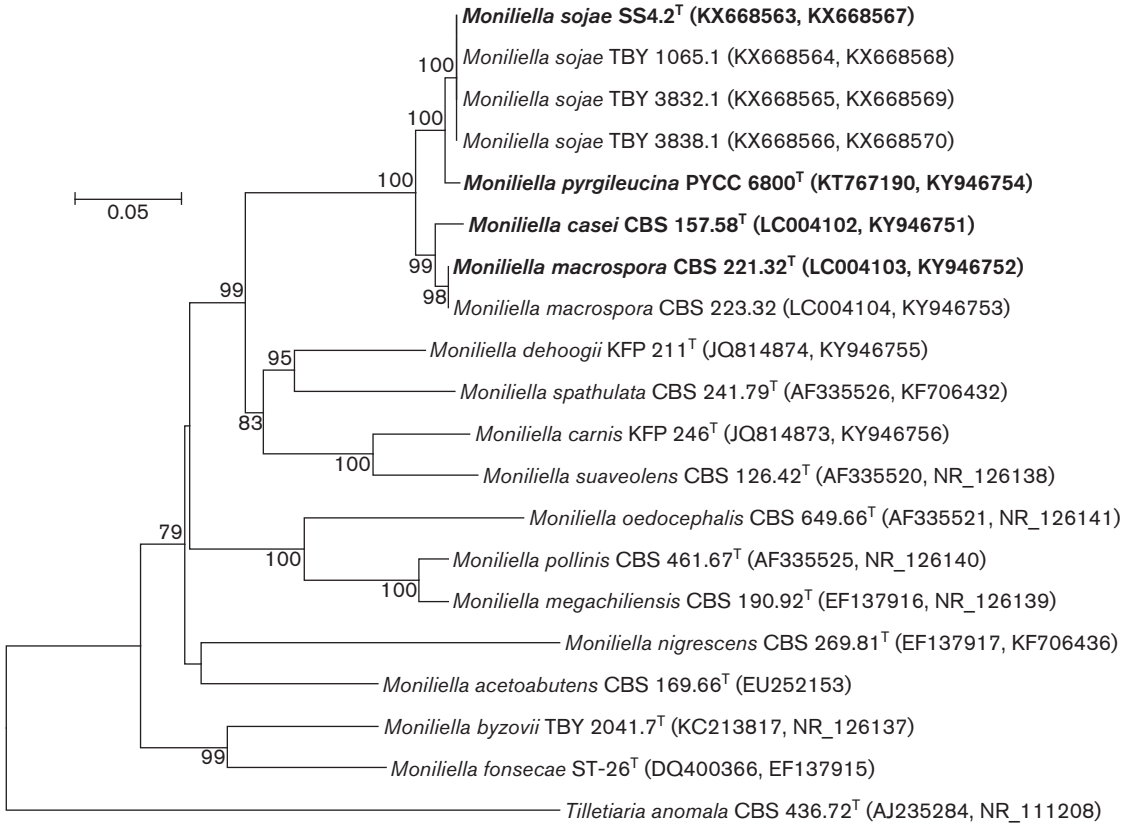

Fig. 2. Neighbour-joining phylogram depicting the relationships between *Moniliella sojae* sp. nov., *Moniliella pyrgileucina* sp. nov., *Moniliella casei* sp. nov., *Moniliella macrospora* comb. nov. and neighbouring taxa. The tree was reconstructed based on concatenate D1/D2-ITS sequences using the maximum composite likelihood distance correction (Tamura *et al.* [29]) in MEGA7 [17]. An alignment of 1181 positions corresponding to 902 nucleotides for *M. sojae* sp. nov. was analysed. All ambiguous positions were removed for each sequence pair. Bootstrap values of >50 %, obtained from 1000 replications, are shown. The D1/D2 sequence for ATCC MYA4962 was taken from the ATCC database (www.atcc.org). *Tilletiaria anomala* CBS 436.72^T was used as an outgroup. GenBank accession numbers of D1/D2 and ITS sequences, respectively, are given in parentheses. Bar, 5 % sequence divergence.

M. suaveolens. The misidentification of CBS 157.58, CBS 221.32 and CBS 223.32 was mentioned earlier [19].

In the ITS–D1/D2 tree, the strains from fermented soybean and the misidentified *M. suaveolens* strains formed three lineages (Fig. 2). The association of PYCC 6800 with the soybean isolates was supported by 100 % bootstrap confidence. Although, phylogenetically, cases could be made to treat strain PYCC 6800 as conspecific (well-supported monophyletic assemblages), the strain differed from soybean isolates (*M. sojae*) by 9 nt in the ITS region and by 5 nt in D1/D2 sequences, well at the species level [20], arguing for separation into distinct species. PYCC 6800 was also distant from soybean isolates in terms of geographical origin and substrate of isolation. PYCC 6800 was isolated from cured cheese (*queijo curado*) in Castelo Branco, Portugal. Phenotypically, PYCC 6800 differs from *M. sojae* strains by five assimilation tests (D-xylose, L-arabinose, melezitose, ribitol and xylitol). The new species, *Moniliella pyrgileucina*, is proposed to accommodate the strain. Similarly, CBS 157.58 differed from the rest by 50–57 nt in the ITS region and by 14–15 nt in D1/D2 sequences. The new species, *Moniliella casei*, is proposed for strain CBS 157.58. Strains CBS 221.32 and CBS 223.32 were identical in both D1/D2 and ITS sequences and differed from their closest relative, *M. casei* CBS 157.58, by 50 nt and 14 nt in ITS and D1/D2, respectively (Table 1). CBS 221.32 and CBS 223.32 were isolated from dried leaves of tobacco in the UK and had been assigned as the type strains of *Monilia macrospora* and *Monilia microspora*, respectively [21]. Later, the species were regarded as synonyms and reassigned to *M. suaveolens* [22]. Since both strains are identical in ITS and D1/D2 sequences and are distinct in sequences from all species of the genus *Moniliella*, the new combination *Moniliella macrospora* is proposed with CBS 221.32 as the type strain.

PHENOTYPIC PROPERTIES

The morphology of *M. sojae* sp. nov., *M. pyrgileucina* sp. nov., *M. casei* sp. nov. and *M. macrospora* comb. nov. was studied using a light microscope (Eclipse E-600; Nikon) with differential interference contrast. Images were taken with a CMOS camera DFK 61AUC02 using the supplied IC Capture AS software (The Imaging Source) with eight frames overlay for noise reduction. The strains showed the characteristic morphological appearance of *Moniliella* species. On nutrient agar media (YM agar, malt agar) the strains formed butyrous colonies with the colour gradually changing from greyish cream to olivaceous and black. Asexual reproduction was by multilateral budding and the formation of true hyphae that broke up into arthroconidia (Figs 3, 4, 5 and 6). As in other species of *Moniliella*, sexual reproduction in *M. sojae* remained unknown. Pairwise mixing of all nine strains on nutrient-rich (YM agar, malt-glucose agar) and poor (yeast carbon base agar, water agar) media yielded no sexual response. Neither conjugation tubes nor teleomorphic states were observed. Similarly, sexual reproduction was not observed for *M. pyrgileucina, M. casei* or *M. macrospora*.

Physiological tests were performed using standard methods [23] for strains of *M. sojae* (SS4.2, TBY 1065.1, TBY 3832.1, TBY 3838.1), *M. pyrgileucina* (PYCC 6800), *M. casei* (CBS 157.58) and *M. macrospora* (CBS 221.32 and CBS 223.32). Carbon assimilation tests were carried out in liquid media and the auxanographic method was used for nitrogen assimilation tests. Carbon and nitrogen sources were from Sigma, and carbon- and nitrogen-containing media were from Difco. All strains were xerotolerant and able to ferment glucose, a combination of characteristics that is common for *Moniliella* species but rare for basidiomycetous yeasts. All strains also assimilated erythritol and nitrate. *M. sojae* strains were identical and differed from *M. pyrgileucina* PYCC 6800, the closest relative, by five assimilation tests (D-xylose, L-arabinose, melezitose, ribitol and xylitol). Although identical in D1/D2 and ITS sequences, the two *M. macrospora* strains differed from each other by four assimilation tests (melezitose, ribitol, xylitol and D-glucitol). It is interesting to note that the species isolated from fermentation substrates (*M. sojae*, *M. pyrgileucina* and *M. casei*) could assimilate DL-lactate, succinate and citrate while the species isolated from tabacoo leaves (*M. macrospora*) did not. *M. sojae* and *M. casei* could be differentiated from each other by the assimilation of D-glucitol.

The strains CBS 221.32 and CBS 223.32 of *M. macrospora* comb. nov. and strain CBS 157.58 of *M. casei* sp. nov. originally belonged to *M. suaveolens*. The standard description of *M. suaveolens* indicates that the species is unable to ferment raffinose [24]. In our tests, CBS 223.32 could strongly ferment raffinose and the other strains demonstrated weak gas production after a week of incubation.

Table 1. Nucleotide difference between *Moniliella* strains in D1/D2 (above diagonal) and ITS (below diagonal) regions

Strain	SS 4.2^T	TBY 1065.1	TBY 3832.1	TBY 3838.1	PYCC 6800^T	CBS 221.32^T	CBS 223.32	CBS 157.58^T
M. sojae SS 4.2^T	–	0	0	0	5	7	7	15
M. sojae TBY 1065.1	0	–	0	0	5	7	7	15
M. sojae TBY 3832.1	0	0	–	0	5	7	7	15
M. sojae TBY 3838.1	0	0	0	–	5	7	7	15
M. pyrgileucina PYCC 6800^T	9	9	9	9	–	2	2	14
M. macrospora CBS 221.32^T	37	37	37	37	44	–	0	14
M. macrospora CBS 223.32	37	37	37	37	44	0	–	14
M. casei CBS 157.58^T	57	57	57	57	56	50	50	–

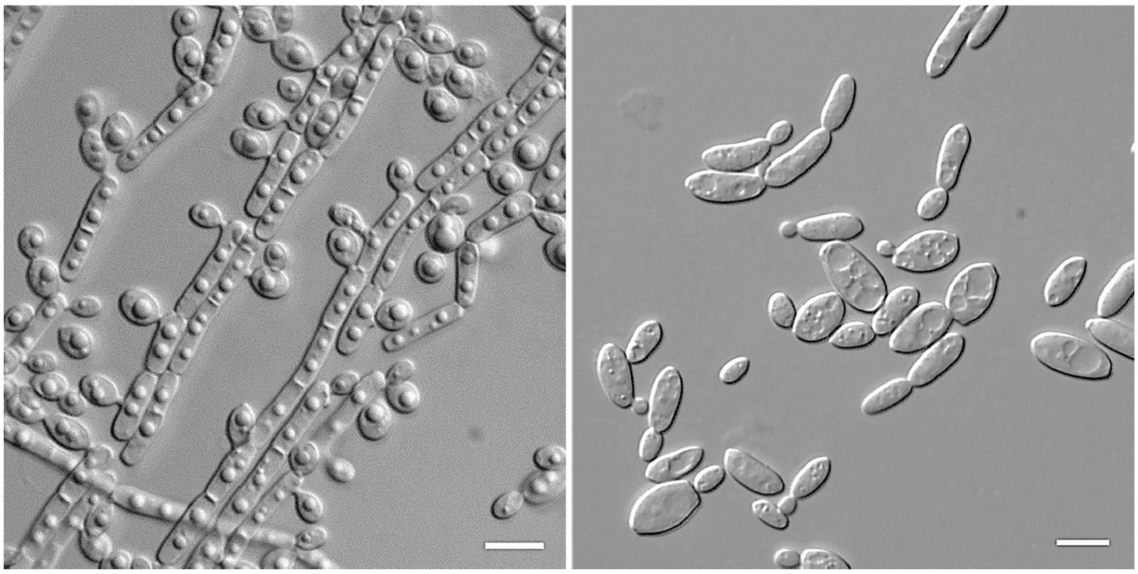

Fig. 3. Microphotographs of *M. sojae* SS 4.2^T grown on cornmeal agar for 5 days (left) and in YM medium for 2 days (right). Bar, 10 µm.

Coenzyme Q type was determined for *M. sojae* strain SS 4.2. For coenzyme Q extraction and purification, the procedure described by Yamada and Kondo [25] was used. Reversed-phase high-performance liquid chromatography (HPLC) was used for identification of coenzyme Q. Reverse-phase HPTLC plates (silica gel 60 for nano thin-layer chromatography, TLC; Merck) were prepared by dipping in n-hexane containing 5 % liquid paraffin. The plates were air-dried. Coenzyme Q samples and standards were spotted on the plates, which were then developed with methanol/isopropanol (1 : 1) saturated with liquid paraffin. Visualization was done by dipping the plates in 0.2 % $KMnO_4$ for 5 min. The major ubiquinone of *M. sojae* SS 4.2 was Q-9.

In a recent screening program for erythritol-producing yeasts, four strains of *M. sojae* were examined. When growing in a liquid medium containing 20 % glucose, all strains demonstrated a moderate level of erythritol accumulation (13.7–33.7 g l^{-1}) [26].

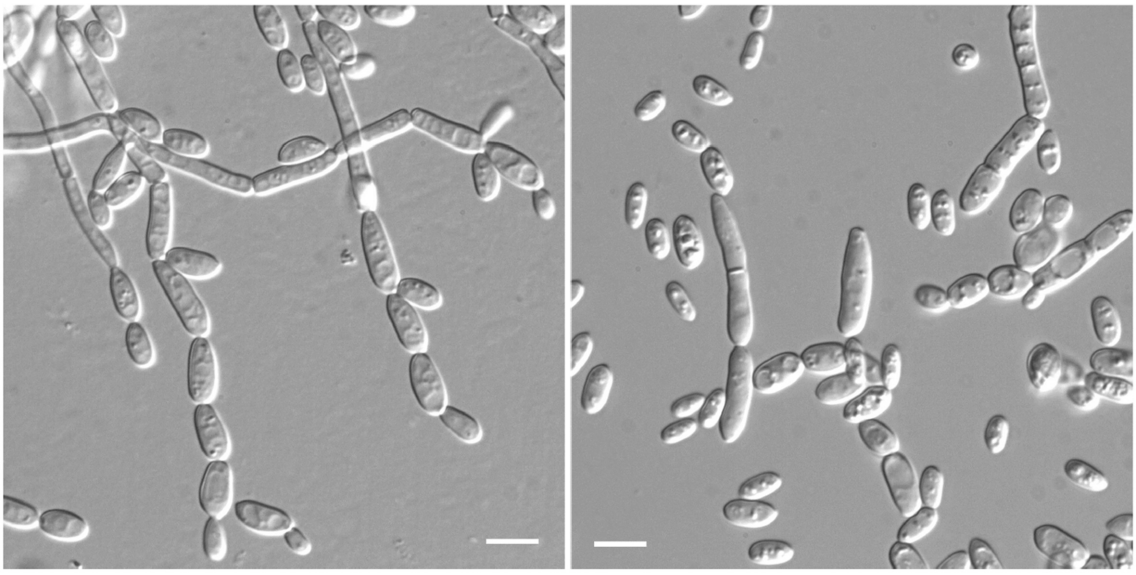

Fig. 4. Microphotographs of *M. pyrgileucina* PYCC 6800^T grown on cornmeal agar for 5 days (left) and in YM medium for 2 days (right). Bar, 10 µm.

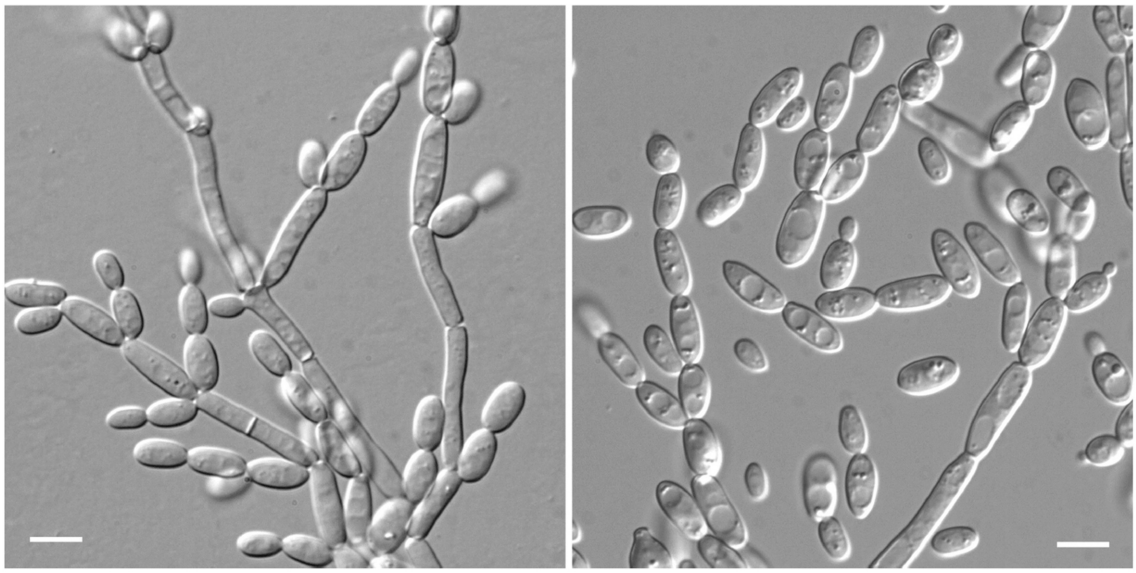

Fig. 5. Microphotographs of strain *M. casei* CBS 157.58^T grown on cornmeal agar for 5 days (left) and in YM medium for 2 days (right). Bar, 10 µm.

DESCRIPTION OF *MONILIELLA SOJAE* THANH, HIEN, YAGUCHI, SAMPAIO *ET* LACHANCE SP. NOV.

Moniliella sojae (so′jae. N.L. gen. n. *sojae* of soybean, referring to the fact that fermented soybean was the source of the first isolates).

MycoBank number: MB 822871.

After 7 days on YM agar at 25 °C colonies are cerebriform, wrinkled, risen, soft and cream to olivaceous in colour. After 3 weeks, colonies turn olivaceous to black and white cottoneous aerial mycelia are formed. In YM broth after 2 days at 25 °C, cells are ovoid to elongate and cylindrical (3.5–8×6–15 µm, elongated to 50 µm) (Fig. 3). A sediment is formed. In Dalmau plates after 7 days on cornmeal agar, pseudohyphae, true mycelia and arthroconidia are formed (Fig. 3).

M. sojae ferments D-glucose, D-galactose, maltose sucrose and raffinose (weak), but not trehalose or lactose. Assimilates D-glucose, inulin, sucrose, raffinose, D-galactose, maltose, cellobiose (weak), salicin (weak), arbutin, D-xylose (weak), L-arabinose (weak), D-ribose, ethanol glycerol, erythritol, D-glucitol, D-mannitol, DL-lactate, succinate, citrate and 2-keto-D-gluconate (weak), but not melibiose, lactose, trehalose, melezitose, methyl α-D-glucoside, starch, L-sorbose, L-rhamnose, D-arabinose, methanol, ribitol, xylitol, propane-1,2-diol, butane-2,3-diol, galactitol, myo-inositol, D-glucono-1,5-lactone, 5-keto-D-gluconate, D-gluconate, D-galacturonate or D-glucosamine. Assimilates nitrate and nitrite, but not glucosamine or imidazole. Grows in media without vitamin. Grows in media containing 1 % acetic acid but not in medium containing 0.01 % cycloheximide. Grows in media containing 60 % glucose or containing 10 % NaCl and 5 % glucose. Grows at 35 °C but not at 37 °C. Does not produce starch-like substances. Urease reaction is positive. The major ubiquinone is Q9. Accumulates erythritol in medium containing 20 % glucose.

The type strain is SS4.2^T (=CBS 126448^T=NRRL Y-48680^T), isolated from fermented soybean during traditional production of *tuong* at Ban Yen Nhan, My Hao, Hung Yen, Vietnam. It is maintained in a metabolically inactive state and ex-types have been deposited in the Yeast Collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands, and the Agricultural Research Service Yeast Collection, US Department of Agriculture, Peoria, IL, USA.

DESCRIPTION OF *MONILIELLA PYRGILEUCINA* THANH, HIEN, YAGUCHI, SAMPAIO *ET* LACHANCE SP. NOV.

Moniliella pyrgileucina (pyr.gi.leu′ci.na. Gr. n. Pyrgileucos; L. adj. f. *pyrgileucina*, referring to Pyrgileucos, the ancient name of the city of Castelo Branco, Portugal, where the strain was isolated).

MycoBank number: MB 823030.

After 7 days on YM agar at 25 °C colonies are cerebriform, wrinkled, risen, soft and creamish to olivaceous in colour. After 3 weeks, colonies are olivaceous and light aerial mycelia may be formed. In YM broth after 2 days at 25 °C, cells are ovoid to elongate and cylindrical (3.0–7.5×5.5–16.0 µm, elongated to 45 µm) (Fig. 4). A sediment is formed. In Dalmau plates after 7 days on cornmeal agar, pseudohyphae, true mycelia and arthroconidia are formed (Fig. 4).

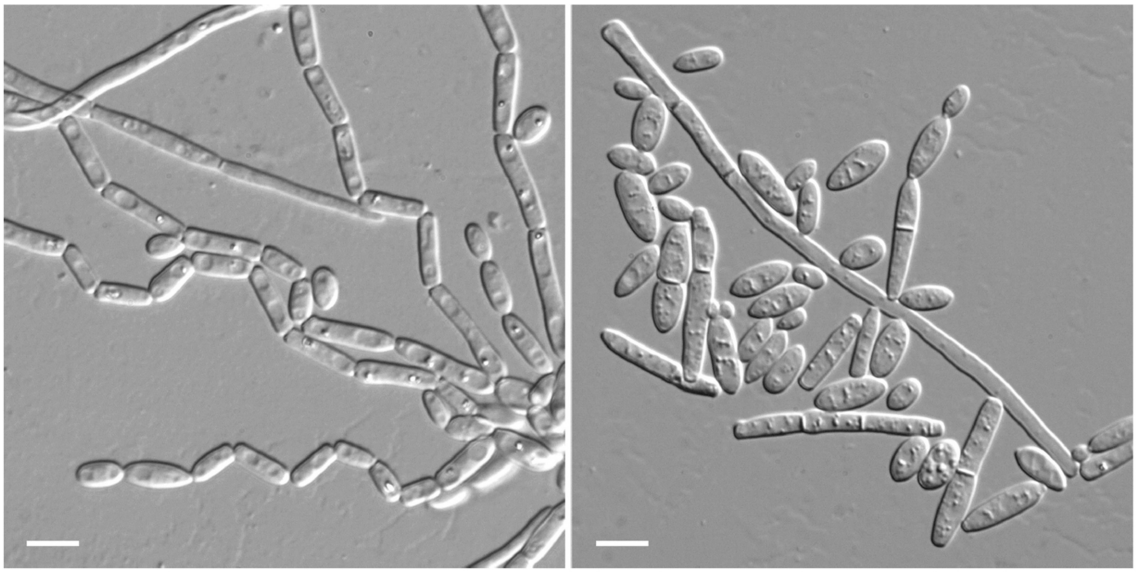

Fig. 6. Microphotographs of strain *M. macrospora* CBS 221.32^T grown on cornmeal agar for 5 days (left) and in YM medium for 2 days (right). Bar, 10 µm.

M. pyrgileucina ferments D-glucose, D-galactose, maltose, sucrose and raffinose (weak), but not trehalose or lactose. Assimilates D-glucose, inulin, sucrose, raffinose, D-galactose, maltose, melezitose, cellobiose (weak), salicin (weak), D-ribose, ethanol, glycerol, erythritol, ribitol, D-mannitol, D-glucitol, DL-lactate, succinate, citrate, 2-keto-D-gluconate (weak), xylitol and arbutin, but not melibiose, lactose, trehalose, methyl α-D-glucoside, starch, L-sorbose, L-rhamnose, D-xylose, L-arabinose, D-arabinose, methanol, propane-1,2-diol, butane-2,3-diol, galactitol, myo-inositol, D-glucono-1,5-lactone, 5-keto-D-gluconate, D-gluconate, D-galacturonate or D-glucosamine. Assimilates nitrate and nitrite, but not glucosamine or imidazole. Grows in media without vitamin. Grows in media containing 1 % acetic acid, but not in medium containing 0.01 % cycloheximide. Grows in media containing 60 % glucose or containing 10 % NaCl and 5 % glucose. Grows at 35 °C but not at 37 °C. Does not produce starch-like substances. Urease reaction is positive.

The type strain is PYCC 6800^T (=CBS 15203^T), isolated from cured cheese (*queijo curado*) in Castelo Branco, Portugal. It is maintained in a metabolically inactive state and ex-types have been deposited in the Yeast Collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands, and the Portuguese Yeast Culture Collection of the Universidade Nova de Lisboa, Caparica, Portugal.

DESCRIPTION OF *MONILIELLA CASEI* THANH, HIEN, YAGUCHI, SAMPAIO *ET* LACHANCE SP. NOV.

Moniliella casei (ca′se.i. L. gen. n. *casei* of cheese, referring to the fact that the yeast was isolated from cheese).

MycoBank number: MB 822872.

After 7 days on YM agar at 25 °C colonies are cerebriform, wrinkled, risen, soft and olivaceous to black in colour. After 3 weeks, colonies turn black and whitish to black aerial mycelia are formed. In YM broth after 2 days at 25 °C, cells are ovoid to elongate and cylindrical (4.0–7.5×6–25 µm, elongated to 60 µm) (Fig. 5). A sediment is formed. In Dalmau plates after 7 days on cornmeal agar, pseudohyphae, true mycelia and arthroconidia are formed (Fig. 5).

Moniliella casei ferments D-glucose, D-galactose, maltose, sucrose and raffinose (weak), but not trehalose or lactose. The yeast assimilates D-glucose, inulin, sucrose, raffinose, D-galactose, maltose, arbutin, D-ribose, ethanol, glycerol, erythritol (weak), D-mannitol, DL-lactate, succinate, citrate, 2-keto-D-gluconate (weak) and D-glucosamine (weak), but not melibiose, lactose, trehalose, melezitose, methyl α-D-glucoside, starch, cellobiose, salicin, L-sorbose, L-rhamnose, D-xylose, L-arabinose, D-arabinose, methanol, propane-1,2-diol, butane-2,3-diol, ribitol, xylitol, D-glucitol, galactitol, myo-inositol, D-glucono-1,5-lactone, 5-keto-D-gluconate, D-gluconate or D-galacturonate. Assimilates nitrate and nitrite, but not glucosamine or imidazole. Grows in media without vitamin. Grows in media containing 1 % acetic acid, but not in medium containing 0.01 % cycloheximide. Grows in media containing 60 % glucose or 10 % NaCl and 5 % glucose. Grows at 35 °C but not at 37 °C. Does not produce starch-like substances. Urease reaction is positive.

The type strain is CBS 157.58^T (=IFM 60348^T), isolated by J. Stadhouders from cheese in The Netherlands. It is maintained in a metabolically inactive state and ex-types have been deposited in the Yeast Collection of the Westerdijk

Fungal Biodiversity Institute, Utrecht, The Netherlands, and at Medical Mycology Research Centre, Chiba University, Inohana, Chuo-ku, Chiba, Japan.

Based on the identity in both D1/D2 and ITS sequences, we consider *Monilia macrospora* Beyma and *Monilia microspora* Beyma synonymous. On the basis of taxonomic priority, we suggest retaining the epithet *macrospora*. The species will be assigned to *Moniliella*. The new combination is as follows.

EMENDED DESCRIPTION OF *MONILIELLA MACROSPORA* (BEYMA) THANH, HIEN, YAGUCHI, SAMPAIO *ET* LACHANCE COMB. NOV.

Moniliella macrospora (Beyma) Thanh, Hien, Yaguchi, Sampaio et Lachance comb. nov. Basionym: *Monilia macrospora* J. F. H. Beyma, Zentralblatt für Bakteriologie und Parasitenkunde Abteilung 2 88 (1933), 127.

MycoBank number: MB 822874.

After 7 days on YM agar at 25 °C colonies are risen, soft, creamish to light olivaceous in colour and radial mycelia are formed. After 3 weeks, white cottoneous aerial mycelia may be formed. In YM broth after 2 days at 25 °C, cells are ovoid to elongate and cylindrical (4.0–7.5×8–20 µm, elongated to 85 µm) (Fig. 6). A sediment is formed. In Dalmau plates after 7 days on cornmeal agar, pseudohyphae, true mycelia and arthroconidia are formed (Fig. 6).

Moniliella macrospora ferments D-glucose, D-galactose, maltose, sucrose and raffinose (positive or weak), but not trehalose or lactose. The yeast assimilates D-glucose, inulin, sucrose, raffinose, D-galactose, maltose, melezitose (variable), arbutin, D-ribose, D-glucosamine (weak), ethanol, glycerol, erythritol, ribitol (variable), xylitol (variable), D-mannitol, D-glucitol (variable) and 2-keto-D-gluconate (weak), but not melibiose, lactose, trehalose, methyl α-D-glucoside, starch, cellobiose, salicin, L-sorbose, L-rhamnose, D-xylose, L-arabinose, D-arabinose, methanol, propane-1,2-diol, butane-2,3-diol, galactitol, *myo*-inositol, DL-lactate, succinate, citrate, D-glucono-1,5-lactone, 5-keto-D-gluconate, D-gluconate or D-galacturonate. Assimilates nitrate and nitrite, but not glucosamine or imidazole. Grows in media without vitamin. Grows in media containing 1 % acetic acid but not in medium containing 0.01 % cycloheximide. Grows in media containing 60 % glucose or 10 % NaCl and 5 % glucose. Grows at 35 °C but not at 37 °C. Does not produce starch-like substances. Urease reaction is positive.

The type strain, CBS 221.32^T (=MUCL 11527), was isolated by Bunting from dried leaf of *Nicotiana tabacum* in England. It is maintained in a metabolically inactive state and ex-types have been deposited in the Yeast Collection of the Westerdijk Fungal Biodiversity Institute Utrecht, The Netherlands, and BCCM/MUCL Agro-food and Environmental Fungal Collection, Croix du Sud, Louvain-la-Neuve, Belgium.

With the description of novel species *M. sojae*, *M. pyrgileucina* and *M. casei*, and the proposal of *M. macrospora* comb. nov., the genus *Moniliella* now comprises 16 species, namely *Moniliella acetoabutens*, *Moniliella byzovii, Moniliella carnis*, *Moniliella casei*, *Moniliella dehoogii*, *Moniliella fonsecae*, *Moniliella macrospora*, *Moniliella megachiliensis*, *Moniliella mellis*, *Moniliella nigrescens*, *Moniliella oedocephalis*, *Moniliella pollinis*, *Moniliella pyrgileucina*, *Moniliella sojae*, *Moniliella spathulata* and *Moniliella suaveolens*. Recently, a strain (Y12=ATCC MYA-4962) of *Moniliella* was isolated from a 20 % biodiesel blend [27]. The strain could degrade biodiesel and cause steel corrosion. In the D1/D2 sequence, the strain was closest to CBS 126.42, the type strain of *M. suaveolens*, but differed from the latter by 12 nt. The provisional name *Moniliella wahieum* was proposed for the strain. Since no valid description was provided, the name is regarded as *nomen nudum*. Multigene analyses indicated that the genus *Moniliella* represents a deeply rooted lineage within *Ustilaginomycotina* and has a sister relationship to both *Ustilaginomycetes* and *Exobasidiomycetes*. A new class *Moniliellomycetes* with order *Moniliellales*, family *Moniliellaceae* was proposed to accommodate the genus [28]. *Moniliella* is a genetically very heterogenous genus and may require revision. Effort is needed in order to find the supporting phenotypic characteristics and to fill the gaps between existing taxa.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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