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TAXONOMIC DESCRIPTION
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Moniliella floricola sp. nov., a species of black yeast isolated from flowers

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Abstract

Moniliella yeasts were isolated from flower samples collected in Vietnam using an enrichment medium containing 50 % (w/w) glucose. The yeasts were identified as *M. byzovii*, *M. dehoogii*, *M. megachiliensis*, *M. mellis*, *M. nigrescens* and *M. spathulata*. A group of 20 strains representing a hitherto undescribed species of *Moniliella* was detected. ITS sequences indicated the presence of four genetic variants differing from each other by 4–14 nt. The strains, however, were identical in the TEF1 sequences and shared 1–2 nt differences in the D1/D2 regions. In the ITS–D1/D2 phylogenetic tree, the strains grouped together and formed a well-supported clade with insect-associated *Moniliella* species, including *M. pollinis*, *M. megachiliensis* and *M. oedocephalis*. The new group was most closely related to *M. pollinis* but differed from the latter by 95 nt (58 substitutions, 37 indels) in the ITS, 36 nt (31 substitutions, five indels) in the D1/D2, and 30 nt (30 substitutions) in the TEF1 sequences. *Moniliella floricola* sp. nov. is proposed to accommodate this group of isolates. The type strain and MycoBank number of *M. floricola* sp. nov. are TBY 30.1^T (=CBS 12758^T=NRRL Y-63660^T) and MB 825274, respectively.

Black yeasts of the genus *Moniliella* are among rare members of the Basidiomycota that demonstrate xerophily and fermentation activity [1]. The genus was initially erected to accommodate two species, *M. acetoabutens* and *M. tomentosa* [2]. Later, based on D1/D2 sequence and phenotypic similarities, species of *Trichosporonoides* were transferred to *Moniliella* [3]. Currently, the genus comprises 16 species, namely *M. acetoabutens*, *M. byzovii*, *M. carnis*, *M. casei*, *M. dehoogii*, *M. fonsecae*, *M. macrospora*, *M. megachiliensis*, *M. mellis*, *M. nigrescens*, *M. oedocephalis*, *M. pollinis*, *M. pyrgileucina*, *M. sojae*, *M. spathulata* and *M. suaveolens*.

Species of *Moniliella* are of economic importance. *M. acetoabutens* and *M. suaveolens* are listed among food spoilage fungi. *M. acetoabutens* can grow in the presence of 4 % acetic acid, a common food preservative, and causes spoilage of pickles, salad dressing and vinegar. *M. suaveolens* causes spoilage of margarine, cheese and bread [4]. *M. carnis* and *M. dehoogii* were isolated from fermented meat and meat processing environments. Most strains of *Moniliella* produce erythritol when growing in medium containing elevated concentrations of glucose [5, 6] and this feature has been utilized in the food industry for the commercial production of erythritol [7]. *Moniliella* strains isolated from traditionally fermented sausage 'nem chua' possessed strong lipolytic activity and were able to convert castor oil into the aromatic compound γ-decalactone [8].

Despite their economic importance, knowledge of the biology, ecology and evolution of *Moniliella* is limited. Although species of *Moniliella*, such as *M. acetoabutens* and *M. byzovii*, produce thick-walled, large chlamydospores resembling teliospores in the Ustilaginomycotina, no sexual reproduction has been recorded and it is not clear if the yeast should be considered as truly asexual. The natural habitat of *Moniliella* is not well understood as most *Moniliella* species have been described based on limited numbers of isolates. Furthermore, 11 of the total 16 *Moniliella* species were initially isolated from artificial substrates (cheese, margarine, sausage, soy paste, jam, etc.). Recent studies have indicated that *Moniliella* might be common inhabitants of flowers and are vectored by insects [3, 9].

Phylogenetically, *Moniliella* represents an early branch in the evolutionary history of Ustilaginomycotina. As such, the monogeneric class *Moniliellomycetes* was proposed to accommodate the genus [10]. *Moniliella* is a genetically highly heterogeneous genus. In the D1/D2 sequences, *Moniliella* species may differ from each other by 21 % or 118 nt as in the case of *M. nigrescens* and *M. fonsecae*. Nuclear DNA G+C content of species in the genus may vary over 16 mol% [11]. Genetic heterogeneity suggests the need for a taxonomic revision, but the splitting of *Moniliella* may require phenotypic support and effort is needed to fill the gaps between existing taxa. In this study, we report on a novel species of *Moniliella* isolated from flowers of *Ipomoea pes-caprae* (beach morning glory, a widely distributed salt-

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Table 1. Strains of *Moniliella floricola* sp. nov. and isolation source

Strain(s)	Flower sample		Place of collection	Year
	Common name	Latin name		
TBY 30.1^T	Beach morning glory	*Ipomoea pes-caprae* (L.) R. Br	Cham Island, Quang Nam, Vietnam	2009
TBY 1897.1	Beach morning glory	*Ipomoea pes-caprae* (L.) R. Br	Cape of Ke Ga, Binh Thuan, Vietnam	2012
TBY 3400, TBY 3401, TBY 3402.2, TBY 3403.2, TBY 3404.1, TBY 3405.2, TBY 3406.1, TBY 3407.2, TBY 3409	Lantana	*Lantana camara* L.	Bai Lu, Nghe An, Vietnam	2013
TBY 3433	Sesame	*Sesamum indicum* L.	Bai Lu, Nghe An, Vietnam	2013
TBY 3444, TBY 3445, TBY 3446.1, TBY 3446.2	Beach morning glory	*Ipomoea pes-caprae* (L.) R. Br	Bai Lu, Nghe An, Vietnam	2013
TBY 4797.2, TBY 4798.2, TBY 4798.5, TBY 4799.1	Lantana	*Lantana camara* L.	Thien Cam, Ha Tinh, Vietnam	2014

tolerant pantropical plant species), *Lantana camara* (lantana, a small perennial invasive shrub that is native to the American tropics) and *Sesamum indicum* (sesame, an oilseed crop species) collected in coastal areas in central Vietnam.

YEAST ISOLATION

Moniliella yeasts were isolated by using an enrichment medium for osmophilic micro-organisms [9]. Approximately 1 g of flowers of each plant species were incubated in 50 ml screw-cap tubes containing 30 ml of 50 % glucose medium [50 % glucose, 0.5 % yeast extract (w/w)]. The tubes were incubated at 28 °C with periodic mixing during the first 3 days to avoid local osmotic pressure drops due to the excretion of water from the plant tissues. After 10 days, the fermentation broths were streaked on malt-glucose agar (1 % malt extract, 1 % glucose, 0.01 % chloramphenicol, 2 % agar) plates, and incubated at 28 °C for 10 days. *Moniliella* colonies can be detected visually based on their characteristic appearance (greyish to olivaceous black colonies, multilateral budding, arthroconidia) [1]. The colonies were purified and maintained on malt-glucose agar.

During the period from 2009 to 2014, over 2000 flower samples collected across Vietnam were analysed. From batch to batch, *Moniliella* was detected in none or in up to 10 % of the examined samples. *Moniliella* species, including *M. byzovii*, *M. dehoogii*, *M. megachiliensis*, *M. mellis*, *M. nigrescens* and *M. spathulata*, were identified. Among the isolates, there was a group of 20 strains representing a new phylogenetic lineage that hereafter is described as *Moniliella floricola* sp. nov. These strains were isolated from four different provinces of Vietnam (Quang Nam, Binh Thuan, Nghe An, Ha Tinh) with furthest distance of 900 km, over a period of 6 years (2009, 2012, 2013, and 2014) and from three plant species (*Ipomoea pes-caprae*, *Lantana camara* and *Sesamum indicum*) (Table 1).

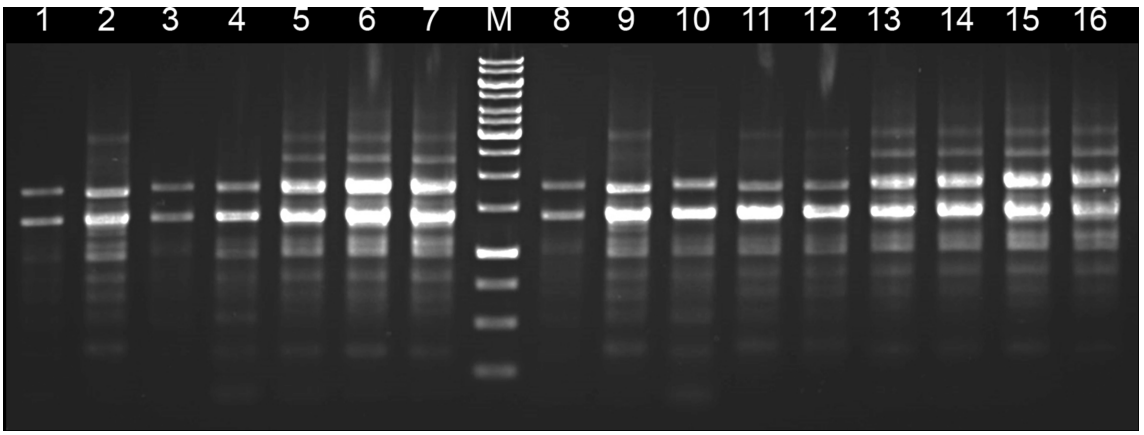

Fig. 1. Microsatellite primed PCR profiles of 16 strains of *M. floricola* sp. nov. generated with primer $(GAC)_5$. M, GeneRuler 1 kb DNA Ladder (Fermentas); 1, TBY 30.1^T; 2, TBY 1897.1; 3, TBY 3444; 4, TBY 3446.2; 5, TBY 3403.2; 6, TBY 3409; 7, TBY 3401; 8, TBY 3445; 9, TBY 3446.1; 10, TBY 3406.1; 11, TBY 3433; 12, TBY 3400; 13, TBY 3407.2; 14, TBY 3404.1; 15, TBY 3405.2; 16, TBY 3402.2.

Table 2. Nucleotide difference between strains of *Moniliella floricola* sp. nov. in the D1/D2 (above diagonal) and ITS (below diagonal) regions. Shaded, groups of strains with identical D1/D2 and five ITS sequences.

Strain	TBY 30.1^T	TBY 1897.1	TBY 3404.1	TBY 4798.2	TBY 3406.1	TBY 3446.1	TBY 4797.2	TBY 4798.5	TBY 4799.1
TBY 30.1^T	–	0	1	1	1	2	2	2	2
TBY 1897.1	0	–	1	1	1	2	2	2	2
TBY 3404.1	7	7	–	0	1	1	1	*1*	1
TBY 4798.2	7	7	0	–	1	1	1	1	1
TBY 3406.1	4	4	5	5	–	0	0	*0*	0
TBY 3446.1	13	13	14	14	14	–	0	0	0
TBY 4797.2	13	13	14	14	14	0	–	0	0
TBY 4798.5	13	13	14	14	14	0	0	–	0
TBY 4799.1	13	13	14	14	14	0	0	0	–

GENETIC CHARACTERIZATION

The 20 *Moniliella* strains were compared genetically by PCR fingerprinting using the microsatellite primer $(GAC)_5$. DNA extraction was performed as previously described [12]. Briefly, freshly grown biomass was collected, and washed twice with 2× SSC (15 mM sodium citrate, 150 mM NaCl, pH 7.0) at 99 °C. Cells were disrupted using a bead beater in the presence of water and phenol/chloroform. After centrifugation, the DNA extract was further purified using a silica gel extraction kit. PCR was carried out with the following thermal programme: 94 °C for 3 min; 30 cycles of 94 °C for 40 s, 52 °C for 40 s and 72 °C for 90 s; and 72 °C for 10 min. PCR products were separated on a 1 % agarose gel. For comparison with known *Moniliella* species, the ITS

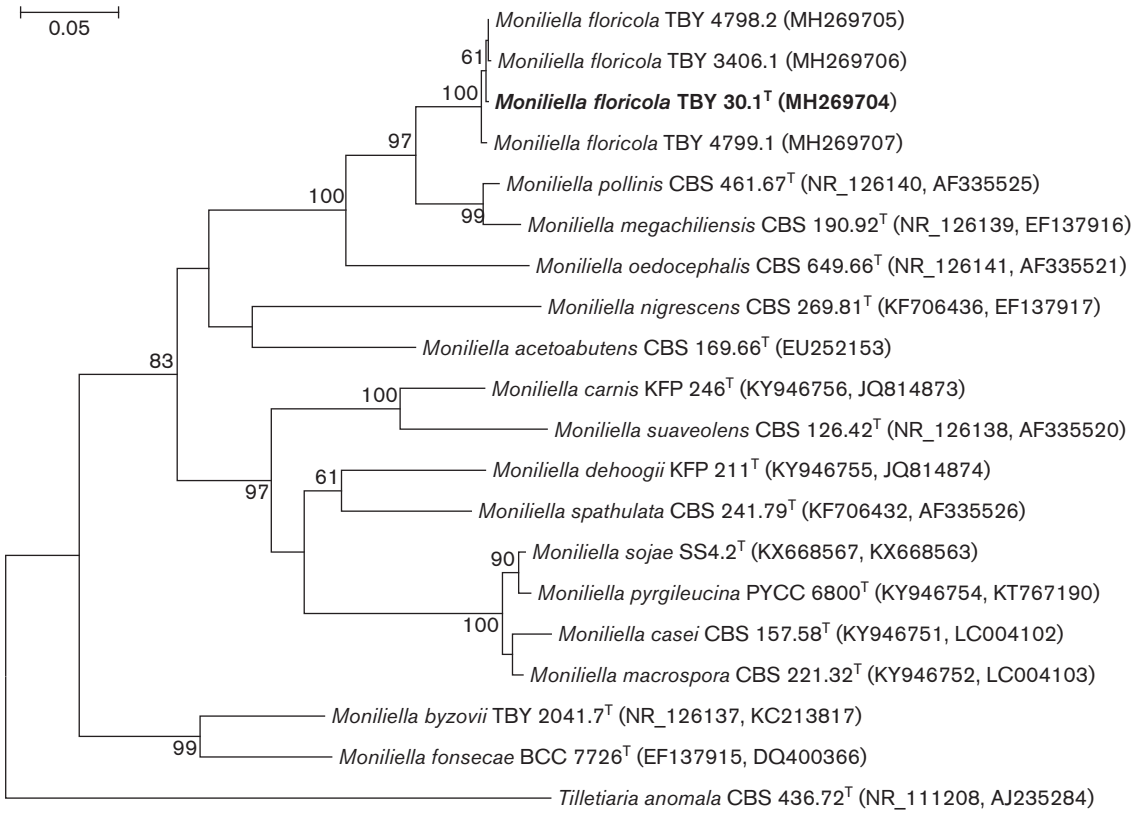

Fig. 2. Phylogram depicting the relationships between *M. floricola* sp. nov. and neighbouring taxa based on concatenated ITS–D1/D2 sequences. The tree was reconstructed using the maximum-likelihood method based on the Tamura–Nei model in MEGA7. An alignment of 1189 positions corresponding to 938 nt for *M. floricola* TBY 30.1^T was taken for analysis. All ambiguous positions were removed for each sequence pair. Bootstrap values of >50 %, obtained from 1000 replications, are shown. *Tilletiaria anomala* CBS 436.72^T was used as an outgroup. GenBank accession numbers of ITS and D1/D2 sequences, respectively, are given in parentheses. Bar, 5 % sequence divergence.

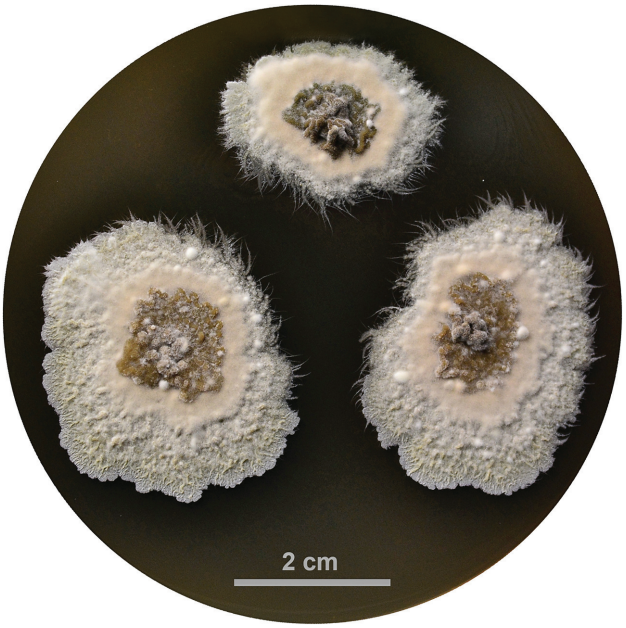

Fig. 3. Three-week-old colonies of *M. floricola* TBY 30.1^T grown on YM agar. Bar, 2 cm.

regions, D1/D2 LSU rRNA and partial TEF1 genes were sequenced. The fragment containing ITS and D1/D2 was amplified using the primer pair ITS1 (TCCGTAGG TGAACCTGCGG) [13] and NL4 (GGTCCGTGTTTCAA-GACGG) [14]. 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; and 72 °C for 10 min. The PCR products were sequenced using the primers ITS1, ITS4 (TCCTCCGCTTA TTGATATGC) [13], NL1 (GCATATCAATAAGCGGAG-GAAAAG) [14] and NL4. Similarly, a fragment of the TEF1 gene was amplified and sequenced using the primers EF1-983F (GCYCCYGGHCAYCGTGAYTTYAT) and EF1-2212R (CCRACRGCRACRGTYYGTCTCAT) [15]. DNA sequencing services were provided by the First BASE Laboratories Sdn Bhd. The obtained and reference sequences were aligned via a fast Fourier transform algorithm using the online version of MAFFT [16] hosted at the European Bioinformatics Institute. The phylogenetic tree was reconstructed using the maximum-likelihood method based on the Tamura–Nei model [17] in the MEGA version 7.0 software package [18]. Bootstrap analyses were performed from 1000 random re-samplings [19].

The PCR fingerprinting patterns generated by the primer $(GAC)_5$ were similar for all strains, indicating that they are genetically related (Fig. 1). The ITS and D1/D2 sequences were obtained for nine strains (TBY 30.1^T, TBY 1897.1, TBY 3404.1, TBY 3406.1, TBY 3446.1, TBY 4797.2, TBY 4798.2, TBY 4798.5 and TBY 4799.1), and partial TEF1 (978 nt) sequences for six strains (TBY 30.1^T, TBY 3404.1, TBY 3406.1, TBY 3446.1, TBY 4798.2 and TBY 4799.1), and deposited at the GenBank with accession numbers MH269704 to MH269707 (for ITS-D1/D2) and MH893626 to MH893631 (for TEF1). The obtained sequences indicated the presence of four genetic variants that differed from each other by 4–14 nt in the ITS region. The differences were large enough to indicate specific status for the genetic variants under question. However, in the D1/D2 sequences, the strains were identical or differed from each other maximally by 2 nt (one substitution, one indel) (Table 2). Similarly, the representatives of the four genetic variants were identical in the TEF1 sequences. Thus, the variants could be considered as conspecific.

In the phylogenetic trees reconstructed based on ITS–D1/D2 or TEF1 sequences, *M. floricola* strains formed a well-supported clade with insect-associated *Moniliella* species, including *M. pollinis*, *M. megachiliensis* and *M. oedocephalis* (Fig. 2). *Moniliella floricola* was most closely related to *M. pollinis* but their type strains (TBY 30.1^T and CBS 461.67^T) differed from each other by 95 nt (58 substitutions, 37 indels) in the ITS, 36 nt (31 substitutions, 5 indels) in the D1/D2, and 30 nt (30 substitutions) in the TEF1 sequences. Among the insect-associated species, *M. pollinis* was isolated from pollen in honeycomb, *M. megachiliensis* from alfalfa leaf-cutting bee and *M. oedocephalis* from the contents of brood cell of *Apis mellifera* [1].

PHENOTYPIC PROPERTIES

The morphology of *M. floricola* strains was studied using a differential interference contrast microscope (Eclipse E-600; Nikon). For image capture, a CMOS camera DFK 61AUC02 and the supplied IC Capture AS software (The Imaging Source) were used. For noise reduction, images were taken with an eight-frames overlay. On YM agar (0.5 % peptone, 0.3 % yeast extract, 0.3 % malt extract, 1 % glucose, 2 % agar), *M. floricola* strains formed butyrous colonies that were greyish cream to olivaceous and later changed to black. Both aerial and substrate hyphae were formed (Fig. 3). The yeast reproduced by multilateral budding and the formation of true hyphae that broke up into arthroconidia (Fig. 4). No sexual response (conjugation tubes or teleomorphic state) was observed when nine representative strains (TBY 30.1^T, TBY 1897.1, TBY 3404.1, TBY 4798.2, TBY 3406.1, TBY 3446.1, TBY 4797.2, TBY 4798.5 and TBY 4799.1) were pair-wise mixed and incubated on nutrient-rich (YM agar) and nutrient-poor (yeast carbone base agar, water agar) media at 28 and 10 °C after 1, 7 and 14 days.

Physiological tests were performed using standard methods [20] for four strains (TBY 30.1^T, TBY 3404.1, TBY 3406.1 and TBY 3446.1) representing the four observed genetic variants. 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 yeast carbon base and yeast nitrogen base were from Difco. *M. floricola* demonstrated physiological characteristics typical of the genus *Moniliella* [1]. All strains were fermentative and able to produce gas from glucose. The tested strains were xerotolerant and able to grow in medium

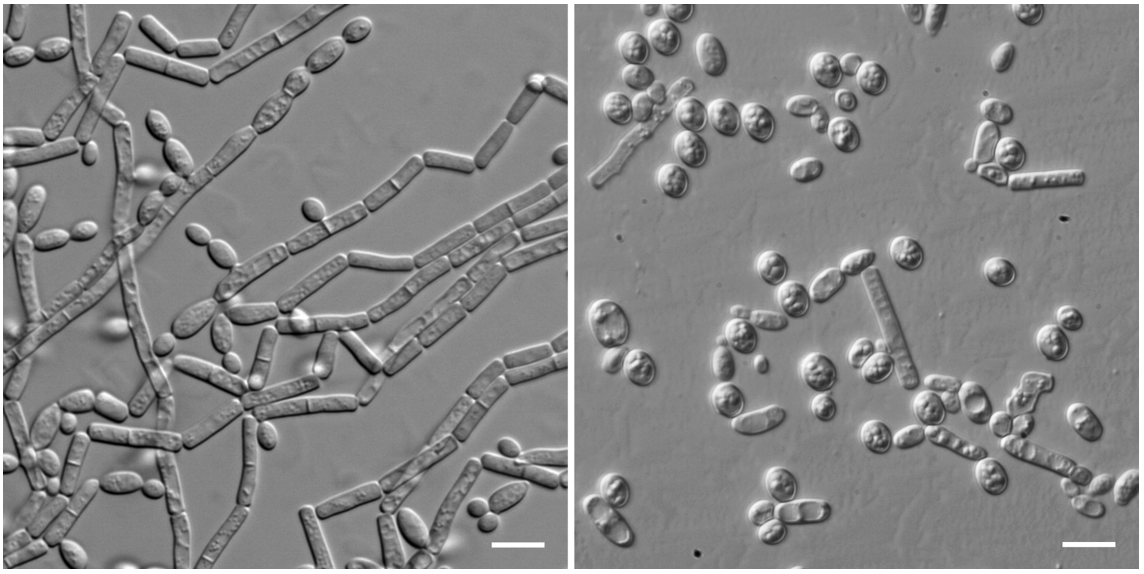

Fig. 4. Photomicrographs of *M. floricola* TBY 30.1^T grown on cornmeal agar for 3 days (left) and in YM broth for 3 days (right). Bars, 10 µm.

containing 60 % glucose. The yeasts were able to assimilate nitrate and to hydrolyse urea. Clear variations (positive/negative) amongst genetic variants were observed in the assimilation of six carbon sources (D-xylose, L-arabinose, arbutin, glycerol, 2-keto-D-gluconate and succinate). A recent screening programme for erythritol-producing yeasts indicated that, in liquid medium containing 20 % glucose, *M. floricola* strains were able to accumulate erythritol at up to 33.3 g l^{-1} [6].

DESCRIPTION OF *MONILIELLA FLORICOLA* THANH *ET* HIEN SP. NOV.

Moniliella floricola [flo.ri′co.la. L. masc. n. *flos*, *floris* flower; N.L. suff. *-cola* (from L. masc. or fem. n. *incola*) inhabitant, dweller; N.L. fem. n. *floricola* a dweller of flowers, referring to the fact that the yeast was isolated from flowers].

MycoBank number MB 825274.

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 aerial and substrate mycelia are formed (Fig. 3). In YM broth after 3 days at 25 °C, cells are ovoid to elongate, and cylindrical (3.0–6.5×4.5–15.0 µm, elongated to 75 µm) (Fig. 4). A sediment is formed. In Dalmau plates after 3 days on cornmeal agar, pseudohyphae, true hyphae and arthroconidia are formed (Fig. 4). Ferments D-glucose, sucrose and maltose but not D-galactose, lactose, raffinose or trehalose. Assimilates D-glucose, inulin (positive or weak), sucrose, maltose, cellobiose, D-xylose (variable), L-arabinose (variable), D-ribose, ethanol, glycerol (variable), erythritol (positive or weak), ribitol (weak or negative), D-mannitol (positive or weak), D-glucitol (weak or negative), DL-lactate (weak or negative), succinate (variable), citrate (positive or weak), 2-keto-D-gluconate (variable), xylitol (weak or negative) and arbutin (variable), but not raffinose, melibiose, D-galactose, lactose, trehalose, melezitose, methyl α-D-glucoside, starch, salicin, L-sorbose, L-rhamnose, D-arabinose, methanol, galactitol, *myo*-inositol, D-gluconate, D-glucosamine, 5-keto-D-gluconate, 1,2-propanediol, 2,3-butanediol, D-glucono-1,5-lactone or D-galacturonate. As a nitrogen source, *M. floricola* assimilates nitrate and nitrite but not glucosamine or imidazole. Growth in vitamin-free medium is positive. Does not grow in medium containing 0.01 % cycloheximide or 1 % acetic acid. Grows at 40 °C but not at 42 °C. Grows in medium containing 60 % glucose but not in medium containing 10 % NaCl and 5 % glucose. Does not produce starch-like substances. Urease reaction is positive. It was reported that *M. floricola* strains accumulate erythritol in medium containing 20 % glucose [6].

The holotype is TBY 30.1^T, isolated from *Ipomoea pes-caprae* (L.) R. Br (beach morning glory) flowers at Cham Island, Quang Nam, Vietnam, in 2009. It is maintained in a metabolically inactive state at the Vietnam Collection of Food Industry Microorganisms, Food Industries Research Institute, Hanoi, Vietnam. The ex-types, CBS 12758^T and NRRL Y-63660^T, 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, respectively.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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