Moniliella byzovii sp. nov., a chlamydospore-forming black yeast isolated from flowers

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Yeast of the genus Moniliella were isolated from 651 flower samples collected in Vietnam, using an enrichment medium containing 50% glucose. Species of the genus Moniliella were found in 5% of the samples and 54 strains were isolated. The strains were identified based on D1/D2 LSU rRNA gene sequences as M. megachiilensis (15 strains), M. dehoogii (14 strains), and M. mellis (2 strains). The remaining 23 strains could not be reliably placed under any known species. Among them, 12 strains isolated from flowers of Ipomoea pes-caprae and Calotropis gigantea were peculiar for the intensive formation of chlamydospores. These strains could be subdivided into pigmented and non-pigmented groups. Both groups were identical in PCR fingerprints generated with primer (GAC)5 and in D1/D2 and ITS sequences. The yeast was closely related to M. fonscaee but differed from the latter by 52 nt (or 10.3% of divergence) in the D1/D2 sequence and 71 nt (or 16.9% of divergence) in the ITS sequence. The name Moniliella byzovii sp. nov. is proposed for this novel species. The type strain is TBY 2041.7T. The MycoBank number is MB 803186.

At the time of writing, the genus Moniliella comprised 11 species, namely M. acetoabutens, M. carnis, M. dehoogii, M. fonscaee, M. megachiilensis, M. mellis, M. nigrescens, M. oedoecephalis, M. pollinis, M. spathulata, and M. suaveolens (de Hoog et al., 2011; Thanh et al., 2012). The genus is not well understood both in terms of taxonomic placement and ecological status (de Hoog et al., 2011). Despite their relative uniformity in morphological appearance and physiological properties, species of the genus Moniliella exhibit a high degree of genetic divergence (Rosa et al., 2009). Most of the species in the genus are only known to occur in manmade substrates (de Hoog et al., 2011). In biotechnology, species of the genus Moniliella are utilized for the commercial production of erythritol (Perko & DeCock, 2007).

In a large survey of yeasts associated with ephemeral flowers and their insects, Lachance et al. (2001) reported on the presence of 28 ‘fermentative yeast-like molds’ among 1596 yeast isolates. Sequencing data of the D1/D2 LSU rRNA gene for the preserved isolates indicated that they represent independent branches within the genus Moniliella (M.A. Lachance, personal communication). In this complementary study, we conducted the selective isolation of members of the genus Moniliella associated with flowers in Vietnam in order to find the missing links between species of the genus Moniliella and to provide new isolates of the genus Moniliella for various biotechnological screening programs.

Yeast isolation

For the isolation of species of the genus Moniliella from flowers, 651 samples were collected across Vietnam during the period from 2008 to 2012. Flowers were stored in sterile plastic bags and analysed within the day of collection. Moniliella yeasts were isolated using an enrichment method for xerophilic micro-organisms. For enrichment, the bottom parts of flowers containing nectaries were transferred into 50 ml Falcon tubes containing 20 ml of enrichment medium [50% glucose, 0.5% yeast extract (w/w)] and incubated at 28°C for 10 days. For yeast isolation, the tubes were shaken thoroughly and 50 μl of culture liquid was spread onto three consecutive malt-glucose agar (1% malt, 1% glucose, 0.01% chloramphenicol, 2% agar) plates, and incubated at 28°C for 10 days. From a limited number (19) of flower samples, nectar could be collected and it was spread directly onto malt-glucose agar without an enrichment step. Colonies with the characteristic appearance of members of the genus Moniliella (greyish to olivaceous black colonies, multilat-terul budding, formation of hyphae and arthroconidia) were collected and purified on malt-glucose agar.

The enrichment procedure succeeded in eliminating common epiphytic yeasts, such as Aureobasidium, Rhodotorula, and Cryptococcus. All samples after enrichment contained yeasts and the most typical were xerotolerant yeasts known as...
to occur in flowers, such as *Kodamaea ohmeri*, *Meyerozyma guilliermondii*, *Candida floricola*, and *Metschnikowia koreensis*. Within the 651 collected samples, *Moniliella* was detected in 34 samples (ca 5%) either as a single colony or as the absolute dominant form on the isolation plates. Similarly, within the 19 collected nectar samples, which were nearly free of epiphytic yeasts, isolates of the genus *Moniliella* were found in only one nectar sample (ca 5%). The occurrence of isolates of the genus *Moniliella* correlated with insect activities rather than the abundance of flowering in the sampling areas. Samples collected in a mountainous area with a temperate climate (0 positive/43 samples) and the northern part of Vietnam with a subtropical climate (14 positives/420 samples) had lower abundance of isolates of the genus *Moniliella* than in the tropical area of southern Vietnam (20 positives/188 samples), where higher insect activity was noticed. When a *Moniliella* biotype was present in a sample, the sample tended to harbour multiple genetic groups of *Moniliella*. In one particular case, four distinct genetic groups of *Moniliella* were found in one flower sample.

In total, 54 strains were isolated. Among them, 15 strains were identified as *Moniliella megachiliensis*, 14 strains as *Moniliella dehoogii*, and two strains as *Moniliella mellis*. *Moniliella megachiliensis* was first isolated from larval guts and frass of the alfalfa leafcutter bee, *Megachile rotundata* (Inglis et al., 1992). Recently, *M. megachiliensis* was found to occur in *Verbascum* flowers from Georgia (Sipiczki, 2012). In our study, *M. megachiliensis* was isolated from flowers of *Ipomoea pes-caprae*, *Luffa aegyptiaca*, *Alstonia megachiliensis* and *Sesamum indicum*. *M. megachiliensis* is currently used for industrial production of erythritol (*Sawada et al.*, 2009). *Moniliella dehoogii* was described recently based on eight strains isolated from meat product and meat processing environments (Thanh et al., 2012). In this study, *M. dehoogii* was found in flowers of *Ipomoea pes-caprae*, *Calotropis gigantea* and *Sesamum indicum*. Two strains of *Moniliella mellis* were isolated from flowers of *Desmos chinensis* and *Calotropis gigantea*. *M. mellis* was previously only known to be associated with honey (de Hoog et al., 2011).

The remaining 23 strains could not be reliably placed under any known species. Among them, a group consisting of 12 strains was peculiar for the intensive formation of chlamydospores. In the primary isolation medium, growth was entirely in the form of chlamydospore chains whereas budding could hardly be detected (similar to that in Fig. 5a). These 12 strains represent a novel species of the genus *Moniliella* and will be described in this study as *Moniliella byzovii* sp. nov. Eleven strains of *M. byzovii* sp. nov. were isolated from flowers of *Ipomoea pes-caprae* (beach morning glory) and one from *Calotropis gigantea* (crown flower) collected at various locations along 30 km coastal line at the Cape of Ke Ga, Phan Thiet, Vietnam in 2012 (Table 1). The strains of *M. byzovii* sp. nov. could be divided into a pigmented group and a non-pigmented group (Fig. 1). Both phenotypic groups could occur in the same flower sample (Table 1). The remaining 11 strains were closely related to *M. mellis* (5 strains), *Moniliella* sp. UWO 95.766.4 (3 strains), *M. pollinis* (2 strains), or basal to *Moniliella* spp. (1 strain). Due to the limited number of strains available and the genetic variability among them, the taxonomy of these strains awaits further study.

**Genetic characterization**

Strains were compared by microsatellite-primed (MSP) PCR using the primer (GAC)\_5\_DNA was required for uniformity of DNA amplification. 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 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 fingerprinting patterns generated by

<table>
<thead>
<tr>
<th>Strain</th>
<th>Pigmentation</th>
<th>Source of collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBY 2041.7_T</td>
<td>Greenish-black</td>
<td>Wilted flower of <em>Ipomoea pes-caprae</em></td>
</tr>
<tr>
<td>TBY 2041.8</td>
<td>None</td>
<td>Wilted flower of <em>Ipomoea pes-caprae</em></td>
</tr>
<tr>
<td>TBY 1932</td>
<td>Greenish-black</td>
<td>Flower of <em>Ipomoea pes-caprae</em></td>
</tr>
<tr>
<td>TBY 1944.11</td>
<td>None</td>
<td>Flower of <em>Calotropis gigantea</em></td>
</tr>
<tr>
<td>TBY 2042.1</td>
<td>Greenish-black</td>
<td>Wilted flower of <em>Ipomoea pes-caprae</em></td>
</tr>
<tr>
<td>TBY 2044.2</td>
<td>None</td>
<td>Wilted flower of <em>Ipomoea pes-caprae</em></td>
</tr>
<tr>
<td>TBY 2045.5</td>
<td>None</td>
<td>Wilted flower of <em>Ipomoea pes-caprae</em></td>
</tr>
<tr>
<td>TBY 2046.1</td>
<td>None</td>
<td>Wilted flower of <em>Ipomoea pes-caprae</em></td>
</tr>
<tr>
<td>TBY 2108.5</td>
<td>None</td>
<td>Wilted flower of <em>Ipomoea pes-caprae</em></td>
</tr>
<tr>
<td>TBY 2108.7</td>
<td>Greenish-black</td>
<td>Wilted flower of <em>Ipomoea pes-caprae</em></td>
</tr>
<tr>
<td>TBY 2108.10</td>
<td>Greenish-black</td>
<td>Wilted flower of <em>Ipomoea pes-caprae</em></td>
</tr>
<tr>
<td>TBY 2085.8</td>
<td>None</td>
<td>Wilted flower of <em>Ipomoea pes-caprae</em></td>
</tr>
</tbody>
</table>
Sequences were aligned using MUSCLE multiple alignment (Edgar, 2004) in the MEGA version 5.1 software package. Bootstrap analyses were performed from 1000 random resamplings. A phylogenetic tree was based on the D1/D2 sequences, M. byzovii sp. nov. and M. fonsecae CBS 10551T formed a well-supported clade (Fig. 3). Strains of M. fonsecae were previously isolated from flowers collected in Thailand, Cuba, and Brazil (Rosa et al., 2009).

Phenotypic properties

The morphology of M. byzovii sp. nov. was studied using a light microscope (Eclipse E-600; Nikon). Photographs were taken with a CCD camera (TK-C1380E; JVC) using an IC TWAN driver (The Imaging Source Europe GmbH) with 8 frame overlay for noise reduction and IrfanView (Irfan Skiljan, Austria) for image acquisition. As mentioned above, on the primary isolation plates (after enrichment) M. byzovii sp. nov. showed filamentous growth in form of chlamydomspore chains. However, upon transferring to fresh medium the yeast resumed typical asexual growth of members of the genus Moniliella (i.e. budding, arthrospore formation). Chlamydomspore formation occurred when the yeast was transferred from nutrient-rich to poor media. Abundant formation of chlamydomspores was observed when M. byzovii sp. nov. was grown on nitrogen-depleted media, such as Yeast Carbon Base agar (Difco). Among members of the genus Moniliella, formation of chlamydomspores is known only for strains of Moniliella acetoabutens (de Hoog et al., 2011).

Since pigmentation is a generic characteristic of Moniliella, the presence of non-pigmented populations within M. byzovii sp. nov. is noteworthy. Both pigmented and non-pigmented phenotypes are stable. No segregation of pigmented phenotype into non-pigmented or vice versa was observed during subculturing or streaking for single colonies. Pairwise mixing of all strains on nutrient-rich (YM agar, malt-glucose agar) and poor (Yeast Carbon Base agar, water agar) media at different temperatures (15 °C and 28 °C) yielded no sexual response. Neither conjugation tubes nor teleomorphic states were observed.

Physiological tests were performed using standard methods (Yarrow, 1998) for strains TBY 2041.7T, TBY 1932, and TBY 2108.7 Carbon assimilation tests were carried out in liquid media and the auxanographic method was used for

**The designated type strain of the novel species, TBY 2041.7T, differed from CBS 10551T, the type strain of M. fonsecae, by 52 nt (or 10.3 % of divergence) in the D1/D2 and 71 nt (or 16.9 % of divergence) in the ITS regions. In a phylogenetic tree based on the D1/D2 sequences, M. byzovii sp. nov. and M. fonsecae CBS 10551T formed a well-supported clade (Fig. 3). Strains of M. fonsecae were previously isolated from flowers collected in Thailand, Cuba, and Brazil (Rosa et al., 2009).**

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**Fig. 1.** Colony morphology of Moniliella byzovii sp. nov. TBY 2041.7T (left) and TBY 2041.8 (right) on malt-glucose agar after 45 days of incubation at 28 °C. Both strains were isolated from the same sample and they shared identical MSP-PCR fingerprinting profile, D1/D2 and ITS sequences.

**Fig. 2.** MSP-PCR profiles of strains of Moniliella byzovii sp. nov. generated with primer (GAC)5. M, GeneRuler 1 kb DNA Ladder (Fermentas); 1–12, strains TBY 2041.8, TBY 2108.5, TBY 2041.7T, TBY 2046.1, TBY 1932, TBY 2108.10, TBY 2108.7, TBY 1944.11, TBY 2042.1, TBY 2085.8, TBY 2045.5 and TBY 2044.2, respectively.
nitrogen assimilation tests. The novel species could be distinguished from known species of the genus Moniliella by phenotypic characteristics (Table 2). It differs from the only known chlamydospore-forming species of the genus Moniliella, M. acetoabutens, by the absence of fermentation of maltose and the absence of assimilation of maltose and lactose (Table 2).

**Description of Moniliella byzovii Thanh, Hien et Thom sp. nov.**

*Moniliella byzovii* [by.zo’vi.i. L. gen. sing. m. n. byzovii of Byzov, in honour of the microbiologist Boris Alexeevich Byzov (Moscow State University) for his contributions to the understanding of microbe-invertebrate interactions].

MycoBank number MB 803186.

After 7 days on malt extract agar at 25 °C colonies are cerebriform, wrinkled, soft, cream or olivaceous to black in colour. In YM broth after 5 days at 25 °C, cells are ovoid to elongate, cylindrical (2.5–4.0 × 3.0–17.0 µm). Sediment is formed. In Dalmau plates after 7 days on cornmeal agar, pseudohyphae, true mycelia and arthroconidia are formed (Fig. 4). In Yeast Carbon Base, intercalary and terminal, hyaline to dark or black, subglobose to globose, 10–16 µm in diameter chlamydospores are formed (Fig. 5). D-Glucose and sucrose are fermented, but not D-galactose, maltose, α,α-trehalose, lactose or raffinose. Positive for assimilation of the carbon compounds D-glucose, sucrose, cellobiose, inulin, erythritol, D-mannitol, D-glucono-1,5-lactone, D-gluconate, citrate (weak) and ethanol. No growth was detected with D-galactose, L-sorbose, D-gluconate, citrate (weak) and ethanol. No growth was detected with D-galactose, L-sorbose, D-gluconate, citrate (weak) and ethanol.

**Table 2. Key characteristics differentiating *M. byzovii* sp. nov. and known species of the genus Moniliella**

T taxa: 1, *M. byzovii* sp. nov.; 2, *M. acetoabutens*; 3, *M. carnis*; 4, M. dehoogii; 5, *M. fonsecae*; 6, *M. megalhiensis*; 7, M. melis; 8, M. nigrescens; 9, *M. oedocephalis*; 10, M. pollinis; 11, M. spathulata; 12, *M. suaveolens*. Characteristics of known species of the genus Moniliella were taken from de Hoog et al., (2011) and Thanh et al., (2012). +, Positive; –, negative; w, weak; V, variable; ND, no data.

The relationships between *M. byzovii* sp. nov. and neighbouring taxa. The tree was constructed based on D1/D2 sequences using the Mega 5.1 software package. An alignment of 562 positions corresponding to 503 nt for *M. byzovii* sp. nov. was taken into analysis. Bootstrap values of >50%, obtained from 1000 replications, are shown. *Phylloporia pectinata* R. Coveny 113 was used as an outgroup. GenBank accession numbers of D1/D2 sequences are given in parentheses. Bar, 5% sequence divergence.
arbutin, melibiose, lactose, raffinose, melezitose, starch, glucose, ribitol, xylitol, D-glucitol, galactitol, myo-inositol, 2-keto-D-gluconate, 5-keto-D-gluconate, D-galacturonate, DL-lactate, succinate, methanol, propane 1,2 diol or butane 2,3 diol. Assimilates nitrate and nitrite, but not glucosamine or imidazole. Positive for growth in vitamin-free medium. Does not grow in media containing 0.01 % cycloheximide or 1 % acetic acid. Grows at 37 °C but not at 40 °C. Grows in media containing 50 % glucose, but not in media containing 60 % glucose or 10 % NaCl and 5 % glucose. Does not produce starch-like substances. Urease reaction is positive.

The type strain, TBY 2041.7^T ( =CBS 12757^T =NRRL Y-63661^T), was isolated from a flower of Ipomoea pes-caprae from Cape of Ke Ga, Phan Thiet, Vietnam and has been deposited in the Yeast Collection of the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands, and the Agricultural Research Service Yeast Collection, US Department of Agriculture, Peoria, IL, USA.

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**References**


