Antonie van Leeuwenhoek (2018) 111:1197–1207
https://doi.org/10.1007/s10482-018-1028-6

ORIGINAL PAPER

Description of *Komagataella mondaviorum* sp. nov., a new sibling species of *Komagataella* (*Pichia*) *pastoris*

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Received: 28 October 2017 / Accepted: 25 January 2018 / Published online: 31 January 2018

Abstract Five methylotrophic strains (UCDFST 71-1024^T, UCDFST 54-11.16, UCDFST 54-11.141, UCDFST 68-967.1 and UCDFST 74-1030) from the Phaff Yeast Culture Collection (University of California Davis, USA) that were originally designated as *Pichia pastoris* were found to represent a novel *Komagataella* species. Strains of *Komagataella mondaviorum* sp. nov. UCDFST 71-1024^T(type strain) = CBS 15017, UCDFST 54-11.16, UCDFST 54-11.141, UCDFST 68-967.1, and UCDFST 74-1030 were isolated in USA, respectively, from cottonwood tree *Populus deltoides* in 1971 (Davis, CA), slime flux of *Quercus* sp. in 1954 (CA), exudate of black oak *Q. kelloggii* in 1954 (Central Sierra Nevada. CA), dry frass from *Salix* sp. in 1968 (Soleduck Road, Olympic National Park, WA) and from flux of hackberry tree *Celtis* sp. in 1974 (CA). The new species was differentiated from *Komagataella kurtzmanii*, *Komagataella pastoris*, *Komagataella phaffii*, *Komagataella populi*, *Komagataella pseudopastoris* and *Komagataella ulmi* by divergence in gene sequences for D1/D2 LSU rRNA, ITS1-5.8S-ITS2, RNA polymerase subunit I and translation elongation factor-1α. *Komagataella mondaviorum* sp. nov. is registered in MycoBank under MB 821789.

Keywords Methanol yeast · Multigenic analysis · New ascosporic yeast · Sibling species of *Komagataella pastoris*

Introduction

The methanol assimilating yeasts of the genus *Komagataella* Y. Yamada et al., better known under the name *Pichia pastoris* (Guilliermond) Phaff, have a long and complex taxonomic history. *P. pastoris* has been also classified in five different genera: *Petasospora*, *Saccharomyces*, *Zygosaccharomyces*, *Zymopichia* and *Zygowillia* (Kurtzman 2011). The genus *Komagataella* was proposed based on the partial 18S and 26S rRNAs sequences (Yamada et al. 1995). However, the genus has been generally accepted only after discovery of *Komagataella phaffii* and recognition of European species *Komagataella pseudopastoris* (Kurtzman 2005, 2011), initially described as *Pichia pseudopastoris* (Dlauchy

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10482-018-1028-6) contains supplementary material, which is available to authorized users.

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et al. 2003). Recently, three more *Komagataella* species have been proposed based on single strains isolated in North America: *Komagataella populi*, *Komagataella ulmi* and *Komagataella kurtzmanii* (Kurtzman 2012; Naumov et al. 2013, 2016). During multigene sequence analysis of methanol assimilating strains maintained at the Phaff Yeast Culture Collection as *Komagataella* (*Pichia*) *pastoris*, a new *Komagataella* species was found. In the present study, we describe the species as *Komagataella mandaviorum* sp. nov.

Materials and methods

Yeast strains, cultivation and phenotypic characterization

The strains and their origins are listed in Table 1. Phenotypic characterization of strains UCDFST 71-1024^T, UCDFST 54-11.16, UCDFST 54-11.141, UCDFST 68-967.1 and UCDFST 74-1030, representing a novel species of the genus *Komagataella*, was carried out according to Yarrow (1998) and Kurtzman et al. (2011). Yeast cells were grown at 25 °C on YPD complete medium (20 g glucose, 20 g peptone, 10 g yeast extract and 20 g agar in 1 l of distilled water). Sporulation and zygote formation were induced at 22 and 25 °C on three different acetate agar media used in genetics and taxonomy of yeasts: (1) 10 g CH_3COONa, 5 g KCl and 20 g agar in 1 l of distilled water; (2) 8.2 g CH_3COONa, 1.8 g KCl, 2.5 g yeast extract, 1 g glucose and 15 g agar in 1 l of distilled water (McClary et al. 1959); (3) 5 g CH_3COONa, 10 g KCl, 10 g glucose and 20 g agar in 1 l of distilled water (Chen et al. 2012). Also, ME medium was used (50 g malt extract and 20 g agar in 1 l of distilled water).

DNA extraction, sequencing and phylogenetic analysis

Genomic DNA was extracted from yeast cells using the Genomic DNA Purification Kit (Fermentas, Lithuania). The genes for the D1/D2 region of the large (26S) ribosomal rRNA subunit, translation elongation factor-1α (EF-1α), RNA polymerase II (subunit RPB1), and ITS1-5.8S-ITS2 were amplified and sequenced using standard oligonucleotide primers (Kurtzman 2009; Kurtzman and Robnett 1998, 2003). Amplification reactions were performed in a volume of 30 µl containing 100 ng of genomic DNA template, *Taq* polymerase (0.05 U, Syntol, Moscow), and the primers (50 pmol each). A Bio-Rad (USA) thermal cycler was programmed for 30 cycles of 45 s at 94 °C, 30 s at 52 °C and 2 min at 72 °C. Amplification products were separated by electrophoresis in 1% agarose gels and detected by staining with ethidium bromide. For sequencing, the amplified products were purified using the GeneClean Purification Kit (Bio101, USA) according to the manufacturer's instructions. Direct sequencing of both strands was performed using an Applied Biosystems 3730 automated DNA sequencer according to the manufacturer's instructions. The sequences obtained were compared with those in the GenBank database using BLAST (http://www.ncbi.nlm.nih.gov). An alignment was done visually with the program BioEdit (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). A neighbour-joining tree using the Kimura 2-parameter correction was generated with the MEGA 6 software package (Tamura et al. 2013). A total of 1000 bootstrap replicates were used for analysis using 2482 aligned nucleotide positions. Alignment gaps were treated pairwise. Type cultures of *Ogataea glucozyma* NRRL YB-2185 and *Pichia membranifaciens* NRRL Y-2026 were used as outgroup species. The nucleotide sequences determined in this study have been deposited in GenBank (Table 1). The reference sequences used in the phylogenetic analysis were retrieved from GenBank under the accession numbers indicated in Table 1.

Results and discussion

The D1/D2 sequences obtained for North American strains UCDFST 71-1024^T, UCDFST 54-11.16, UCDFST 54-11.141, UCDFST 68-967.1 and UCDFST 74-1030 were compared with the corresponding sequences from the GenBank database. The D1/D2 sequences of strains UCDFST 54-11.16, UCDFST 54-11.141 and UCDFST 68-967.1 were identical and differed from the corresponding sequences of UCDFST 71-1024^T and UCDFST 74-1030 by one nucleotide substitution. Strains UCDFST 71-1024^T and UCDFST 74-1030 had D1/D2 sequences, which differed from the corresponding sequences of the type strains of *K. pseudopastoris* NRRL Y-27603^T and *K. populi* NRRL YB-455^T by one and two nucleotides, respectively (Table 2). On

Table 1 List of *Komagataella* strains and their sequence accession numbers

Species	Strain designation[a]			Source and place of isolation	GenBank accession numbers[b]			
	UCDFST	NRRL	CBS		D1/D2 LSU	ITS	EF-1α	RPB1
Komagataella kurtzmanii	76-20^T	Y-63667^T	12817^T	Fir (*Abies* sp.) flux, Catalina mountains, Southern AZ, USA	KC715720	KC771256	KC715721	KC715722
Komagatalla mondaviorum	71-1024^T	Y-63969^T	15017	Exudate of cottonwood tree (*Populus deltoides*), Davis, CA, USA	**MF276795**	**MF276791**	**MF278887**	**MF278892**
	74-1030	Y-63970	15018	Flux of hackberry tree *Celtis* sp., CA, USA	**MF276796**	**MF276792**	**MF278888**	**MF278893**
	54-11.16			Slime flux of *Quercus* sp., CA, USA	**MF661895**	**MF663746**	**MF667016**	**MF667019**
	54-11.141			Exudate of black oak *Q. kelloggii*, Central Sierra Nevada. CA, USA	**MF661896**	**MF663747**	**MF667017**	**MF667020**
	68-967.1			Dry frass from *Salix* sp., Soleduck Road, Olympic National Park, WA, USA	**MF661897**	**MF663748**	**MF667018**	**MF667021**
Komagataella pastoris		Y-1603^T	704^T	Exudate of chestnut tree (*Castanea* sp.), France	U75963	JQ398742	EF552478	GQ327955
Komagataella phaffii		Y-7556^T	2612^T	Exudate of black oak (*Quercus kelloggii*), CA, USA	AF017407	JQ398743	EF552480	GQ327957
Komagataella populi		YB-455^T	12362^T	Exudate of cotton wood tree (*Populus deltoides*), Peoria, IL, USA	JN234404	JQ398744	JN234408	JN234410
Komagataella pseudopastoris		Y-27603^T	9187^T	Rotted willow tree (*Salix alba*), Hungary	AF403149	JQ398745	EF552479	GQ327956
		Y-27604	9188	Rotted willow tree (*Salix alba*), Hungary	AY091589	**MF276794**	**MF278890**	**MF278895**
		Y-27602	9186	Rotted willow tree (*Salix alba*), Hungary	KY108155	KY103881	**MF278891**	**MF278896**
Komagataella ulmi		YB-407^T	12361^T	Exudate of elm tree *Ulmus americana*, Peoria, IL, USA	JN234403	JQ398746	JN234407	JN234409

Table 1 continued

Species	Strain designation[a]			Source and place of isolation	GenBank accession numbers[b]			
	UCDFST	NRRL	CBS		D1/D2 LSU	ITS	EF-1α	RPB1
Ogataea glucozyma		YB-2185^T	5766^T	Insect frass, Engelmann spruce (*Picea engelmannii*), Wyoming, USA	U75520	DQ414539	EU014736	GQ327959
Pichia membranifaciens		Y-2026^T	107^T	Substrate unknown	U75725	NR_111195	EF552451	GQ327958

[a]UCDFST, Phaff Yeast Culture Collection, University of California Davis, Davis, CA, USA; NRRL, ARS Culture Collection, National Center for Agricultural Utilization Research, Peoria, Illinois, USA; CBS, Westerdijk Fungal Biodiversity Institute (formerly called Centraalbureau voor Schimmelcultures), Utrecht, The Netherlands; VKPM, All-Russian Collection of Industrial Microorganisms, Moscow, Russia. T—type strain. UCD-FST 76-20 = VKPM Y-727

[b]Sequences with accession numbers in bold were generated in this study

the other hand, UCDFST 71-1024^T and UCDFST 74-1030 shared identical D1/D2 sequences with *K. pseudopastoris* strains NRRL Y-27602 and NRRL Y-27604. The D1/D2 sequences of strains UCDFST 54-11.16, UCDFST 54-11.141 and UCDFST 68-967.1 differed by two nucleotide substitutions from the corresponding sequences of the type strains of *K. populi* NRRL YB-455^T and *K. pseudopastoris* NRRL Y-27603^T, and by one substitution from *K. pseudopastoris* NRRL Y-27602 and NRRL Y-27604 (Table 2).

To elucidate the taxonomic status of UCDFST 71-1024^T, UCDFST 54-11.16, UCDFST 54-11.141, UCDFST 68-967.1 and UCDFST 74-1030 we conducted comparative analyses of D1/D2, ITS1-5.8S-ITS2, EF-1α and RPB1 nucleotide sequences. The mitochondrial SSU rRNA gene was not used in multigene sequence analysis, since this molecular marker does not distinguish three out of the six known *Komagataella* species: *K. phaffii*, *K. ulmi* and *K. kurtzmanii* (Kurtzman 2012; Naumov et al. 2013). Based on the multigene sequence comparisons of the thirteen *Komagataella* strains listed in Table 1, a phylogenetic tree was generated (Fig. 1). Strains UCDFST 71-1024^T, UCDFST 54-11.16, UCDFST 54-11.141, UCDFST 68-967.1 and UCDFST 74-1030 formed a clearly separated clade from the closest relatives *K. pseudopastoris* and *K. populi* (Fig. 1). The five strains shared identical RPB1 sequences and differed by not more than one substitution in D1/D2, by one substitution and two indels in ITS, and by two substitutions in EF-1α, suggesting they are conspecific. It should be noted that *K. pseudopastoris* is characterized by intraspecific sequence divergences. NRRL Y-27602 differed from the type strain NRRL Y-27603^T and NRRL Y-27604 by 5 and 11 nucleotide substitutions, respectively, in EF-1α and RPB1, and by two substitutions and four indels in ITS1-5.8S-ITS2. On the other hand, the number of nucleotide substitutions in ITS1-5.8S-ITS2, EF-1α and RPB1 sequences among *K. populi*, *K. pseudopastoris* and the strains under examination was markedly higher and can be interpreted as interspecific sequence divergences (Table 2). Note that only D1/D2 and ITS are usually used for description of new yeast species. Phylogenetic tree based on the analysis of concatenated D1/D2 and ITS sequences is depicted in Fig. S1 (available in the online Supplementary Material). The five *Komagataella* strains studied is clearly differentiated by divergence in nucleotide sequences for ITS1-5.8S-ITS2. The results indicate that strains UCDFST 71-1024^T, UCDFST 54-11.16, UCDFST 54-11.141, UCDFST 68-967.1 and UCDFST 74-1030 represent a distinct species. The novel species differs from its closest relatives, *K. populi* and *K. pseudopastoris,* by 10–14 nucleotide substitutions in the EF-1α gene, by 27–32 nucleotide substitutions in the RPB1 gene, and by more than 7 nucleotide substitutions and 5 indels in the ITS–5.8S region (Table 2).

Based on the multigene sequence analysis, we propose to describe a novel species: *Komagataella mondaviorum* sp. nov. (type strain UCDFST

Table 2 Genetic divergence among the type strains of *Komagataella* species based on pairwise comparison of nucleotide sequences for D1/D2 domain, ITS1-5.8S-ITS2 region, translation elongation factor-1α and subunit RPB1 of RNA polymerase II

Species pair[a]	D1/D2[b] (341 bp)	ITS1-5.8S-ITS2 (277 bp)	EF-1α (876 bp)	RPB1 (828 bp)
K. mondaviorum/K. pastoris	6s + 4i	17s + 7i	25s	81s
K. mondaviorum/K. phaffii	7s + 6i	21s +7i	27s	90s
K. mondaviorum/K. kurtzmanii	5s + 5i	14s + 2i	27s	88s
K. mondaviorum/K. ulmi	5s + 6i	11s + 11i	26s	73s
K. mondaviorum/K. populi	2s	7s + 6i	10s	32s
K. mondaviorum/K. pseudopastoris	1s	12s + 5i	14s	27s
K. pastoris/K. phaffii	6s + 4i	21s + 11i	10s	55s
K. pastoris/K. kurtzmanii	5s + 3i	21s + 8i	10s	52s
K. pastoris/K. pseudopastoris	7s + 4i	33s + 14i	23s	82s
K. pastoris/K. populi	4s + 6i	18s + 9i	28s	83s
K. pastoris/K. ulmi	7s + 3i	23s + 21i	3s	21s
K. phaffii/K. kurtzmanii	4s + 1i	13s + 9i	4s	9s
K. phaffii/K. pseudopastoris	7s + 6i	32s + 5i	27s	94s
K. phaffii/K. populi	6s + 6i	24s + 8i	33s	97s
K. phaffii/K. ulmi	4s + 3i	21s + 14i	13s	52s
K. kurtzmanii/K. pseudopastoris	6s + 5i	29s + 4i	27s	90s
K. kurtzmanii/K. populi	5s + 5i	21s + 13i	32s	93s
K. kurtzmanii/K. ulmi	4s + 4i	16s + 16i	13s	49s
K. populi/K. pseudopastoris	3s	23s + 12i	13s	11s
K. populi/K. ulmi	5s + 7i	35s + 10i	29s	73s
K. pseudopastoris/K. ulmi	6s + 7i	20s + 15i	24s	74s

[a]Nucleotide differences between UCDFST 71-1024^T and UCDFST 74-1030: D1/D2 = 0, ITS = 1s + 2i, EF-1α = 1s, RPB1 = 0; UCDFST 71-1024^T and UCDFST 54-11.16: D1/D2 = 1s, ITS = 1s, EF-1α = 1s, RPB1 = 0; UCDFST 74-1030 and UCDFST 54-11.16: D1/D2 = 1s, ITS = 2i, EF-1α = 2s, RPB1 = 0; UCDFST 54-11.16, UCDFST 54-11.141 and UCDFST 68-967.1 have identical D1/D2, ITS, EF-1α and RPB1 sequences. NRRL Y-27603^T and NRRL Y-27604: D1/D2 = 1s, ITS = 0, EF-1α = 0, RPB1 = 0; NRRL Y-27603 and NRRL Y-27602: D1/D2 = 1s, ITS = 2s + 4i, EF-1α = 5s, RPB1 = 11s; UCDFST 71-1024^T and NRRL Y-27602: D1/D2 = 0, ITS = 10s + 9i, EF-1α = 11s, RPB1 = 30s; UCDFST 54-11.16 and NRRL Y-27603^T: D1/D2 = 2s, ITS = 11s + 5i, EF-1α = 13s, RPB1 = 27s; UCDFST 54-11.16 and NRRL Y-27604: D1/D2 = 1s, ITS = 11s + 5i, EF-1α = 13s, RPB1 = 27s; UCDFST 54-11.16 and NRRL Y-27602: D1/D2 = 1s, ITS = 9s + 9i, EF-1α = 10s, RPB1 = 30s

[b]s = substitutions; i = indels

71-1024^T, other investigated strains UCDFST 54-11.16, UCDFST 54-11.141, UCDFST 68-967.1 and UCDFST 74-1030).

Using induced complementary auxotrophic mutants and selective growth of prototrophic hybrids on minimal medium, we demonstrated earlier that *K. kurtzmanii*, *K. pastoris*, *K. phaffii*, *K. populi*, *K. pseudopastoris* and *K. ulmi* possess a common mating type system allowing them to be crossed (Naumov 2015). Due to postzygotic isolation, the resulting interspecies hybrids are sterile, having non-viable ascospores (Naumov et al. 2016). Biogeographical factor may play a role in genetic isolation of *Komagataella* species. *K. pastoris* and *K. pseudopastoris* are known from Europe, whereas *K. kurtzmanii*, *K. mondaviorum* sp. nov, *K. phaffii*, *K. populi*, and *K. ulmi* are isolated in North America (Dlauchy et al. 2003; Kurtzman 2011; present study).

Multigene phylogenetic analysis conducted by us separated the seven *Komagataella* species into two main groups (Fig. 1). The first combines *K. pastoris, K. kurtzmanii*, *K. phaffii* and *K. ulmi*. The second includes *K. mondaviorum, K. populi* and *K. pseudopastoris*. There are numerous strains of *Komagataella* isolated from various tree exudates (*Ulmus fulva*, *U. caprinifolia*, *Populis fremontii*,

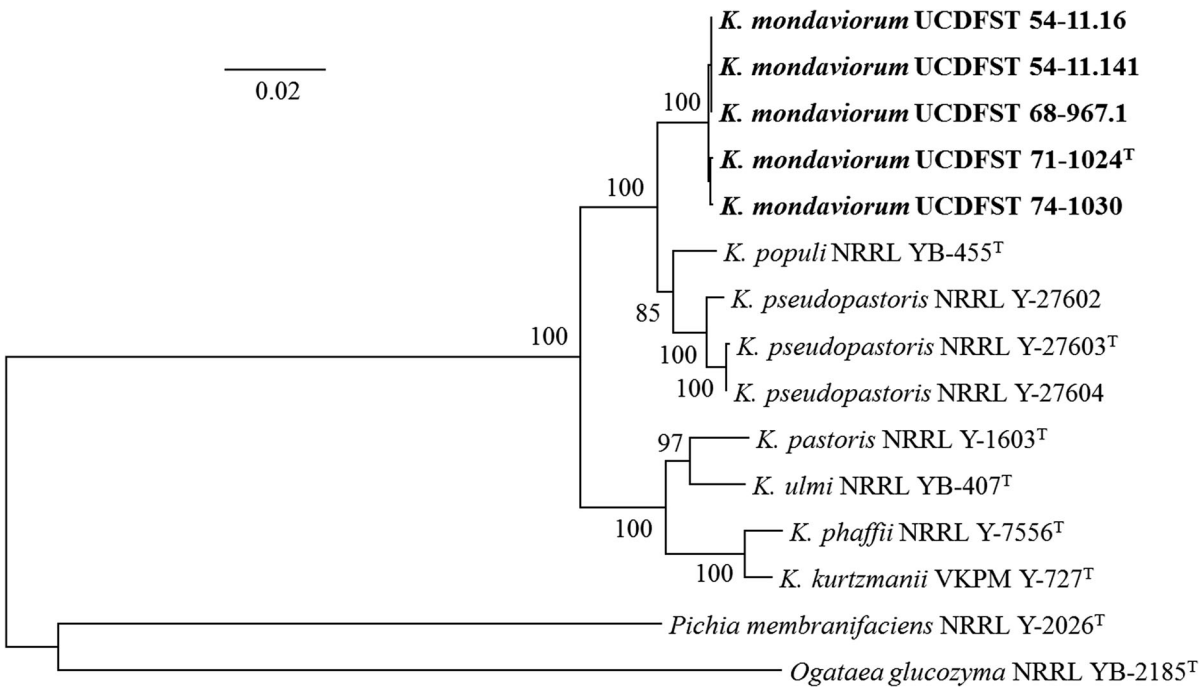

Fig. 1 Neighbour-joining tree showing phylogenetic relationship between seven species of the genus *Komagataella* based on combined sequences for D1/D2 and ITS1-5.8S-ITS2 rDNA, translation elongation factor-1α, and RNA polymerase II (subunit RPB1). The analysis is based on 2482 aligned positions. Bootstrap percentages > 70% for 1000 replicates are shown. *Pichia membranifaciens* and *Ogataea glucozyma* were used as the outgroup. Bar, 20 estimated base substitutions per 1000 nucleotide positions. T—type culture

P. trichocarpa, *Salix* sp., *Quercus agrifolia*, *Q. emorye*, *Q. kelloggii* and *Q. suber*) in North America (Miller et al. 1962; Phaff et al. 1972; Ganter et al. 1986) and Europe (Dlauchy et al. 2003). Large-scale screening of methanol-assimilating ascomycetous yeasts using a methanol-enrichment technique failed to isolate *Komagataella* strains in Thailand and Brazil (Limtong et al. 2013; Santos et al. 2015). Extremely rare isolates of *Komagataella* yeasts were documented from Japan (Phaff et al. 1972; Kodama 1974) and from Argentina (Spencer et al. 1995, 1996).

The seven species of the genus *Komagataella* are phenotypically very similar (Table 3). Some physiological differences are strain-variable. Taking into account that *K. kurtzmanii*, *K. populi* and *K. ulmi* are represented by single strains, it seems difficult to separate all seven *Komagataella* species from one another solely by conventional physiological tests. Consequently, multigene sequence comparisons should be used for reliable species identification. Of the four molecular markers used in this study, the ITS1-5.8S-ITS2, EF-1α and RPB1 gave congruent resolutions for species differentiation. It should be underlined that the key taxonomic molecular marker (D1/D2 LSU rRNA) fails to separate all *Komagataella* species (Table 2). *K. mondaviorum* sp. nov., *K. populi* and *K. pseudopastoris* are indistinguishable by D1/D2 sequences. Recent study of several thousand strains maintained in the CBS-KNAW collection showed that 9.5% of yeast species could not be distinguished by LSU (Vu et al. 2016).

The ITS region, currently used as one of the universal DNA barcode marker for species and genera discrimination in fungi (Schoch et al. 2012; Vu et al. 2016), is characterized by a significant interspecies divergence and a low level of intraspecies polymorphism. Indeed, all seven *Komagataell*a species can be delineated by the ITS sequences, which differ by 7–35 substitutions and numerous indels. Note that sequence divergence of the ITS1-5.8S-ITS2 between *K. pseudopastoris* strains does not exceed two nucleotide substitutions (Table 2), and there was no intraspecific variation for the ITS sequences in *K. pastoris* and *K. phaffii* (Kurtzman 2009). Thus, multigene sequence

Table 3 Physiological characteristics of *Komagataella mondaviorum* sp. nov, and other species of the genus *Komagataella*

Physiological test[a]	Kmo[b]	Kku[c]	Kpa	Kph	Kpo	Kul	Kps
Fermentation							
Glucose	+	+	+	+	+	+	+
Galactose	−	−	−	−	−	−	−
Maltose	−	−	−	−	−	−	−
Sucrose	−	−	−	−	−	−	−
Trehalose	−	−	−	−	w	w	−
Lactose	−	−	−	−	−	−	−
Raffinose	−	−	−	−	−	−	−
Growth							
Glucose	+	+	+	+	+	+	+
Galactose	−	−	−	−	−	−	−
L-Sorbose	−	−	−	−	−	−	−
D-Glucosamine	−	−	−	−	−	−	−
D-Ribose	−	−	−	−	−	−	−
D-Xylose	−	−	v	−	−	−	s
L-Arabinose	−	−	−	−	−	−	−
D-Arabinose	−	−	−	−	−	−	s/-
L-Rhamnose	+	+	+	+	+	+	+
Sucrose	−	−	−	−	−	−	−
Maltose	−	−	−	−	−	−	−
Trehalose	+/w	−	+	+	+	+	+
Methyl-α-D-glucoside	−	−	−	−	−	−	−
Cellobiose	−	−	−	−	−	−	−
Salicin	−	−	−	−	−	−	−
Arbutin	n	−	−	−	n	n	−
Melibiose	−	−	−	−	−	−	−
Lactose	−	−	−	−	−	−	−
Raffinose	−	−	−	−	−	−	−
Melezitose	−	−	−	−	−	−	−
Inulin	−	−	−	−	−	−	−
Soluble starch	−	−	−	−	−	−	−
Glycerol	+	+	+	+	+	+	+
Erythritol	−	−	−	−	−	−	−
Ribitol	−	−	−	−	−	−	−
Xylitol	−	−	−	−	n	n	−
L-Arabinitol	−	−	−	−	n	n	−
D-Glucitol	+	−	+	+	+	+	+
D-Mannitol	v	−	+	+	+	+	+
Galactitol	−	−	−	−	−	−	−
myo-Inositol	−	−	−	−	−	−	−
D-Glucono-1,5-lactone	−	−	−	−	n	n	−
2-Keto-D-gluconate	−	−	−	−	−	−	−
5-Keto-D-gluconate	−	−	−	−	−	−	n
D-Gluconate	−	−	−	−	−	−	−

Table 3 continued

Physiological test[a]	Kmo[b]	Kku[c]	Kpa	Kph	Kpo	Kul	Kps
D-Glucuronate	−	−	−	−	n	n	−
D-Galacturonate	−	−	−	−	n	n	−
DL-Lactate	+	+	+	+	w	+	+
Succinate	+	+	+	+	+	+	+
Citrate	−	−	v	v	−	−	−
Methanol	+	+	+	+	+	+	+
Ethanol	+	+	+	+	+	+	+
N-Acetyl-D-glucosamine	−	−	−	−	−	−	−
Nitrate	−	−	−	−	−	−	−
Nitrite	−	−	−	−	n	n	−
Ethylamine	+	+	+	+	n	n	+
L-Lysine	+	+	+	+	n	n	+
Glucosamine	−	−	n	n	n	n	−
Imidazole	−	−	−	−	n	n	−
w/o vitamins	−	−	−	−	−	−	+/w
Cycloheximide 0.1%	+	+	+	+	n	n	+
Acetic acid 1%	−	−	−	−	n	n	−
10% NaCl/5% Glucose	−	−	v	−	−	−	−
Growth on 50% w/w glucose yeast extract agar	−	−	−	−	−	n	−
Growth at 35 °C	−	−	+	+	+	+	+
Growth at 37 °C	−	−	+/w	+	w	w	n

[a]− negative, + positive, w weak, s slow, v variable, n no data

[b]Species: Kmo, *Komagataella mondaviorum* sp. nov. (UCDFST 71-1024[T], UCDFST 54-11.16, UCDFST 54-11.141, UCDFST 68-967.1 and UCDFST 74-1030); Kku, *K. kurtzmanii*; Kpa, *K. pastoris;* Kph, *K. phaffii*; Kpo, *K. populi*; Kul, *K. ulmi*; Kps, *K. pseudopastoris*

[c]Data for species Kku are from Naumov et al. (2013); Kpa, Kph, Kpo, Kul are from Kurtzman (2012); Kps are from Dlauchy et al. (2003). Growth of species Kpa, Kph, Kpo, Kul and Kps at 35 °C was studied by us, using the corresponding type cultures

divergence separating *Komagataella mondaviorum* sp. nov. from the other members of the genus is similar to those of the other six *Komagataella* species.

Description of *Komagataella mondaviorum* G. I. Naumov, E. S. Naumova and K. L. Boundy-Mills sp. nov.

Komagataella mondaviorum (mon.da.vio'rum. N. L. gen. masc. plur. n. *mondaviorum* is named in honor of the late Robert and Margrit Mondavi, honoring their tremendous impact on the CA wine industry and their generous and forward-thinking support of facilities and programs at the University of California Davis).

Growth on 5% malt extract agar

After 3 days on 5% malt extract (ME) agar at 25 °C, cells divide by multilateral budding and are spherical (2–6 μm) to ovoid (2–5 × 4–7 μm), occur singly and in pairs (Fig. 2a). Colony growth is white, butyrous and with a smooth semi-glistening surface.

Dalmau plate culture on morphology agar

After 7 days on morphology agar at 25 °C, growth under the coverglass formed neither hyphae nor pseudohyphae.

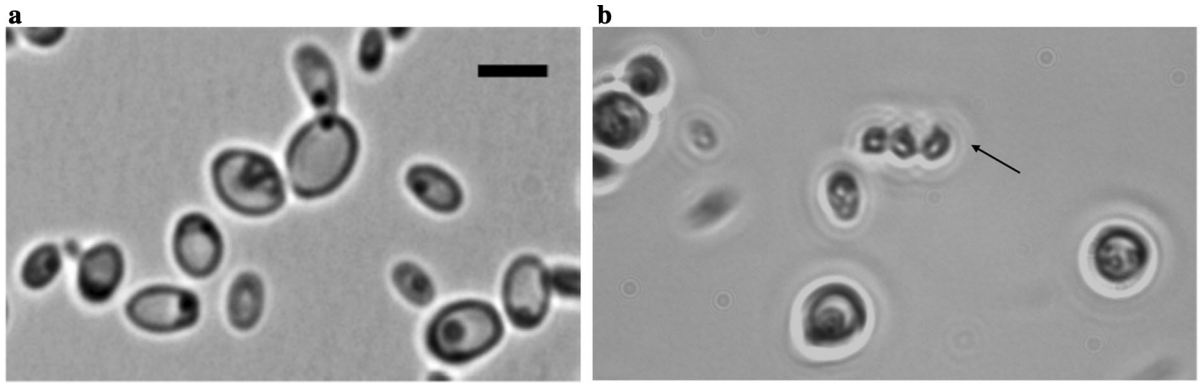

Fig. 2 *Komagataella mondaviorum* sp. nov. UCDFST 71-1024^T **a** Budding cells at 25 °C on 5% ME agar at 3 days. Bar, 5 μm. **b** The arrow indicates a deliquescent ascus with three ascospores on acetate medium at 6 days incubation at 22 °C

Formation of ascospores

Ascosporulation occurs on different acetate agar media after 6 days at 22 °C. The best sporulation is observed on the medium containing 5 g CH3COONa, 10 g KCl, 10 g glucose and 20 g agar in 1 l of distilled water. Asci may be unconjugated or show conjugation between a cell and its bud or between independent cells. One to four hat-shaped ascospores are formed in each ascus and they are soon liberated (Fig. 2b). In view of conjugation between cells and their buds, the species appears to be homothallic.

Fermentation and growth reactions

Glucose is fermented. Galactose, maltose, sucrose, trehalose, lactose and raffinose are not fermented. Carbon compounds: glucose, L-rhamnose, trehalose, glycerol, D-glucitol, D-mannitol (variable), DL-lactate, succinate, methanol and ethanol are assimilated; no growth occurs on galactose, L-sorbose, D-glucosamine, D-ribose, D-xylose, L-arabinose, D-arabinose, sucrose, maltose, methyl α-D-glucoside, cellobiose, salicin, melibiose, lactose, raffinose, melezitose, inulin, soluble starch, erythritol, ribitol, xylitol, L-arabinitol, galactitol, myo-inositol, D-glucono-1,5-lactone, 2-keto-D-gluconate, 5-keto-D-gluconate, D-gluconate, D-glucoronate, D-galacturonate, citrate and N-acetyl-D-glucosamine. Nitrogen compounds: ethylamine, L-lysine and cadaverine are assimilated; no growth occurs on potassium nitrate, sodium nitrite, D-glucosamine and imidazole. Growth on vitamin-free medium is negative. Growth with 0.1% cycloheximide is positive. Growth is absent with 1% acetic acid, on YM agar with 10% NaCl and on 50% w/w glucose/yeast extract (0.5%) agar. Growth at 35°°C is negative. Physiological data are from strains UCDFST 71-1024^T, UCDFST 54-11.16, UCDFST 54-11.141, UCDFST 68-967.1 and UCDFST 74-1030.

The type strain is UCDFST 71-1024^T isolated in 1971 by H.J. Phaff from exudate of cottonwood (*Populus deltoides*) in Davis, CA, USA. The strain was originally designated as *P. pastoris.* It is preserved as a lyophilized preparation in the Phaff Yeast Culture Collection, University of California, Davis, CA, USA. Ex-type culture has been deposited in the CBS yeast collection of the Westerdijk Fungal Biodiversity Institute (Utrecht, the Netherlands) under the designation CBS 15017^T, in the All-Russian Collection of Industrial Microorganisms (Moscow, Russia) under the designation VKPM Y-4330^T and in the USDA-ARS Culture Collection, National Center for Agricultural Utilization Research (Peoria, Illinois, USA) under the designation NRRL Y-63969^T. The Mycobank number is MB 821789.

This study demonstrates the importance of preserving microbes in professionally managed microbe culture collections to enable future discoveries (Boundy-Mills et al. 2016). Because many yeast strains and associated data were preserved in the Phaff Yeast Culture Collection by foresighted researchers, the yeasts continue to be instrumental for discoveries and innovation, such as recent discoveries regarding oleaginous yeast species (Garay et al. 2016), glycolipid-secreting yeasts (Garay et al. 2017) and novel species of industrially important methylotrophic yeasts *Komagataella* and *Ogataea*

(Kurtzman 2009; Naumov 2015; Naumov et al. 2013, 2016, 2017; Yamada et al. 1995).

Acknowledgements The authors thank Yuriy A. Rybakov, Sergey E. Cheperegin, Erin Cathcart and Russell Fry for technical assistance. This material is based upon work supported by the Russian Foundation for Basic Research under Grant Number 18-54-52002 MHT_a and the National Science Foundation under Grant Number 1349395, which funded, respectively, nuclear genes (EF-1α and RPB1) and ribosomal sequence analyses of yeasts from the Phaff Yeast Culture Collection. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation.

Conflicts of interest The authors declare no conflict of interest.

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