Metschnikowia cubensis sp. nov., a yeast species isolated from flowers in Cuba

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A novel yeast species is described from 19 strains isolated from flowers and insects in three provinces of Cuba. The species is so far known only from Cuba. Characteristic asci and ascospores as well as phylogenetic analysis of the rDNA sequence place the novel species in the genus *Metschnikowia*. The novel species belongs to the New World subclade of large-spored species of *Metschnikowia*. Mating tests with other members of the subclade resulted in the formation of sterile asci without ascospores, showing that the Cuban strains represent a distinct biological species. Intraspecific matings lead to the production of fertile asci containing large needle-shaped ascospores. The novel species was further distinguished from its close relatives by rDNA sequences and PCR fingerprinting using primers derived from mini- and microsatellites. We propose the name *Metschnikowia cubensis* sp. nov. and designate MUCL 45753^T (=CRGF 279^T =CBS 10832^T, h⁺) as the type strain and MUCL 45751 (=CRGF 278 =CBS 10833, h⁻) as the allotype.

Flowers have been known to harbour a significant yeast community since the studies of Boutroux (1884). Members of the genus *Metschnikowia* were among the first yeasts recovered from this habitat and were thought to be vectored by flower-associated insects (Reukauf, 1911–1912). They are among the best-studied yeasts with respect to their biogeography and ecology (Lachance *et al.*, 2001, 2003). The genus *Metschnikowia* is characterized morphologically by elongated asci containing long needle-shaped ascospores. Within the genus, a subgroup of nutritionally homogeneous insect-associated *Metschnikowia* species is recognizable by the formation of generally large ascospores, 100–250 µm length, in contrast to only 20–60 µm in most other *Metschnikowia* species (Marinoni & Lachance, 2004; Miller & Phaff, 1998). The term ‘large-spored *Metschnikowia* species’ has been coined based on this character. Members of the group have so far been isolated only from the Americas and Hawai’i, and each species is limited in geographical distribution. Some species were shown to be endemic to the Hawaiian islands (Lachance *et al.*, 2005), and their local distribution is assumed to be

due to their close association with small nitidulid beetles and allopatric or peripatric speciation (Lachance *et al.*, 2001; Marinoni & Lachance, 2004). Some *Candida* species are members of the *Metschnikowia* clade as seen in phylogenetic analyses of rDNA sequences (Nguyen *et al.*, 2006). Of these, *Candida ipomoeae* is an integral member of the New World large-spored subclade.

Cuba is recognized as a biodiversity hot spot, with approximately 6500 vascular plant species, of which more than 50% are endemic (Capote *et al.*, 1989; <http://www.biodiversityhotspots.org/xp/Hotspots/caribbean/>). The National Conservation Strategies for Fungi in Cuba reported the presence of only 10 species of the Saccharomycetales (Mena *et al.*, 2003). This lack of knowledge of the yeasts of this region prompted us to investigate their diversity. The study has led to the finding of several undescribed species (Fidalgo-Jiménez *et al.*, 2005). Nineteen strains were found to represent a novel species of the genus *Metschnikowia*, for which the name *Metschnikowia cubensis* sp. nov. is proposed.

Isolation and preservation

Nineteen strains of *M. cubensis* were found in about 500 samples from flowers of more than 120 plant species and some flower-associated insects taken in Cuba between 2001 and 2005. The samples yielded more than 400 yeast strains.

The GenBank/EMBL/DDBJ accession number for the large-subunit rDNA sequence of strain MUCL 45753^T is EU143316.

Further details on strain collection and results of mating tests are available as supplementary material with the online version of this paper.

Table 1. Origins of isolates considered in this study

Numbers following isolation sources signify individual flowers or insects that were sampled in the same location. The locations of the collection sites are given as: country, province, region/park/reserve, sampling location. ^T, ex type; ^I, ex isotype.

Strain	Mating type	Isolation source	Location
<i>M. cubensis</i> sp. nov.			
MUCL 44438, CRGF 82	h ⁻	Flower of <i>Talipariti elatum</i> , Malvaceae	Cuba, La Habana, Boyeros, Instituto de Ecología y Sistemática
MUCL 44441, CRGF 83	h ⁻	Flower of <i>T. elatum</i>	As above
MUCL 45753 ^T , CRGF 279 ^T , CBS 10832 ^T	h ⁺	Flower of <i>T. elatum</i>	Cuba, Sancti Spiritus, Alturas de Banao, path El Regalo
MUCL 45751, CRGF 278, CBS 10833	h ⁻	Flower of <i>Gesneria humilis</i> , Gesneriaceae	Cuba, Sancti Spiritus, Alturas de Banao, house Maria Antonia
MUCL 49175, CRGF 826	h ⁺	Flower of <i>Bidens alba</i> var. <i>radiata</i> , Asteraceae	Cuba, Pinar del Río, Mil Cumbres, on the way to the start of the pipe of the hydroelectric plant
MUCL 46753, CRGF 752	h ⁺	Flower of <i>Tabebuia</i> sp., Bignoniaceae	Cuba, Pinar del Río, Mil Cumbres, Reserva San Marcos
MUCL 51011, CRGF 833	h ⁻	Individual 1 of <i>Conotelus</i> sp. in flower 1 of <i>Ipomoea acuminata</i> , Convolvulaceae	Cuba, Sancti Spiritus, Topes de Collantes, start of path to the Salto de Cabuni
MUCL 51012, CRGF 842	h ⁺	Individual 1 of <i>Conotelus</i> sp. in flower 1 of <i>I. acuminata</i>	As above
MUCL 51013, CRGF 834	h ⁺	Individual 3 of <i>Conotelus</i> sp. in flower 1 of <i>I. acuminata</i>	As above
MUCL 51014, CRGF 835	h ⁺	Flower 1 of <i>I. acuminata</i>	As above
MUCL 51015, CRGF 836	h ⁻	Ant in flower of <i>T. elatum</i>	Cuba, Sancti Spiritus, Topes de Collantes, south of Hotel Escambray
MUCL 49859, CRGF 828	h ⁺	Pollen of <i>T. elatum</i>	As above
MUCL 51016, CRGF 837	h ⁻	Pollen of <i>T. elatum</i>	As above
MUCL 49860, CRGF 829	h ⁺	Flower 1 of <i>I. acuminata</i>	Cuba, Sancti Spiritus, Topes de Collantes, ~500 m from Hotel Escambray
MUCL 51017, CRGF 838	h ⁻	Flower 2 of <i>I. acuminata</i>	As above
MUCL 51018, CRGF 839	h ⁻	Flower 1 of <i>I. acuminata</i>	Cuba, Sancti Spiritus, Topes de Collantes, ~1500 m from Hotel Escambray
MUCL 49858, CRGF 827	h ⁻	Flower of <i>T. elatum</i>	Cuba, Pinar del Río, Mil Cumbres, near cabin Los Pinos
MUCL 51019, CRGF 840	h ⁺	Fallen flower of <i>T. elatum</i>	Cuba, Pinar del Río, Viñales valley, camp Cueva Los Portales
MUCL 51020, CRGF 841	h ⁻	Flower 5 of blue <i>Ipomoea</i> sp.	Cuba, Pinar del Río, Viñales valley, roadside between Viñales and Pons, near path 'Maravillas de Viñales'
<i>Metschnikowia</i> sp.			
MUCL 46232, UWOPS 00-154.1	h ⁻	<i>Conotelus</i> sp. collected on <i>Merremia tuberosa</i>	Costa Rica, Guanacaste, Dos Ríos
<i>M. lochheadii</i>			
MUCL 46297 ^T , UWOPS 00-133.2 ^T	h ⁺	<i>Conotelus mexicanus</i> collected on <i>Tabebuia rosea</i>	Costa Rica, Guanacaste
MUCL 46298 ^I , UWOPS 99-661.1 ^I	h ⁻	<i>C. mexicanus</i> collected on <i>Brugmansia candida</i>	USA, Hawaii, Maui Island
<i>C. ipomoeae</i>			
MUCL 46294 ^T , UWOPS 91-672.1 ^T	Unknown	<i>Exalloscaptomyza calliginosa</i> collected on <i>I. acuminata</i>	USA, Hawaii, Hawaii Island

The distances between the three larger collection areas are between 130 and 440 km. The strains and their isolation substrates are listed in Table 1. More detailed information about the collections is provided in Supplementary Table S1, available in IJSEM Online.

For yeast isolation, flowers were placed in sterile plastic bags or sterile tubes depending on their size. For large

flowers (e.g. Malvaceae), 150 µl sterile water was added into each flower to release yeast cells adhering to their surface. Small flowers (e.g. Asteraceae) were taken with sterile forceps and immersed in 1000 µl sterile water. In both cases, 100 µl of the wash fluid was diluted 10-fold and 100 µl of this dilution was spread on DYPA (2 % glucose, 1 % peptone, 0.5 % yeast extract, 2 % agar) supplemented

with 0.02 % chloramphenicol. Insects were placed on Petri dishes with the same medium, allowed to move around for 2–5 min and then removed. The cultures were incubated at ambient temperature and examined every 12 h for 72 h. Representative colonies of each morphological type were purified and maintained on DYPA. All strains have been deposited in the Collection of Fungal Genetic Resources (CRGF), Cuba, with duplicates at the MUCL. They are preserved on agar slants under oil and under water at CRGF and cryopreserved at -130°C , lyophilized, on agar slants under oil and under water at MUCL. The type strains were also deposited at the Centraalbureau voor Schimmelcultures (CBS).

Growth characteristics

The strains were characterized by 96 morphological and physiological tests, performed with the microplate-based ALLEV system (Robert *et al.*, 1997), following the methods of Kreger-van Rij (1987) and Van der Walt & Yarrow (1984). The strains showed some slightly variable growth reactions, which are represented in the species description. Measurements of cells and ascospores were obtained based on micrographs with the BioloMICS software (BioAware) and are given in the description.

The mating type for MUCL 45753^T h⁺ and MUCL 45751 h⁻ was determined by mixing active cultures of the two strains on yeast carbon base (Difco) supplemented with 0.01 % ammonium sulphate and 1.5 % agar, followed by periodic examination by phase-contrast microscopy. Quantitative assessment of ascospore formation for selected strains was performed as follows. Cells of active cultures were suspended in sterile water and the cell density was adjusted visually to McFarland standard 2 ($\sim 20 \times 10^6$ cells ml⁻¹). Five hundred microlitre aliquots of each mating partner were mixed and four 50 µl aliquots of the mixtures were spot-inoculated onto GYA (1 % glucose, 0.01 % yeast extract, 2 % agar) and the plates were incubated at 18 °C. Slides were prepared using cotton blue (5 mg cotton blue ml⁻¹ in lactic acid) and examined every 12 h. For each slide, 50 asci were scored for the presence of ascospores and the results after 72 h incubation are reported in Supplementary Table S2. Mating types were assigned based on the formation of sterile asci in crosses with authentic strains of other species and are thus consistent with those of all other large-spored *Metschnikowia* species. Species delineation was based on the development of fertile asci with two ascospores in compatible mating reactions. A minimum percentage of 70 % fertile asci was found for the strain combination MUCL 46753^T h⁺ × MUCL 45751 h⁻. All other intraspecies combinations showed more than 80 % fertile asci (Supplementary Table S2). The combination of *M. cubensis* strains with compatible mating types of *Metschnikowia lochheadii* (MUCL 46297 h⁺, MUCL 46298 h⁻) and *Metschnikowia* sp. UWOPS 00-154.1 produced sterile asci.

DNA-based studies

Extraction of high-molecular-mass DNA was performed by a combination of the Qiagen DNeasy procedure and the Invisorb Spin Plant Mini kit (Invitek) with modifications. From a culture grown on DYPA for 72 h at 25 °C, three 10 µl loops of cells were resuspended in 600 µl sorbitol buffer (1 M sorbitol, 100 mM sodium EDTA, 14 mM β-mercaptoethanol) and 200 U lyticase (L4025; Sigma). The samples were shaken overnight at 30 °C, the suspension was centrifuged for 2 min at 12 000 r.p.m., the supernatant was discarded, 400 µl lysis buffer P and 20 µl proteinase K were added and finally the mixture was homogenized with a pipette tip and incubated for 30 min at 65 °C. The solution was then transferred onto a spin filter and centrifuged for 5 min at 12 000 r.p.m. and 6 µl RNase (100 mg ml⁻¹ in molecular biology grade water) was then added to the filtrate, which was vortexed briefly and incubated for 5 min at room temperature. Binding buffer P (200 µl) was added and the sample was vortexed briefly and then transferred to a fresh spin filter and centrifuged for 1 min at 12 000 r.p.m. Wash buffer I (550 µl) was then added to the spin filter, which was centrifuged as before; the filtrate was discarded, followed by a similar treatment using wash buffer II and a final treatment with 400 µl wash buffer II and centrifugation for 2 min at 12 000 r.p.m. The DNA was eluted with 100 µl 65 °C molecular biology grade water for 3 min and centrifuged for 1 min at 10 000 r.p.m. This DNA solution, stored at 4 °C for short-term preservation and at -20°C for long-term preservation, was used for PCR fingerprinting and DNA sequence determinations.

PCR fingerprinting was performed using the minisatellite-specific oligonucleotide derived from the core sequence of the bacteriophage M13 (Vassart *et al.*, 1987) with the sequence 5'-GAGGGTGGCGGTTCT-3' and the microsatellite-specific oligonucleotides (GACA)₄, (GTG)₅ and (ATG)₅ as single PCR primers. PCR amplifications were performed in 25 µl reaction volumes containing 10 ng genomic DNA, 0.13 µM primer, 0.2 mM dNTPs, 4.5 mM MgCl₂ and 2.5 U AmpliTaq DNA polymerase (Applied Biosystems). Amplifications were carried out in an Eppendorf Mastercycler programmed for 35 cycles (20 s at 94 °C, 60 s at 50 °C, 20 s at 72 °C), followed by 6 min final extension at 72 °C and cooling to 4 °C. Amplified DNA fragments were separated by electrophoresis in 1.4 % agarose dissolved in TBE buffer, stained with 0.6 × GelRed (approx. 0.8 µg ml⁻¹; Biotium) and photographed under UV light (Fig. 1). The species selection for PCR fingerprint analysis was based on the smallest differences in the D1/D2 large-subunit (LSU) rDNA sequence among type strains of *M. cubensis* vs *Candida ipomoeae* with five substitutions and two indels, vs *Metschnikowia* sp. UWOPS 00-154.1 with three substitutions and four indels and vs *M. lochheadii* with seven substitutions and one indel. The DNA banding patterns with the primers M13, (GTG)₅ and (ATG)₅ were generally similar within *M. cubensis* and *M. lochheadii*, and dissimilar between the four species

Fig. 1. PCR fingerprinting profiles with primers M13, (GACA)₄, (GTG)₅ and (ATG)₅ for selected strains of *Metschnikowia cubensis* sp. nov. and type strains of the most closely related species. Lanes: M, markers; 1, MUCL 49175; 2, MUCL 46753; 3, MUCL 45753^T; 4, MUCL 45751; 5, MUCL 44441; 6, MUCL 44438; 7, *Metschnikowia* sp. UWOPS 00-154.1; 8, *M. lochheadii* UWOPS 00-133.2^T; 9, *M. lochheadii* UWOPS 99-661.1^I; 10, *Candida ipomoeae* UWOPS 91-672.1^T; –, negative control. ^T, ex type; ^I, ex isotype.

compared. The DNA amplified with the primer (GACA)₄ shows a large degree of intraspecies variation in *M. cubensis* and almost identical patterns for MUCL 44441 and MUCL 44438 that were isolated in close proximity to each other, suggesting that (GACA)₄ might be most useful for differentiation at the population level.

Phylogenetic relationships

Comparison of D1/D2 sequences of the type and allotype strains originally indicated that *M. cubensis* may be closely related to *M. lochheadii*, *Metschnikowia* sp. strain UWOPS 00-154.1, and *C. ipomoeae*. However, the phylogenetic signals of the D1/D2, internal transcribed spacer (ITS) and intergenic spacer (IGS) components of the nuclear rDNA have been shown not to be perfectly congruent in the large-spored group (Marinoni & Lachance, 2004). Separate phylogenetic analyses of various partitions of the rDNA repeat showed that the LSU, including the D1/D2 region, favoured a sisterhood of *M. cubensis* with *C. ipomoeae* and that the D1/D2 LSU alone lead to the placement of these two taxa next to *M. lochheadii* and UWOPS 00-154.1. Small-subunit (SSU) rDNA trees indicated a sister relationship of *M. cubensis* and *Metschnikowia borealis*, with 70% bootstrap support. No other bootstrap-supported groups could be detected in these analyses, indicating a low information content of SSU sequences

for this comparison. As none of the rDNA partitions resolved the relationships, the tree presented in Fig. 2 is based on the complete transcribed region of the rDNA. The IGS sequences could not be aligned in a satisfactory manner and were deleted from the analysis. The sequences

Fig. 2. Neighbour-joining phylogram of selected large-spored *Metschnikowia* species showing the placement of *M. cubensis* based on 5638 aligned nucleotide positions of rDNA sequences including the external transcribed spacer, SSU gene, internal transcribed spacers 1 and 2, 5.8S gene and the LSU gene. Shown is the tree obtained in MEGA 3.1 under Kimura's two-parameter model with gapped alignment positions deleted during the pairwise sequence comparisons. The analysis was replicated 1000 times and only bootstrap values greater than 70% are shown. The root was positioned on the midpoint of the longest path between two taxa. Bar, 0.5% sequence divergence.

were obtained as described in Rosa *et al.* (2007). Analyses by maximum-parsimony (results not shown) and neighbour-joining (Fig. 2) suggest that *M. cubensis* occupies a fairly basal position within the New World large-spored species. A possible sisterhood with *M. borealis*, an eastern North American endemic, is not supported by bootstrap analysis in the parsimony analysis and received intermediate bootstrap values in the neighbour-joining analysis. The neighbour-joining bootstrap values should be interpreted with caution, as varying rates of nucleotide substitution might lead to incorrect branching patterns receiving high bootstrap values.

Geographical distribution and ecology

The low frequency with which the species was found indicated that its true specific habitat has not yet been identified unequivocally. Strains were obtained either from flowers found in clearings of tropical wet forest, at forest edges, in abandoned gardens or from insects found in flowers (Table 1). These habitats were characterized by a general abundance of ascomycetous yeasts. The relatively large distances between the three larger collection areas, Sierra del Escambray, Cordillera de Guaniguanico and La Habana, and the frequent occurrence of similar disturbed habitats suggest that the species is widely distributed throughout central and western Cuba. On the Peninsula de Guanahacabibes in the far west of Cuba and in the Cuchillas del Toa near the north-eastern coast, where similar numbers of samples were collected and analysed by similar methods, no isolates of *M. cubensis* have yet been found. Both mating types were present in all collection areas with the exception of La Habana, from where only two isolates are known. From two samples, from *Conotelus* sp. and pollen, respectively, two isolates each with opposite mating types were obtained. This and the balanced occurrence of the two mating types among the isolates suggests that the species forms one sexually reproducing population. *M. cubensis* was isolated from flowers of five plant species and associated insects with a repeated occurrence on *Talipariti elatum*, a tree with hibiscus-like flowers formerly classified as *Hibiscus elatus*, and on *Ipomoea acuminata*. The isolation plates of one *Conotelus* sp. individual, the *Ipomoea acuminata* flower containing *Conotelus* sp. and a fallen *Talipariti elatum* flower were noted for particularly large numbers of 100–200 yeast colonies of homogeneous morphology (Supplementary Table S1). A specific association of New World large-spored *Metschnikowia* species with floricolous beetles (*Conotelus* spp., Nitidulidae) has been reported (Lachance *et al.*, 2001, 2003). Beetles of this genus have been found in all sampled localities in North, Central and South America and have been shown to harbour large populations of large-spored *Metschnikowia* species. Although the current study focused on yeasts of flowers and Cuban *Conotelus* sp. were not sampled repeatedly, it is likely that they are involved in the transmission of *M. cubensis*.

Species identification

The genus *Metschnikowia* is morphologically and physiologically homogeneous (Miller & Phaff, 1998). Identification of *M. cubensis* is therefore not possible based on these features owing to the similarity with related species. The novel species is included in the genus *Metschnikowia* based on the large cylindrical asci with one or two needle-shaped ascospores and phylogenetic analysis of rDNA sequences (Fig. 2). *M. cubensis* can be separated from related *Metschnikowia* species by reproductive isolation in compatible mating reactions, PCR-fingerprinting profiles produced with the primers M13, (GTG)₅ and (ATG)₅, and by sequencing of D1/D2 LSU or other rDNA components.

Latin diagnosis of *Metschnikowia cubensis* Fidalgo-Jiménez, Daniel, Evrard, Decock et Lachance sp. nov.

Post dies tres in medio DYPA cultura parva, convexa, aliquando cum margina filamentosa, candida aut cremea, lucida et rugosa, aliquando cerebriformis. Cellulae globosae ad ellipsoidae (2–4 × 2–5 µm). Cultura in agar PDA post dies 7 pseudomycelium formatur. Mycelium verum formatum tuborum longorum. Post dies unus, cellulae stirpium interfertilium mixtarum in agar carbonis fundamento tubi junctionis formantur. Post dies tres, magni asci cylindrati (80–120 µm) possunt videri. Asci stabiles sunt. Ascosporae aculeatae. Glucosum, galactosum et trehalosum (omnia variabile) fermentantur. Glucosum, galactosum, L-sorboseum, D-xylosum, sucrosum (aliquando exigue), maltosum, trehalosum, cellobiosum, salicinum, arbutinum (aliquando exigue), melezitolum, glycerolum (aliquando exigue), xylitolum (aliquando exigue), glucitolum, mannitolum, gluconolactolum (variabile), 2-ketogluconatum, D-gluconatum (aliquando exigue), acidum succinicum (aliquando exigue), ethanolum (aliquando exigue), propanum-1,2-diolum (aliquando exigue), assimilantur at non D-glucosaminum, D-ribosum (variabile), L-arabinosum, D-arabinosum, L-rhamnosum, methyl-α-D-glucosidum, melibiosum, lactosum, raffinoseum, inulinum, erythritolum, ribitolum (aliquando exigue), L-arabinitolum, galactitolum, myo-inositolum, 5-ketogluconatum (aliquando exigue), D-glucuronatum, acidum lacticum (aliquando exigue), acidum citricum (aliquando exigue), methanolum nec butanum-2,3-diolum (aliquando exigue). Ethylaminum, lysinum et cadaverinum assimilantur at non natrium nitricum nec natrium nitrosum, creatinum (variabile), creatininum, glucosaminum aut imidazolium. Ad crescentiam biotinum et thiaminum externa necessaria sunt. Augmentum in 30 °C, at non 37 °C.

Typus MUCL 45753^T e flore *Talipariti elatum* et allotypus MUCL 45751 e flore *Gesneria humilis*, Cuba. In collectione zymotica Centraalbureau voor Schimmelcultures, Trajectum ad Rhenum, sub no. CBS 10832^T et CBS 10833 depositae sunt.

Fig. 3. Phase-contrast micrographs of *M. cubensis* MUCL 45753^T. (a) Vegetative cells on DYPA at 25 °C after 72 h. (b, c) Strain MUCL 45753^T in mixture with allotype MUCL 45751 on GYA at 18 °C after 72 h showing a sterile ascus (b) and an ascus with two ascospores (c). Bars, 10 µm.

Description of *Metschnikowia cubensis* Fidalgo-Jiménez, Daniel, Evrard, Decock et Lachance sp. nov.

Metschnikowia cubensis (cu.ben'sis. N.L. nom. fem. sing. adj. *cubensis* of Cuba, referring to the country where all currently known strains were recovered).

Mycobank number: MB 511354.

After 72 h on DYPA at 25 °C, colonies are small, circular, convex, occasionally with a mycelial margin, white to cream, glistening and rugose. Some colonies are cerebriform. No pigment is observed. Cells are ovoidal to ellipsoidal, occur singly or in pairs and measure 2–4 × 2–5 µm (Fig. 3). Vegetative reproduction by multilateral budding is observed. In Dalmau plate cultures on PDA (potato dextrose agar 3.9%, agar 0.2%) after 7 days at 25 °C, pseudomycelium is formed and, more rarely, also true hyphae with rare septa are present. After 12 h on GYA at 18 °C, mixed cultures of complementary mating types show conjugated cells, zygotes and immature asci. After 3 days, mature and immature asci are present. Asci are large and cylindrical, usually retaining the original zygote. Mature asci contain two needle-shaped ascospores measuring 80–120 µm in length (Fig. 3). Evanescence of asci is not observed after 30 days of incubation. Glucose, galactose and trehalose fermentation is variable. Reactions are listed as observed in the type strain; reactions of additional strains are indicated in parentheses. Glucose, galactose, L-sorbose, xylose, sucrose (sometimes weak), maltose, trehalose, cellobiose, salicin, arbutin (sometimes weak), melezitose, glycerol (sometimes weak), xylitol (sometimes weak), glucitol, mannitol, D-glucono-1,5-lactone (variable), 2-keto-D-gluconate, D-gluconate (sometimes weak), succinate (sometimes weak), ethanol (variable and weak) and propane-1,2-diol (variable and weak) are assimilated, but not D-glucosamine, D-ribose (variable), L-arabinose, D-arabinose, L-rhamnose, methyl α-D-glucoside, melibiose, lactose, raffinose, inulin, erythritol, ribitol

(sometimes weak), L-arabinitol, galactitol, *myo*-inositol, 5-ketogluconate (sometimes weak), D-glucuronate, D-galacturonate, DL-lactate (sometimes weak), citrate (sometimes weak), methanol or butane-2,3-diol (sometimes weak). Ethylamine, L-lysine and cadaverine are utilized as nitrogen sources, but not nitrate, nitrite, creatine (variable), creatinine, glucosamine or imidazole. Biotin and thiamine are required for growth. Arbutin hydrolysis is variable. Growth is not observed in the presence of 0.1% cycloheximide or 1% acetic acid. Grows in the presence of 50% glucose. Starch formation, acetic acid production and urea hydrolysis are not observed. The Diazonium blue B reaction is negative. Grows at 30 °C but not at 37 °C.

The type strain is strain MUCL 45753^T (h⁺) (=CRGF 279^T =CBS 10832^T), isolated as I-206 by one of the authors (A.F.-J.) from a flower of *Talipariti elatum* on 27 January 2004 at vereda El Regalo, Alturas de Banao, Province Santí Spiritus, Cuba. The allotype strain is strain MUCL 45751 (h⁻) (=CRGF 278=CBS 10833), isolated as I-254 by one of the authors (A.F.-J.) from a flower of *Gesneria humilis* on 29 January 2004 at casa Maria Antonia, Alturas de Banao, Province Santí Spiritus, Cuba.

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