Metschnikowia shivogae sp. nov., a yeast species associated with insects of morning glory flowers in East Africa

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The novel species *Metschnikowia shivogae* is described to accommodate three isolates recovered from insects of morning glory flowers at two localities in East Africa. The isolates differ slightly in rDNA ITS and D1/D2 large-subunit sequences and one isolate featured a two-base heterogeneity that might be the result of recombination between two variant rDNAs. *M. shivogae* is a sister species to *Metschnikowia aberdeeniae* and shares the same habitat. The reproductive boundaries of *M. aberdeeniae*, which were not clear in the past, have now been elucidated further. The type strain of *Metschnikowia shivogae* sp. nov. is strain SUB 04-310.1^T (h^+ ; =CBS 10292^T =NRRL Y-27924^T) and the allotype is strain UWOPS 07-203.2 (h^- ; =CBS 10770 =NRRL Y-48447).

The yeast species *Metschnikowia aberdeeniae* was described on the basis of five strains isolated from insects of flowers of morning glory (*Ipomoea hildebrandtii*, Convolvulaceae) in sites located near the Serengeti National Park, Tanzania (Lachance *et al.* 2006). This species is part of a growing clade of yeasts that form large needle-shaped ascospores and that occur with high frequency worldwide in nitidulid beetles and other insects that visit ephemeral flowers. The large-spored *Metschnikowia* species are haplontic and heterothallic, such that ascus formation must be preceded by conjugation between genetically compatible, independent isolates. Six strains isolated with *M. aberdeeniae* were related to the species, but their reproductive boundaries were uncertain. Two were identical to the type in their ITS/5.8S and D1/D2 large subunit (LSU) rDNA sequences (sequence type A). Three differed by three substitutions (type B) and one by many more (about 8.6%; type C). All these strains were of mating type h^+ and, when crossed with the type of *M. aberdeeniae* (h^-), produced mixtures of sterile and single-spored asci. As h^- strains of the divergent biotypes were not available, within-group fertility could not be assessed. Recently, additional isolates in the same clade have been recovered in the vicinity of Kabarnet, Kenya, from small clavicorn beetles collected in morning glory flowers. Eight were assigned to *M. aberdeeniae* and

two were found to be conspecific with the divergent isolate from Tanzania. We now describe the latter as *Metschnikowia shivogae*, named in honour of the family of William Shivoga, Dean of the Faculty of Environment and Resources Development of Egerton University, Egerton, Kenya. Professor Shivoga and his family went far beyond the call of duty in the process of collecting the new yeast strains discussed in this paper.

Isolation and characterization

The strains used in this study are listed in Table 1. Collection details for the Tanzanian isolates were given by Lachance *et al.* (2006). The Kenyan collection was conducted at two nearby locations along the road to Cheremon Field Station of Egerton University, Marigat, which is located about 265 km north-west of Nairobi, near the town of Kabarnet. The sites were separated by 400 m. Ten samples were plated on acidified YM agar by allowing the insect to walk over the agar for 5–10 min. Three of the samples consisted of the meloid beetle *Mylabris tristigma* and gave rise only to unidentified moulds. The remainder were small clavicorn beetles tentatively assigned to the Nitidulidae. Drosophilids were also present in the same flowers. All yeasts were 'barcoded' by PCR amplification and rDNA sequencing as described previously (Lachance *et al.* 2005). Morphology and physiology were characterized by standard methods (Yarrow 1998). Mating reactions were obtained by mixing actively growing cells in pairs on

The GenBank/EMBL/DDBJ accession numbers for the rDNA sequences of strains SUB 04-310.1^T, UWOPS 07-204.1 and UWOPS 07-203.2 are respectively DQ266431, EU220026 and EU220027.

Table 1. Selected properties of *Metschnikowia* isolates from East Africa

The accession numbers are those in the yeast culture collection of the Department of Biology, Syracuse University, Syracuse, NY, USA (SUB), the culture collection of the Department of Biology, University of Western Ontario (UWOPS), the Centraalbureau voor Schimmelcultures, Utrecht, Netherlands (CBS), and the ARS-USDA Culture Collection, Peoria, IL, USA (NRRL). ^AAllotype.

Strain	Mating type	Source	Sequence type†
<i>Metschnikowia aberdeeniae</i>			
UWOPS 07-202.1	<i>h</i> ⁺	Kenya, 2007	A
UWOPS 07-202.3	<i>h</i> ⁺	Kenya, 2007	A
UWOPS 07-203.1	<i>h</i> [−]	Kenya, 2007	A
UWOPS 07-204.2	<i>h</i> ⁺	Kenya, 2007	A
UWOPS 07-205.1	<i>h</i> [−]	Kenya, 2007	A
UWOPS 07-207.1	<i>h</i> ⁺	Kenya, 2007	A
UWOPS 07-207.2	<i>h</i> ⁺	Kenya, 2007	A
UWOPS 07-208.1	<i>h</i> ⁺	Kenya, 2007	A
SUB 04-304.1*	<i>h</i> ⁺	Tanzania, 2004	A
SUB 05-213.1 ^T = CBS 10289 ^T = NRRL Y-27921 ^T	<i>h</i> [−]	Tanzania, 2005	A
SUB 05-213.2 ^A = CBS 10290 ^A = NRRL Y-27922 ^A	<i>h</i> ⁺	Tanzania, 2005	A
<i>Metschnikowia shivogae</i> sp. nov.			
SUB 04-310.1 ^T = CBS 10292 ^T = NRRL Y-27924 ^T	<i>h</i> ⁺	Tanzania, 2004	C1
UWOPS 07-203.2 ^A = CBS 10770 ^A = NRRL Y-48447 ^A	<i>h</i> [−]	Kenya, 2007	C2
UWOPS 07-204.1	<i>h</i> ⁺	Kenya, 2007	C3

*Species assignment previously uncertain (Lachance *et al.* 2006).

†Sequence types A, B and C were defined by Lachance *et al.* (2006) and refer to the ITS/5.8S and LSU D1/D2 rDNA regions. Subtypes C1, C2 and C3 were identified in this study (see text for details).

yeast carbon base agar supplemented with 0.01 % ammonium sulphate. Asci were detected at low magnification 1 day after mixing. The presence of ascospores was assessed after 2–3 days at higher magnifications. The strains were preserved in liquid nitrogen in the UWOPS yeast collection, Department of Biology, University of Western Ontario, and suitable vouchers were deposited in the collection of the Yeast Division of the Centraalbureau voor Schimmelcultures, Utrecht, Netherlands, and the ARS (NRRL) Culture Collection, Peoria, IL, USA, as indicated.

Species delineation

None of the Kenyan isolates had rDNA sequences of type B (Lachance *et al.* 2006), so it was not possible to improve our understanding of sexual fertility within that phylotype. The eight strains with type A sequences included representatives of both mating types. They were mixed in all possible combinations with the type and allotype of *M. aberdeeniae*, as well as strain SUB 04-304.1. The latter had been provisionally excluded from the species on the basis of the formation of mostly sterile asci. In this new experiment, we found that the proportion of asci containing two spores (as opposed to one or no spore) varied more as a function of individual strains and did not appear to be correlated with pairwise compatibility. Lachance & Bowles (2004) found the ability to produce a substantial proportion of two-spored asci to be a good estimate of spore viability in other large-spored *Metschnikowia* species.

We therefore recognize all strains with sequence type A as members of *M. aberdeeniae*. The Kenyan strains UWOPS 07-203.2 and UWOPS 07-204.1 were similar but not identical in ITS/5.8S and D1/D2 LSU rDNA sequences to the Tanzanian strain SUB 04-310.1^T (type C sequences). The three strains were mixed in all possible reciprocal combinations and with the type and allotype of *M. aberdeeniae*. Abundant two-spored asci were found in crosses between the strains with type C sequences, but crosses with the other two strains (type A) gave rise only to sterile asci interspersed with a few single-spored asci. On this basis, we conclude that the three type C strains represent a distinct species, which we name *Metschnikowia shivogae* sp. nov.

Some of the sequence differences among the three strains assigned to *M. shivogae* are of special interest. In the ITS1 region, sequences C1 and C3 have the doublets TT and GA, respectively, whereas sequence C2 has both (KW), indicating a possible recombination event involving the ribosomal repeats. In the ITS2 region, sequence C1 differs from the other two by one substitution and one insertion. In the D1 domain, it is sequence C3 that differs from the other two, by one substitution. Finally, the D2 domain of sequence C2 differs from the other two, also by one substitution. The topology of the tree presented in Fig. 1 reflects some of these differences. Additional sampling over a larger geographical area should yield interesting data as to the significance of rDNA polymorphisms in *M. shivogae*.

Fig. 1. Neighbour-joining phylogram depicting the relationships among strains of *M. shivogae* sp. nov. and related taxa based on ITS/5.8S and D1/D2 LSU rDNA sequences. The tree was rooted by the equal distance method. Bootstraps from 1000 pseudo-replicates with values of 50% or above are shown.

Morphology, physiology and identification

The cells and asci (Fig. 2) of *M. shivogae* are somewhat similar to those of *M. aberdeeniae*, but tend to resemble most those of species in the *Metschnikowia arizonensis* clade (Lachance & Bowles 2002, 2004; Lachance *et al.* 2006). This is of interest for the following reason. The floricolous beetle-associated large-spored *Metschnikowia* clade consists of four subclades, one of which contains the two African species. *M. arizonensis* and a few other species found in various Neotropical regions form another subclade (represented in Fig. 1 by *Metschnikowia colocasiae*). The largest subclade includes *Metschnikowia hawaiiensis* and a dozen close relatives. Last is a single species of Australian origin, *Metschnikowia hibisci*. Members of the *M. hawaiiensis* subclade tend to form the largest asci, with very long ribbon-like ascospores and a significant amount of periplasm, similar to what is seen in *M. aberdeeniae*. It therefore comes as a surprise that the asci of *M. shivogae* would instead resemble those found in the *M. arizonensis* clade, which are not as large and where much of the ascus volume is occupied by rather fusiform ascospores.

The growth responses are typical of most *Metschnikowia* species, although growth on some media is less vigorous. Specifically, growth on galactose, β -glucosides, mannitol, glucitol and D-glucosamine as carbon sources or lysine as nitrogen source, as well as glucose fermentation, were weaker compared with *M. aberdeeniae*. These differences are unsuitable for unambiguous identification, which should rely on mating tests or DNA sequencing.

Latin diagnosis of *Metschnikowia shivogae* Lachance, Bowles et Starmer sp. nov.

In medio YM *post dies tres* cellulae singulae, binae, aut in catenis brevis, ovoidae aut elongatae (2–3 $\times$ 4–9 μ m). *Cultura in agar*o malti *post dies 14* infima-convexa tumulosaque, glabra aut nitens, candida et butyrosa. *In agar*o carbonis *fundamento post dies 14* pseudomycelium nec mycelium verum non-formatur. *Post dies unus*, cellulae stirpum interfertiliu mixtarum in agar carbonis *fundamento tubi junctionis* formantur. *Post dies tres*, magni asci cylindrati possunt videri. Asci stabiles sunt. Ascosporae aculeatae (1.5–2 $\times$ 45–60 μ m). Glucosum (exigue) fermentatur. Glucosum, sucrosu, galactosu (exigue), trehalosu, maltosu, melezitosu, cellobiosu (exigue), salicinu (exigue), L-sorbosu, D-xylosu, ethanolu (exigue), glycerolu (lente), xylitolu, mannitolu (exigue), glucitolu (exigue), acidu succinicu (exigue), 2-ketogluconatu (lente) et N-acetylglucosaminu assimilantur, at non-inulinu, raffinosu, melibiosu, lactosu, methyl- α -D-glucosidu, amylu solubile, L-rhamnosu, L-arabinosu, D-arabinosu, D-ribosu, methanolu, 1-propanolu, 2-propanolu, 1-butanolu, erythritolu, ribitolu, galactitolu, meso-inositolu, acidu lacticu, acidu citricu, acidu gluconicu, glucono- δ -lactonu, glucosaminu, acetonu, ethyl acetu nec hexadecanu. Ethylaminu, lysinu (exigue) et cadaverinu assimilantur at non-natriu nitricu nec natriu nitrosu. Ad crescentiam vitaminae externae necessariae sunt. Augmentu in 32 $^{\circ}$ C, at non 34 $^{\circ}$ C. Habitat insectos e floribus *Ipomoea* spp. in Tanzania et Kenya. *Typus* SUB 04-310.1^T (h^{+}). *Allotypus* UWOPS 07-203.2 (h^{-}). In collectione zymotica Centraalbureau voor Schimmelcultures, Trajectum ad *Rhenum*, sub no. CBS 10292^T et CBS 10770 depositae sunt.

Description of *Metschnikowia shivogae* Lachance, Bowles et Starmer sp. nov.

Metschnikowia shivogae (shi.vo'gae. N.L. gen. masc. sing. n. *shivogae* of Shivoga, in honour of William Shivoga and his family, in recognition of their contribution to the discovery of this species).

On YM agar after 3 days at 25 $^{\circ}$ C, cells are ovoid to elongate, occur singly, in parent–bud pairs or in short

Fig. 2. Phase-contrast micrographs of asci of *Metschnikowia shivogae* after 2 days on YCBAS agar resulting from crosses between strains SUB 04-310.1^T and UWOPS 07-203.2 (a) and strains UWOPS 07-204.1 and UWOPS 07-203.2 (b). Bar, 10 μ m.

chains and measure $2\text{--}3 \times 4\text{--}9\text{ }\mu\text{m}$. After 2 weeks, colonies are low-convex and umbonate, semi-glossy to glossy, white and butyrous. In Dalmau plate culture on yeast carbon base agar after 2 weeks, pseudohyphae or true hyphae are not formed. On yeast carbon base agar at $18\text{ }^{\circ}\text{C}$, mixtures of cells of complementary mating types give rise to zygotes and asci after 12–16 h. After 3 days, mature asci containing two acicular ascospores ($1.5\text{--}2 \times 45\text{--}60\text{ }\mu\text{m}$) are formed in a persistent ascus that retains conspicuous vestiges of the conjugated parent cells (Fig. 2). Fermentation: in glucose, a small amount of gas develops after 10–12 days. Glucose, sucrose, galactose (weak), trehalose, maltose, melezitose, cellobiose (very weak), salicin (weak), sorbose, xylose, ethanol (very weak), glycerol (slow), xylitol, mannitol (weak), glucitol (weak), succinic acid (weak), 2-ketogluconic acid (slow) and *N*-acetyl-D-glucosamine are assimilated, but not inulin, raffinose, melibiose, lactose, methyl α -D-glucoside, starch, L-rhamnose, L-arabinose, D-arabinose, D-ribose, methanol, 1-propanol, 2-propanol, 1-butanol, erythritol, ribitol, galactitol, inositol, lactic acid, citric acid, gluconic acid, glucono- δ -lactone, D-glucosamine, acetone, ethyl acetate or n-hexadecane. Ethylamine, lysine (weak) and cadaverine are utilized as nitrogen sources, but not sodium nitrate or nitrite. No growth in the absence of vitamins. Grows in the absence of amino acids. Grows at $32\text{ }^{\circ}\text{C}$ but not at $34\text{ }^{\circ}\text{C}$. Gelatin and casein hydrolysis is negative. Hydrolysis of Tween 80 is positive. Acid production on chalk agar is negative. Grows in the presence of 10% NaCl but not 15%. Growth in the presence of 50% glucose is slow. Growth in the presence of 10 mg cycloheximide l^{-1} is negative. Growth in the presence of 75 mg CTAB l^{-1} is positive. Starch production and diazonium blue B reaction are negative.

The known habitat is in insects associated with flowers of *Ipomoea* spp. in Tanzania and Kenya. The type strain is strain SUB 04-310.1^T (h^{+}) (=CBS 10292^T =NRRL Y-27924^T), recovered from a specimen of the meloid beetle *Mylabris tristigma* collected in Mugumu, Tanzania, near the Serengeti National Park. The allotype is strain UWOPS

07-203.2 (h^{-}) (=CBS 10770 =NRRL Y-48447), isolated from a clavicorn beetle collected near Kabarnet, Kenya. The name is registered under MycoBank number MB 511466.

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