Metschnikowia hawaiensis sp. nov., a Heterothallic Haploid Yeast from Hawaiian Morning Glory and Associated Drosophilids

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A new haploid, heterothallic yeast species was isolated repeatedly from morning glory (Ipomoea acuminata) flowers and from two associated drosophilid species, Scaptomyza calliginosa and Drosophila florica, in a Hawaiian kipuka. Haploid strains of this organism multiply asexually by budding and, under nutrient deprivation, by the formation of long germ tubes that develop into branching true mycelia. Mating compatibility is controlled by two alleles of a single locus. Plasmogamy between compatible strains is followed by the development of very large elongate ascii bearing vestiges of the zygotides and the formation in each ascus of two unusually large aciculate ascospores similar to those formed by members of the genus Metschnikowia. Membership in the genus Metschnikowia is supported by the physiological profile of the yeast, which is typical of the genus but not identical to the profile of any previously described species. The name Metschnikowia hawaiensis is proposed to emphasize the geographic origin of the new species, not its habitat, which has not been determined precisely. The holotype strain of M. hawaiensis is strain UWO(PS) 87-2167.2 (= ATCC 76059 = CBS 7432), and the isotype strain is strain UWO(PS) 87-2201.2 (= ATCC 76058 = CBS 7433).

The morning glory Ipomoea acuminata (Vahl) R & S and the two drosophilid flies Scaptomyza calliginosa Hardy and Drosophila florica, Sturtevant constitute a unique ecosYSTEM that is apparently restricted to a single Hawaiian locality (7). Bird Park, which is located near the Kilauea Crater on the Island of Hawaii, is a kipuka, that is, a characteristic island (literally hole) of vegetation amid recent lava flows. The vegetation is a diverse combination of hardwood trees, bushes, grasses, and large patches of morning glories. Soon after opening, the short-lived flowers are visited by a number of insects, in particular two drosophilid fly species which colonize the corollas, ultimately ovipositing and leaving. Usually one larva (occasionally several larvae) develops in each closed flower, presumably feeding on yeasts that were inoculated by the visiting flies (3).

Four yeast species dominate this complex habitat (3). The most abundant is a new species that has been isolated in large numbers from the flies and is provisionally referred to as Candida species L because of its powerful lipolytic activity, which contrasts with an otherwise restricted carbon compound assimilation spectrum. The morning glory flowers abound with a form of Metschnikowia reukaufii that differs from the more usual isolates of this species by the formation of dry, convoluted colonies on agar media. Two other species are found in smaller numbers and are distributed more or less equally among flies and flowers. One of these has been identified as Candida azyma, and the other is a new, heterothallic, haplontic Metschnikowia species. In this paper the name Metschnikowia hawaiensis is proposed for this new species.

MATERIALS AND METHODS

Yeast isolation and identification. Yeasts were isolated from flowers by direct streak plating of inner corolla scrapings onto acidified YM agar (Difco Laboratories, Detroit, Mich.) and from insects by allowing single flies to walk on plates for 12 h. Yeasts were identified by using standard methods (11). Growth responses were studied by replica plating. The strains have been maintained in YM agar (Difco) and 10% glycerol in liquid nitrogen.

Nucleic acid analyses. Nuclear DNA base compositions were determined by using the buoyant density method in CsCl as described by Price et al. (9), except that the buoyant density of the Micrococcus luteus reference DNA was calculated to be 1.7310 rather than 1.7311 g/cm3. DNA extraction, purification, and reassociation were carried out by using the procedures of Price et al. (9), except that purified DNA was concentrated by electrophoresis in a concentrator (ISCO, Lincoln, Nebr.) and the reference DNA was labeled by using the random primed labeling method developed by Feinberg and Vogelstein (1, 2). This method involves incubating purified, sheared, denatured DNA (the template) with a mixture of all possible hexanucleotides containing [32P]dATP and labeling-grade Klenow enzyme, the large fragment of DNA polymerase I. This enzyme lacks 5'-3' exonuclease, which would rapidly degrade the primer and then begin to degrade the labeled cDNA product. We found that at the recommended incubation temperature (room temperature or higher) for the labeling reaction (2), very high values for zero-time binding (9) of the tracer DNA were obtained (30 to 40%). This appeared to be due to the formation of snapback hairpin loops (8), and the resulting tracer then behaved like double-stranded DNA on hydroxyapatite during separation of duplexed DNA. However, when the reaction mixture was incubated at 14 to 15°C for 1 h (8, 10), the level of zero-time binding was reduced to 7 or 8% and the tracer DNA was used as such. The labeling procedure which we used has several advantages over the ionization procedure used previously. The main advantage is that the reference DNA to be labeled does not have to be banded in CsCl for ultimate purity prior to labeling. Specific activities close to 108 dpm/mg can be obtained with minimum quantities of template DNA (0.1 µg or less), dATP, dCTP, dGTP, dTTP, and Klenow enzyme were purchased from Boehringer Mannheim Biochemicals, Indianapolis, Ind.; [32P]dATP was obtained from Amersham Corp., Arlington.
RESULTS

A total of 19 strains that putatively belonged to the new species were isolated, but only 13 were maintained in a viable state long enough to preserve them permanently in liquid nitrogen and conduct characterization studies. The designations, mating types, and isolation sources of these strains are shown in Table 1.

**Latin diagnosis of Metschnikowia hawaiiensis** sp. nov. In medio liquido post dies 3 cellulae singulae, binae, aut in catenis brevis; cellulae ovoideae (2.4 x 4.6 μm). Post unum mensem sedimentum formatur. Cultura in agaro malti post dies 14 (17°C), magna, infimo-convexa, tumulosa, glabra, candida, et butyrosea. Interdum cellulae cum extuberatione formantur.

**Description of Metschnikowia hawaiiensis.** In 0.5% yeast extract–2% glucose broth after 3 days at 25°C, the cells are short ovoidal to ovoidal, occur singly, in parent-bud pairs, or as parent cells with several buds, and measure 2 to 4 by 6 μm (Fig. 1A). A sediment is formed after 1 month.

On malt agar after 2 weeks at 17°C, colonies are large, low convex to umbonate, glabrous, smooth, white, and butyros. Some cells develop long tubular outgrowths instead of mycelium (Fig. 2 through E). After 1 day, tubular asci, some more than 200 μm long, are formed (Fig. 3). Most asci retain signs of the original conjugating pair of parent cells. Two large acicular ascospores are produced per mature ascus. Evanescentia is not observed.

In fermentation tests a full tube of gas develops in about 5 days with glucose or trehalose.

The following carbon compounds are assimilated: sucrose, galactose, trehalose, maltose, melezitose, cellobiose, salicin, L-sorbos, D-xyllose, ethanol, glycerol, ribitol (exigue), xylitol, mannitol, glucitol, acidum succinicum, acidum citricum, acidum malicum (exigue), acidum gluconum (exigue aut lente), glucono-δ-lactonum (lente), 2-ketogluconatum, glucosamarinum (variable et exigue), N-acetylglucosaminum, acidum tannicum, et hexadecanum (vel exigue) assimilantur, at non inulimum, raffinosum, melibiosum, lactosum, methyl-α-D-glucosidum, amyllum solubile, L-rhamnosum, L-arabinosum, L-arabinosum, D-rabinosum, D-rubinosum, methanolum, 2-propanolum, 1-butanolum, erythritolum, galactitolum, meso-inositolum, acidum lacticum, acetonum, nec ethyl acetat.

Ethylaminum, lysinum, et cadaverinum assimilantur at non natrium nitricum nec natrium nitrosum.

Ad crescendum vitaminea externae necessariae sunt. Augmentum in 30°C at non in 37°C.

**Table 1.** Strains of M. hawaiiensis, their mating types, and isolation sources

<table>
<thead>
<tr>
<th>Strain #</th>
<th>Mating type</th>
<th>Isolation source</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>UWO(PS) 87-2164.2</td>
<td>h</td>
<td>Morning glory flower</td>
<td>Senescent flower</td>
</tr>
<tr>
<td>UWO(PS) 87-2167.2</td>
<td>h⁺</td>
<td>Morning glory flower</td>
<td>Senescent flower</td>
</tr>
<tr>
<td>UWO(PS) 87-2168.2</td>
<td>h⁻</td>
<td>Morning glory flower</td>
<td>Senescent flower</td>
</tr>
<tr>
<td>UWO(PS) 87-2199.2</td>
<td>h⁻</td>
<td>S. calliginosa male</td>
<td>Senescent flower</td>
</tr>
<tr>
<td>UWO(PS) 87-2202.1</td>
<td>h⁻</td>
<td>S. calliginosa female</td>
<td>Weak mating reaction</td>
</tr>
<tr>
<td>UWO(PS) 87-2203.2</td>
<td>h⁻</td>
<td>D. florica female</td>
<td>Weak mating reaction</td>
</tr>
<tr>
<td>UWO(PS) 87-2210.3</td>
<td>h⁺</td>
<td>S. calliginosa male</td>
<td>Weak mating reaction</td>
</tr>
<tr>
<td>UWO(PS) 87-2218.2</td>
<td>h⁻</td>
<td>Morning glory flower</td>
<td>Young flower</td>
</tr>
<tr>
<td>UWO(PS) 87-2245.1</td>
<td>h⁺</td>
<td>S. calliginosa female</td>
<td>Weak mating reaction</td>
</tr>
<tr>
<td>UWO(PS) 87-2249.1</td>
<td>h⁻</td>
<td>S. calliginosa female</td>
<td>Weak mating reaction</td>
</tr>
<tr>
<td>UWO(PS) 87-2249.3</td>
<td>h⁻</td>
<td>S. calliginosa male</td>
<td>Weak mating reaction</td>
</tr>
<tr>
<td>UWO(PS) 87-2263.1</td>
<td>h⁺</td>
<td>Morning glory flower</td>
<td>Senescent flower</td>
</tr>
<tr>
<td>UWO(PS) 87-2287.2</td>
<td>h⁻</td>
<td>Morning glory flower</td>
<td>Senescent flower</td>
</tr>
</tbody>
</table>

* a UWO(PS), University of Western Ontario Department of Plant Sciences Culture Collection, London, Ontario, Canada.

b Isotype strain.
FIG. 1. Phase-contrast micrographs of vegetative growth of *M. hawaiiensis*: budding (A and C), protuberance formation (A through C), and hyphal growth (C and D).

Growth does not occur in vitamin-free medium.
Growth occurs in amino-acid-free medium.
Growth occurs at 30°C but not at 37°C.
Acid formation on chalk agar is weak or negative.
Urease activity is negative.
Gelatin liquefaction and casein hydrolysis are negative.
Lipolytic activity on Tween 80 agar is weak.
Amyloid compounds are not produced.
Growth occurs on 50% glucose-yeast extract agar.
Growth occurs slowly on YM agar supplemented with 15% sodium chloride.
Growth does not occur in the presence of 10 μg of cycloheximide per ml.
Growth occurs in the presence of 16 μg of digitonin per ml.

The guanine-plus-cytosine content of the nuclear DNAs of strains UWO(PS) 87-2167.2T (T = type strain) and UWO(PS) 87-2203.2 is 46.6 mol%.

The habitat is morning glory (*I. acuminata*) flowers and associated drosophilids on the island of Hawaii.

The holotype strain of *M. hawaiiensis*, strain UWO(PS) 87-2167.2, was isolated from the corolla of a senescent morning glory (*I. acuminata*) flower in Bird Park on the island of Hawaii. Its mating type is arbitrarily designated h⁺.

The isotype strain, strain UWO(PS) 87-2203.2, was isolated from a female *D. floricola* specimen that was collected from a morning glory flower in the same locality. These strains have been deposited in the Collection of the Yeast Division of the Centraalbureau voor Schimmelcultures, Delft, The
FIG. 2. Phase-contrast micrographs of ascus formation in *M. hawaiiensis*: formation of conjugation tube (A), early (B) and late (C) stages of isogamous conjugation, and ascal development from a zygote (D and E).

Netherlands, as strains CBS 7432^T^ and CBS 7433, respectively, and in the American Type Culture Collection, Rockville, Md., as strains ATCC 76059^T^ and ATCC 76058, respectively.

The epithet of *M. hawaiiensis* (ha.wai.i'en'sis, L. nom. fem. adj. hawaiiensis, of Hawaii) refers to the island where the yeast was isolated.

**DISCUSSION**

The morphology of the vegetative cells and asci and the physiological profile of the yeast which we studied are consistent with its being a typical *Metschnikowia* species, but assignment to one of the previously described species was not possible because of several differences between the

FIG. 3. Phase-contrast micrographs of mature asci of *M. hawaiiensis*. (A) Two aciculate ascospores that clearly intersect each other near the center of the ascus. (B) Large, two-spored ascus shown against a background of vegetative cells for size comparison.
morning glory strains and each of the previously described taxa (Table 2). \textit{M. hawaiiensis}, like \textit{Metschnikowia australis}, occurs in nature as separate haploid mating types. It exhibits strong assimilation of citric acid, a response which is occasionally observed in \textit{Metschnikowia pulcherrima}. The dimensions of the mature ascii of \textit{M. hawaiiensis} are remarkable, but not completely unprecedented, since \textit{Metschnikowia bicuspidata} has been known to produce ascii that are up to 50 \(\mu\)m long. Every strain of the new species grew rather strongly on 50% glucose, a characteristic found sporadically in strains of other terrestrial species and also in \textit{Metschnikowia zobellii}, a marine species. Overall, the characteristics of \textit{M. hawaiiensis}, including DNA base composition (Table 3), suggest that this organism is most closely related to \textit{M. pulcherrima}, but these two species differ in ploidy, in assimilation of \(\alpha\)-methylglucoside, and in formation of chlamydospores (the last two characteristics are almost invariably positive in \textit{M. pulcherrima} but are negative in all strains of \textit{M. hawaiiensis}). To obtain conclusive evidence that \textit{M. hawaiiensis} represents a new species, a DNA-DNA hybridization experiment was designed to compare \textit{M. hawaiiensis} with two other terrestrial species isolated from flowers, \textit{M. pulcherrima} and \textit{M. reukaufii}. \textit{Metschnikowia lunarosa} was not included because its cell morphology is unique. The aquatic species were also omitted because Mendonça-Hagler et al. (4) observed no close relationship between species belonging to the aquatic and terrestrial groups of the genus \textit{Metschnikowia}; this conclusion was also supported by the data of Meyer and Phaff (5), who used labeled \textit{M. pulcherrima} DNA. Table 3 shows that the DNAs of the two mating types of \textit{M. hawaiiensis} had a reassociation value of 100% but only insignificant relative binding values with DNAs from the other two \textit{Metschnikowia} species. \textit{Pichia opuntiae} was used as a negative control. The habitat of \textit{M. hawaiiensis} is uncertain. It was isolated in low and relatively equal numbers from morning glory flowers and associated drosophilid flies, in contrast with \textit{M. reukaufii}, which occurs in very high numbers in older morning glory flowers but in low numbers in flies (3). The flies may be the hosts of the new species, or alternatively they may serve as vectors between the morning glories and other plants which are the actual hosts.

### ACKNOWLEDGMENTS

This work was supported by grants from the Natural Science and Engineering Research Council of Canada (to M.-A.L.) and from the National Science Foundation (to W.T.S. and H.J.P.). We are indebted to John R. Blue for his expert technical assistance with the DNA base composition determinations and DNA reassociation experiments. Field assistance by Jane Bowles and David Droney and the kind cooperation of the staff at the Volcano Research Station are gratefully acknowledged.

### LITERATURE CITED


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**TABLE 2.** Diagnostic properties of \textit{Metschnikowia} species and varieties\(^{a}\)

| Taxon         | Assimilation of: | Growth on 50% glucose agar | Glucose fermentation | No. of spores | Sexuality | Ploidy | Chlamydosporae | Habitat     | Guanine plus- cytosine content (mo%)
<table>
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</tr>
</thead>
<tbody>
<tr>
<td>\textit{M. krissii}</td>
<td>-(^{b})</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>?</td>
<td>2N</td>
<td>Aquatic</td>
<td>45.4</td>
</tr>
<tr>
<td>\textit{M. zobellii}</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>or + (w)</td>
<td>+</td>
<td>1</td>
<td>?</td>
<td>2N</td>
</tr>
<tr>
<td>\textit{M. bicuspidata}</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>2</td>
<td>Homothallic</td>
<td>2N</td>
</tr>
<tr>
<td>\textit{M. reukaufii}</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>v</td>
<td>-</td>
<td>2</td>
<td>Heterothallic</td>
<td>2N</td>
</tr>
</tbody>
</table>

\(^{a}\) Data from references 4 and 6 and this study.

\(^{b}\) +, Positive; -, negative; v, variable; (w), weak; (s), slow.

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**TABLE 3.** Nuclear DNA base compositions and results of reannealing between \(^{32}\)P\(\)DNA from \textit{Metschnikowia} UWO(PS) 87-2167.2\(^{a}\) and DNAs from four yeast strains and calf thymus\(^{a}\)

| Organism or tissue\(^{b}\) | Guanine- plus-cytosine content (mo%)\(^{a}\) | Actual binding (%)\(^{a}\) | Relative binding (%)\(^{a}\)
<table>
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<tbody>
<tr>
<td>\textit{M. hawaiiensis} UWO(PS) 87-2167.2(^{a})</td>
<td>47</td>
<td>77</td>
<td>(100)</td>
</tr>
<tr>
<td>\textit{M. hawaiiensis} UWO(PS) 87-2203.2</td>
<td>47</td>
<td>78</td>
<td>100</td>
</tr>
<tr>
<td>\textit{M. reukaufii} UCD-FST 62-331(^{T})</td>
<td>41</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>\textit{M. pulcherrima} UCF-FST</td>
<td>46</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>\textit{P. opuntiae} UCD-FST 84-860.2</td>
<td>34</td>
<td>-1</td>
<td>0</td>
</tr>
<tr>
<td>Calf thymus</td>
<td>-1</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

\(^{a}\) \(^{32}\)P\(\)DNA (<0.1 \(\mu\)g) and unlabeled DNA (200 \(\mu\)g) were incubated for 25 h in 45 \(\mu\)l of 280 mM phosphate buffer (pH 6.8) at 65°C.

\(^{b}\) UCD-FST, Culture Collection of the Department of Food Science and Technology, University of California, Davis.

\(^{c}\) Average of three or more buoyant density determinations.

\(^{d}\) Average for three samples corrected for zero-time binding (8%) and for self-renaturation (4%) of labeled DNA (9).

\(^{e}\) Data from reference 4.