Antonie van Leeuwenhoek (2007) 92:37–42
DOI 10.1007/s10482-006-9132-4

ORIGINAL PAPER

# *Candida gelsemii* sp. nov., a yeast of the Metschnikowiaceae clade isolated from nectar of the poisonous Carolina jessamine

**Jessamyn S. Manson · Marc-André Lachance · James D. Thomson**

Received: 2 October 2006 / Accepted: 26 October 2006 / Published online: 5 January 2007


**Abstract** A new yeast species, *Candida gelsemii*, is described to accommodate three isolates recovered in Georgia, USA, from the toxic nectar of the Carolina jessamine (*Gelsemium sempervirens*). The species resembles other members of the Metschnikowiaceae clade that have been recovered from nectar, but differs in a number of morphological and physiological characteristics. Analysis of rDNA sequences places the new species well into the clade, but in a basal position with respect to a group of *Metschnikowia* and *Candida* species known to occur in association with nectars and bees, as well as marine invertebrates. The type is strain UWOPS 06–24.1$^{T}$ (CBS 10509$^{T}$, NRRL Y-48212$^{T}$.

**Keywords** *Candida gelsemii* · Metschnikowiaceae · *Gelsemium sempervirens* · Nectar alkaloids · Gelsemine · New yeast species

J. S. Manson · J. D. Thomson
Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, Ontario, Canada M5S 3G5

M.-A. Lachance (✉)
Department of Biology, University of Western Ontario, London, Ontario, Canada N6A 5B7
e-mail: lachance@uwo.ca

## Introduction

Floral nectars often contain yeasts that are vectored by pollinating and non-pollinating insects (Lachance et al. 2001; Rosa et al. 2003; Brysch-Herzberg 2004). The role of the yeasts in this ecosystem is poorly understood, but it is clear that the composition of the yeast community is highly dependent on the types of insects involved. Whereas nitidulid beetles carry yeasts with affinities in the genera *Metschnikowia, Kodamaea*, and *Wickerhamiella*, bees tend to vector other yeasts related to the genera *Metschnikowia* and *Starmerella*. Even within the Metschnikowiaceae clade, the species associated with nitidulids and those associated with bees are not the same. It was therefore of interest to examine the nectar of the Carolina jessamine (*Gelsemium sempervirens*). This perennial vine is endemic to the southeastern United States. It is also a distylous species, making it an obligate outcrosser. It blooms in the early spring, producing a multitude of fragrant tubular yellow flowers which attract a diverse range of pollinators including eusocial, solitary, and nectar-robbing bees (Ornduff 1970; Adler and Irwin 2005, 2006). Most importantly, the nectar of this plant contains gelsemine, an alkaloid that is highly toxic to vertebrates (Burrow and Tyrl 2001). The alkaloid, presumed to be a deterrent for herbivores, is reported to deter pollinators and nectar robbers at natural

concentrations (Adler and Irwin 2005). Adaptive hypotheses for the presence of alkaloids in floral nectar include providing the nectar with antimicrobial properties to prevent the proliferation of organisms such as floral yeasts (Adler 2000).

In the course of determining whether the yeast community of the nectar is also affected by gelsemine, we sampled nectars from *G. sempervirens* and also some sympatric azaleas that appeared to have a similar array of bees foraging for nectar. At the time of collecting, both plant species were visited by queens of the native bumblebees *Bombus impatiens* and *B. bimaculatus*, as well as introduced honeybees (*Apis mellifera*). In the process, strains of *Metschnikowia reukaufii* were recovered from azalea nectar whereas the jessamine nectars yielded strains of *Candida rancoensis* as well as a new asexual species with metschnikowiaceous affinities, which we now describe as *Candida gelsemii*.

## Material and methods

Nectar samples were collected on March 30$^{th}$ and 31$^{st}$ 2006 in four sites located near the campus of Georgia Southern University in Statesboro, Georgia. Other isolation details are given in Table 1. In each case, approximately 2µl of nectar was placed onto a plate of YM agar supplemented with 100 mg/l chloramphenicol and the nectar was streak–diluted with a sterile loop. Mould colonies were removed periodically with a knife. The plates were returned to the laboratory (UWO) and colonies were picked for purification and identification by rDNA partial sequencing (Kurtzman and Robnett 1998). Sequence editing, alignment, and analysis were conducted with DNAMAN version 4.1. The sequences were queried against the GenBank database, using the Megablast algorithm of Zhang et al. (2000). Strain characterization followed standard methods (Yarrow 1998). Growth responses were determined by replica plating as detailed by Lachance (1987). Replica plating was also used to evaluate the effect of gelsemine on yeast growth. The potential effect of gelsemine on yeasts was also assessed by agar diffusion. An ethanol solution of gelsemine was added to sterile discs of Whatman 3 mm paper so that each disk contained 35, 3.5, and 0.35µg, respectively. The air-dried discs were then placed individually on the surface of YM agar plates inoculated with dilute yeast suspensions. The plates were examined periodically for evidence of inhibition zones.

**Table 1** Summary of yeasts recovered from nectar of flowers obtained in the vicinity of Georgia Southern University campus in Statesboro, GA

| Yeast species | Strain number | Plant species (*N*) | Locality |
|---|---|---|---|
| *Candida gelsemii* | 06–11.1 | *Gelsemium sempervirens* (34) | 25.5 km east of campus |
| | 06–17.1 | | 25 km east of campus |
| | 06–24.1 | | 1.6 km east of campus |
| *Candida rancoensis* | 06–22.1 | | |
| | 06–26.1 | | |
| *Metschnikowia reukaufii* | 06–29.1 | Horticultural azalea (6) | University campus |
| | 06–32.1 | | |

## Results and discussion

### Species delineation, phylogenetic placement and phenotypic variability

The three isolates of *Candida gelsemii* were similar but not identical in sequences, morphology, and growth responses. The ribosomal internal transcribed spacer (rDNA ITS) sequences of strains 06–17.1 and 06–24.1 differed by a single indel and that of strain 06–11.1 differed from the other two by eight substitutions and one or two indels. However, the nearly identical large subunit rDNA D1/D2 regions (strain 06–11.1 differs by a single substitution in the D1 domain) and a comparison of morphology and physiology within the greater context of other related yeast species supports assigning the isolates to a single species. Extensive attempts to obtain evidence of a sexual cycle were not successful, and so a biological species concept cannot be applied at present. A Megablast search of the GenBank database using the D1/D2 domains identified the nearest known relative as *Metschnikowia bicuspidata*, with a

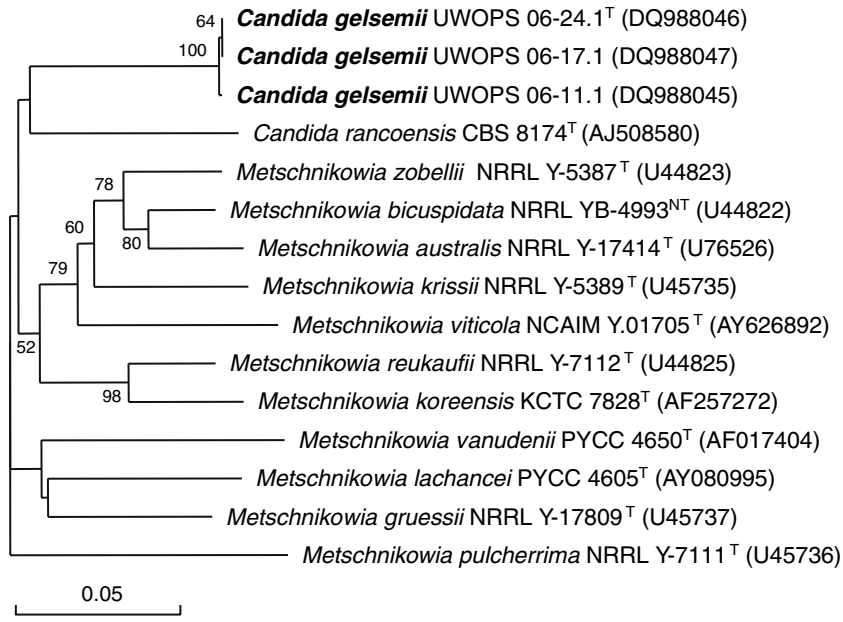


**Fig. 1** Phylogram of *Candida gelsemii* and closest relatives based on a neighbour–joining analysis (K2P transform) of D1/D2 LSU rDNA sequences. Bootstrap values (1000 pseudoreplications) of 50% or greater are shown. Strain numbers and sequence accession numbers are given. The superscripts identify type (T) and neotype strains (NT)

divergence of 57 substitutions. Fig. 1 shows that the new species occupies a somewhat basal position with respect to that group, which contains a number of species that are often isolated from nectars or other plant components, as well as a small subclade of species thought to be parasitic on certain aquatic invertebrates (*i.e., M. bicuspidata* and allies; Miller and Phaff 1998). Sisterhood of *C. gelsemii* and *C. rancoensis* is not well supported by the data. However, addition of any other species to the sequence analysis did not alter the presumed monophyly of the ingroup species included in Fig. 1 (*Metschnikowia pulcherrima* was the outgroup).

Considering the narrow distribution of the known isolates of *C. gelsemii*, their phenotypic variation is significant. As shown in Fig. 2, our attempts to obtain ascus formation on dilute V8 agar, although unsuccessful, demonstrated that the three strains are distinct with respect to cell size and propensity to differentiate into chlamydospores that are often referred to as ''pulcherrima cells'', in reference to the resting, pre-ascal cells formed by *M. pulcherrima* (Miller & Phaff 1998). The large lipid globules seen in strain 06–24.1 are most typical of this. The formation of bilobate lipid globules by some of the cells is not typical, however. The variation observed at the physiological level, detailed in Table 2, was not obviously correlated with the extent of divergence in ITS sequences.

Physiologically, *C. gelsemii* superficially resembles phylogenetic congeners such as *Metschnikowia lachancei* and *Metschnikowia vanudenii*, and to a lesser extent *M. bicuspidata* and *Metschnikowia gruesii*, most of which have been isolated from in floral nectars (Miller & Phaff 1998; Giménez-Jurado et al. 2003). *M. bicuspidata* is of marine origin. The most important differences from other nectar isolates were the weak or negative growth at 30°C or in the presence of 50% glucose, and the rather weak fermentation. Such differences would not constitute strong key characters for identification.

## Ecology

The presence of a highly potent toxic alkaloid in the nectar of *G. sempervirens* might constitute a selective factor that favours the presence of resistant yeast species over the more frequently isolated nectar species such as *Metschnikowia reukaufii*. To test the hypothesis that gelsemine might be such a niche determinant, we tested its effect on the growth of the yeasts listed in Table 1 as well as others, using two approaches. Two

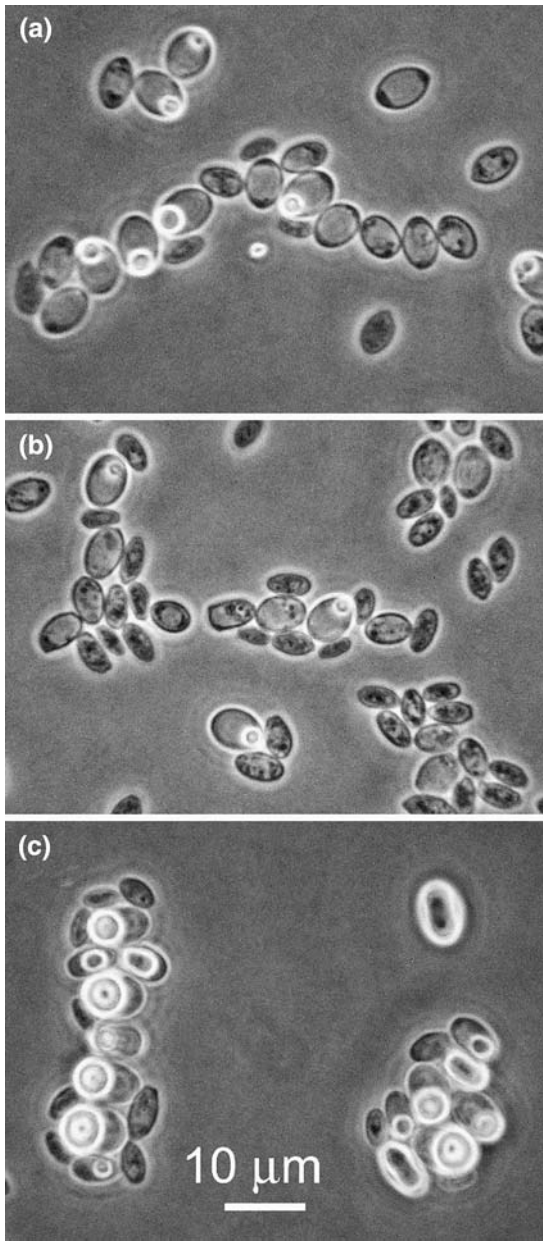


**Fig. 2** *Candida gelsemii* after 1 month on dilute V8 agar (1/20) at 18°C. Strains 06–11.1 (a), 06–17.1 (b), and 06–24.1 (c), showing various degrees of differentiation into ''pulcherrima cells''. Asci were not formed on these and other sporulation media

concentrations of synthetic gelsemine, 100 ng/µl and 250 ng/µl, were added to YM agar and used in the replica plate series used to characterize the isolates as well as others that came from nectar of a tropical palm. These concentrations simulate

**Table 2** Growth responses that exhibit variation among the three known strains of *C. gelsemii*

| Growth test | Strain | | |
|---|---|---|---|
| | 06–11.1 | 06–17.1 | 06–24.1$^T$ |
| Galactose | w | – | – |
| Trehalose | – | – | w |
| Cellobiose | s | – | s |
| Salicin | w | – | w |
| Succinic acid | – | w | – |
| Glucosamine | w | – | – |
| 30°C | w | – | – |
| 10% NaCl | s | + | s |

levels of gelsemine that occur in the nectar of natural *G. sempervirens* populations and are known to deter several species of flower visitors, including the bumble bee *B. impatiens*, an important pollinator of *G. sempervirens* (Adler and Irwin 2005, 2006, Manson, personal observation). To ensure that these concentrations were neither insufficient nor excessive, disks impregnated with gelsemine were also applied to lawns of yeast on agar. The species tested included *M. pulcherrima, M. reukaufii, Debaryomyces melissophilus*, and *Starmerella bombicola*. In all cases, no significant effect was detected, indicating that neither yeasts from jessamine nectar nor those from the nectar of other plants experienced reduced growth due to the presence of gelsemine. The mechanism of tolerance to this alkaloid is unknown but appears to be generalized within a broad range of yeasts and suggests that predictions of the toxicity of nectar alkaloids to microbial communities may be incorrect (Adler 2000). We propose that perhaps, the yeasts found in jessamine nectar may instead act as a detoxifying agent selectively carried by visitors to that plant as a co-evolved adaptation. We hope to test that hypothesis in the future.

*Candida gelsemii* Lachance sp. nov.

In medio liquido, post dies 3, cellulae ovoidae, singulae aut binae gemmationis causa (3–7 × 5–10µm). Annulum nullum et pellicula nulla. In medio agaro, coloniae infimo-convexae, tumulosae, cum marginem integram. Superficia glabra aut papillata aut perforata. Catenae simplices cellularum formantur. Asci non formantur. Glucosum fermentatur (exigue). Glucosum, sucrosum,

maltosum, melezitosum, cellobiosum (variabile aut lente), salicinum (variabile aut exigue), glycerolum (exigue), glucitolum (exigue), acidum gluconicum, glucono-Δ-lactonum (exigue), N-acetyl glucosaminum et hexadecanum (exigue) assimilantur, at non inulinum, raffinosum, melibiosum, galactosum (aliquando exigue), lactosum, trehalosum (aliquando exigue), α-methyl-D-glucosidum, sorbosum, rhamnosum, xylosum, L-arabinosum, D-arabinosum, ribosum, methanolum, 1-propanolum, 2-propanolum, 1-butanolum, erythritolum, ribitolum, xylitolum, galactitolum, mannitolum, inositolum, acidum lacticum, acidum succinicum (aliquando exigue), acidum citricum, acidum malicum, 2-keto-gluconatum, nec glucosaminum (aliquando exigue). Ethylaminum, L-lysinum et cadaverinum assimilantur at non nitratum nec nitritum. Ad crescentiam vitamina necessaria sunt. Augmenta ad 4°C (exigue), 24°C; 30°C variabile. Habitat nectarum *Gelsemium sempervirens*, Georgia, USA. Typus, stirps UWOPS 06–24.1$^{T}$. In collectione zymotica Centraalbureau voor Schimmelcultures, Trajectum ad Rhenum, sub no. CBS 10509$^{T}$ deposita est.

Description of *Candida gelsemii* Lachance sp. nov.

In 2% glucose 0.5% yeast extract after 3 days, the cells are ovoid, occur singly or in bud-mother cell pairs, and measure 3–7 × 5–10μm. Neither a ring nor a pellicle is formed. On agar media, the colonies are low-convex, slightly umbonate with an entire margin. The surface is glabrous and can be papillate or pitted. On Dalmau plates with YCB agar supplemented with 0.01% ammonium sulfate, after 2 weeks, a few chains of undifferentiated cells may be formed. The cultures were examined individually or mixed in pairs on YCB agar with 0.01% ammonium sulfate and dilute (1/20) V8 agar. Mating or ascus formation were not observed. Resting cells with conspicuous lipid globules may be formed on dilute V8 (Fig. 2). Glucose is fermented weakly. Glucose, sucrose, maltose, melezitose, cellobiose (variable or slow), salicin (variable or weak), glycerol (weak), glucitol (weak), gluconic acid, glucono-Δ-lactone (weak), N-acetyl glucosamine, and hexadecane (weak) are assimilated, but not inulin, raffinose, melibiose, galactose (sometimes weak), lactose, trehalose (sometimes weak), α-methyl-D-glucoside, sorbose, rhamnose, xylose, L-arabinose, D-arabinose, ribose, methanol, 1-propanol, 2-propanol, 1-butanol, erythritol, ribitol, xylitol, galactitol, mannitol, inositol, lactic acid, succinic acid (sometimes weak), citric acid, malic acid, 2-keto-gluconic acid, or glucosamine (occasionally weak). Ethylamine, L-lysine, and cadaverine are utilized as sole nitrogen sources, but not nitrate or nitrite. Growth in vitamin-free medium negative. Growth in amino acid-free medium positive. Growth at 4°C weak, at 24°C positive, at 30°C negative or weak. Gelatin hydrolysis weak. Casein hydrolysis positive. Tween 80 hydrolysis positive. Acid production on chalk agar negative. Growth in YM agar with 10% NaCl positive or slow; 15% negative. Growth in 50% W/W glucose, 1% yeast extract agar negative. Growth in the presence of 10 mg/L cycloheximide negative. Growth in the presence of 75 mg/L CTAB positive. Starch production negative. Diazonium Blue B reaction negative. The habitat is nectar of *Gelsemium sempervirens* in Georgia, USA. The type culture is strain UWOPS 06–24.1$^{T}$ isolated from nectar. The type was deposited in the culture collection of the Centraalbureau voor Schimmelculture, Utrecht, the Netherlands (CBS 10509, = NRRL Y-48212). Mycobank 46490.

*Etymology*: gel.se'mi.i, L. gen. sing. neut. n., gelsemii, of *Gelsemium*, referring to the plant from which the isolates were obtained.

**Acknowledgements** This work was funded by grants from the Natural Science and Engineering Research Council of Canada (MAL and JDT). We thank Sheila Colla and Erin Willis for their assistance during field collections, and Lissa Leege for directing us towards our field sites.

## References

Adler LS (2000). The ecological significance of toxic nectar. Oikos 91:409–420

Adler LS, Irwin RE. (2005) Ecological costs and benefits of defenses in nectar. Ecology 6:2968–2978

Adler LS, Irwin RE (2006) Comparison of pollen transfer dynamics by multiple floral visitors: Experiments with pollen and fluorescent dye. Annals of Botany 97:141–150

Brysch-Herzberg M (2004) Ecology of yeasts in plant–bumblebee mutualism in Central Europe. FEMS Microbiol Ecol 50:87–100

Burrow GE, Tyrl RJ (2001) Toxic Plants of North America, 1st edn. Iowa State University Press, Ames

Kurtzman CP, Robnett CJ (1998) Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. Antonie van Leeuwenhoek 73:331–371

Lachance MA (1987) Approaches to yeast identification. In: Berry DR, Russell I, Stewart GG (eds) Yeast biotechnology. Allen & Unwin London, pp. 33–51

Lachance MA, Starmer WT, Rosa CA, Bowles JM, Barker JSF, Janzen DH (2001) Biogeography of the yeasts of ephemeral flowers and their insects. FEMS Yeast Res 1:1–8

Miller MW, Phaff, HJ (1998) *Metschnikowia* Kamienski. In: Kurtzman CP, Fell JW (eds) The yeasts, a taxonomic study, 4th edn. Elsevier, Amsterdam, pp. 256–267

Ornduff R (1970). Systematics and Breeding System of Gelsemium (Loganiaceae). J Arnold Arbor 51:1–17

Rosa CA, Lachance MA, Silva JOC, Teixeira ACP, Marini MM, Antonini Y, Martins RP (2003) Yeast communities associated with stingless bees. FEMS Yeast Res 4:271–275

Yarrow D (1998). Methods for the isolation and identification of yeasts. In: Kurtzman CP, Fell JW (eds) The yeasts, a taxonomic study, 4th edn. Elsevier, Amsterdam, pp. 77–100

Zhang Z, Schwartz S, Wagner L, Miller W (2000) A greedy algorithm for aligning DNA sequences. J Comput Biol 7:203–214