Candida adriatica sp. nov. and *Candida molendinolei* sp. nov., two yeast species isolated from olive oil and its by-products

Neža Čadež,¹ Peter Raspor,¹ Benedetta Turchetti,^{2,3} Gianluigi Cardinali,³ Gino Ciafardini,⁴ Gianluca Veneziani⁵ and Gábor Péter⁶

Correspondence

Neža Čadež
neza.cadez@bf.uni-lj.si

¹Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, 1000 Ljubljana, Slovenia

²DBVPG Industrial Yeasts Collection, University of Perugia, Borgo XX Giugno 74, I-06121 Perugia, Italy

³Department of Applied Biology, University of Perugia, Borgo XX Giugno 74, I-06121 Perugia, Italy

⁴Department of Animal, Plant and Environmental Sciences, Agriculture Faculty, University of Molise, Via De Sanctis, I-86100 Campobasso, Italy

⁵Department of Economics and Food Science, University of Perugia, Borgo XX Giugno 74, I-06121 Perugia, Italy

⁶National Collection of Agricultural and Industrial Microorganisms, Faculty of Food Sciences, Corvinus University of Budapest, Somlói út 14-16, H-1118 Budapest, Hungary

Thirteen strains isolated from virgin olive oil or its by-products in several Mediterranean countries were found to be phenotypically and genetically divergent from currently recognized yeast species. Sequence analysis of the large subunit (LSU) rDNA D1/D2 domain and internal transcribed spacer regions/5.8S rDNA revealed that the strains represented two novel species described as *Candida adriatica* sp. nov. (type strain ZIM 2334^T=CBS 12504^T=NCAIM Y.02001^T) and *Candida molendinolei* sp. nov. (type strain DBVPG 5508^T=CBS 12508^T=NCAIM Y.02000^T). Phylogenetic analysis based on concatenated sequences of the small subunit rRNA gene, the D1/D2 region of the LSU rDNA and the translation elongation factor-1 α gene suggested that *C. adriatica* sp. nov. and *C. molendinolei* sp. nov. should be placed within the *Lindnera* and *Nakazawaea* clades, respectively.

Olive oil is a typical product of the Mediterranean area and is well known for its high nutritional value. Extra virgin olive oil is obtained from the fruit of the olive tree (*Olea europaea* L.) solely by milling and cold-pressing of olive flesh. Although the freshly extracted virgin olive oil is bitter and has green olive fruit characteristics, it improves after a period of storage. This improvement of flavour is assigned to enzymic activities of either plant or microbial origin during extraction or the sedimentation phase (Ciafardini & Zullo, 2002; Servili *et al.*, 2004; Vichi *et al.*, 2011). Yeasts have been found to be the predominant micro-organisms associated with olive oil (Ciafardini & Zullo, 2002), which suggests that they may play a role in flavour improvement

or be involved in the production of undesired acidity due to their lipolytic activity (Ciafardini *et al.*, 2006a, b; Koidis *et al.*, 2008).

During a survey of yeasts other than *Candida boidinii*, *Candida wickerhamii* and *Saccharomyces cerevisiae* associated with extra virgin olive oils (V. Režić-Dereani & N. Čadež, unpublished results), two groups of isolates showing a distinct physiological profile were found. Based on the degree of divergence in the D1/D2 region of the large subunit (LSU) rDNA, we predicted that these groups represent two novel yeast species. Because initially only one to two isolates were recovered for each species from Croatian olive oil, additional strains from Slovenian, Israeli and Italian olive oils (Zullo *et al.*, 2010) or concentrate from olive vegetation water in Italy were collected. For phylogenetic placement of the isolates, sequences of the D1/D2 regions of the LSU rDNA, the translation elongation factor-1 α (EF-1 α) gene and small subunit (SSU) rDNA were determined. Thirteen strains, which are hereby described and represent two novel species (*Candida adriatica* sp. nov. and *Candida*

Abbreviations: EF-1 α , translational elongation factor-1 α ; ITS, internal transcribed spacer; LSU, large subunit; SSU, small subunit.

The GenBank/EMBL/DDBJ accession numbers for the sequences determined in this study are listed in Table 1.

Three supplementary figures are available with the online version of this paper.

molendinolei sp. nov.), clustered into the two distinct clades of *Lindnera* and *Nakazawaea*, respectively.

The yeast strains listed in Table 1 were isolated either by dilution of spoiled olive oils or by membrane filtration of extra virgin olive oil through nitrocellulose filters with 0.2 µm pore size. The dilutions or membrane filters were transferred onto YPD (Sigma) agar plates. For the isolation of strains NCAIM Y.01995, NCAIM Y.01996 and NCAIM Y.01997, 0.1 ml olive oil samples were transferred to the surface of Rose-Bengal chloramphenicol (RBC) agar (Merck) and streaked using a loop. The agar plates were incubated at 25 °C for 5 days in darkness. Individual colonies were picked up and purified by repeated streaking on GPY agar before further study. DBVPG 5508^T, DBVPG 5509 and DBVPG 55010 were isolated by transferring a crude phenolic concentrate separated from olive vegetation water as described by Servili *et al.* (2011) onto the surface of malt extract agar [3 % MEA; 3 % malt extract (Biotech), 0.5 % peptone (Costantino), 2 % agar (Biotech), pH 5.4] following the methods described above. The strains were characterized by standard methods described by Kurtzman

et al. (2011). Sporulation was investigated on acetate (1.4 % sodium acetate, 0.04 % glucose), cornmeal, potato-glucose, 2 % malt extract, 5 % malt extract, glucose-peptone-yeast extract (GPY), yeast extract-malt extract (YM), V8 and diluted (1:9) V8 agars at 15 and 25 °C for up to 5 weeks. For macromorphological analysis, 3 % MEA and YM agar [0.3 % yeast extract (Costantino), 0.5 % peptone, 0.3 % malt extract, 1 % glucose (Carlo Erba Reagents), 2 % agar] were used. Lipolytic activity was examined on tributyrin agar (Fluka) incubated for 7 days at 25 °C, as well as according to the method described by Phaff *et al.* (1997). Proteolytic activity was tested on milk agar (Fluka) for 7 days at 25 °C.

The D1/D2 regions of the LSU rDNA and the internal transcribed spacer (ITS) regions were sequenced as described previously (Cadez *et al.*, 2003). The SSU rDNA and EF-1 α gene were amplified by primers NS1 and NS8 (White *et al.*, 1990) and YTEF-1G and YTEF-6G (Kurtzman & Robnett, 2003), respectively. Prior to sequencing, the fragments were inserted into pGEM-T vector system (Promega) as detailed in Cadez *et al.* (2006). All sequences were determined by a commercial sequencing facility (Macrogen, Korea). Sequences

Table 1. List of strains isolated, their origin and GenBank/EMBL/DDBJ accession numbers

Culture collections are abbreviated as follows: CBS, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; DAPES, Collection of Department of Animal, Plant and Environmental Science, Campobasso, Italy; DBVPG, Industrial Yeast Collection, Perugia, Italy; LCF, Internal Yeast designation of the Cardinali Laboratory; NCAIM, National Collection of Agricultural and Industrial Micro-organisms, Budapest, Hungary; ZIM, Collection of Industrial Micro-organisms, Ljubljana, Slovenia. ND, Not determined.

Species/strain designation	Origin	Accession number*			
		SSU	EF-1 α	D1/D2	ITS
<i>Candida adriatica</i> sp. nov. (Group A)					
ZIM 2334 ^T (=CBS 12504 ^T =NCAIM Y.02001 ^T)	Sediment of extra virgin olive oil, Slovenian Istria, Slovenia	HE574675	HE574667	HE574661	HE574654
DAPES 1933	Extra virgin olive oil, Italy	HE603645	HE603646	JN637464	JN637464
LCF 963	Extra virgin olive oil, Italy	ND	ND	JN637463	JN637463
NCAIM Y.01995 (=ZIM 2328)	Unfiltered extra virgin olive oil, Italy	HE574674	HE574666	HE574660	HE574653
ZIM 2219	Spoiled virgin olive oil, Island of Korčula, Croatia	HE574673	HE574665	FR690076	HE574652
<i>Candida molendinolei</i> sp. nov. (Group B)					
DBVPG 5508 ^T (=CBS 12508 ^T =NCAIM Y.02000 ^T)	Crude phenolic concentrate from olive vegetation water, Perugia, Italy	HE574679	HE574671	JN688665	JN688668
DBVPG 5509	Crude phenolic concentrate from olive vegetation water, Perugia, Italy	ND	ND	JN688666	JN688669
DBVPG 5510	Crude phenolic concentrate from olive vegetation water, Perugia, Italy	HE574680	HE574672	JN688667	JN688670
NCAIM Y.01996 (=ZIM 2330)	Unfiltered extra virgin olive oil, Italy	HE574677	HE574669	HE574663	HE574657
NCAIM Y.01997 (=ZIM 2329)	Spoiled olive oil with a twig of the olive tree in a bottle, Israel	ND	ND	HE574664	HE574658
ZIM 2279	Extra virgin olive oil, Split, Croatia	ND	ND	FR690077	HE574656
ZIM 2280	Extra virgin olive oil, Split, Croatia	HE574676	HE574668	HE574662	HE574655
ZIM 2320	Extra virgin olive oil, Slovenian Istria, Slovenia	HE574678	HE574670	FR690078	HE574659

*Gene sequences: SSU, nuclear small subunit rDNA; EF-1 α , translation elongation factor-1 α gene; D1/D2, large subunit rDNA domains; ITS, internal transcribed spacer including the 5.8S rDNA region.

were aligned using CLUSTAL_X (Thompson *et al.*, 1997). Most parsimonious trees were generated by the PAUP* 4.0b10 software package (Swofford, 2002) using a heuristic search procedure with 1000 random addition replicates and tree bisection-reconnection branch swapping. The stability of the branches was assessed by bootstrap analysis (Felsenstein, 1985) in which 1000 replicates were set in PAUP*. Relationships between strains were determined by the network creation method Minimum Spanning Tree, based on the number of nucleotide changes in the ITS/5.8S rDNA (positions 37–620) and D1/D2 domains of the LSU rDNA (positions 25–558), using BioNumerics 6.6.

Thirteen strains isolated from olive oil or its by-products in several Mediterranean countries segregated into groups A and B based on their LSU rDNA D1/D2 and ITS/5.8S rDNA sequences (Table 1). A connected network based on ITS–D1/D2 sequences was constructed by using the minimum spanning method to show relationships between strains of groups A and B (see Fig. S1 in IJSEM Online). Group A consisted of five strains; strain ZIM 2334^T, isolated from olive oil in Slovenia, was placed in a central position between strains from Croatia and Italy. Only two Italian strains (DAPES 1933 and LCF 963) shared identical sequences. Group B was genetically more homogeneous as only two genetically divergent Croatian isolates (ZIM 2279 and ZIM 2280) differed from the main group. The latter comprised six strains isolated from various Mediterranean countries. Additionally, a similar ITS sequence belonging to strain ITEM10464, which was isolated from wastewater of olive oil production in Italy (Bleve *et al.*, 2011), is deposited in GenBank (FN376420) and differs in one nucleotide substitution from sequences in Group B. The strain was not studied here because it was discovered too late for inclusion in the present study. Furthermore, Romo-Sánchez *et al.* (2010) isolated yeast strains from olive paste or pomace in Spain that may be similar to strains of Group B but, due to the low quality and shortness of their sequences (GQ340436, GQ340443, GQ340444, GQ340448), the level of similarity was difficult to establish.

A few physiological discrepancies were found among strains of both groups but they were mainly limited to the intensity and speed of the conventional physiological test reactions. On the other hand, there was a notable difference in the morphological appearance of strains NCAIM Y.01996, ZIM 2320, ZIM 2279 and ZIM 2280 on MEA and YM agars as their texture was pasty and not as viscous as other strains of Group B (see Fig. S2).

A BLAST similarity search with D1/D2 sequences revealed that Group A was most closely related to the species of the *Lindnera* clade (Kurtzman *et al.*, 2008), comprising mostly species belonging to the genus *Lindnera* Kurtzman *et al.* nom. illegit., a homonym of the plant genus *Lindnera* Fuss (Minter, 2009). For this reason, *Cyberlindnera* (Minter, 2009) is used in this report instead of *Lindnera*, as proposed by Kurtzman (2011c). Group A differed from the type

strains of *Cyberlindnera fabianii* and *Cyberlindnera japonica* by 20 substitutions. Based on the extent of divergence in D1/D2 regions as well as additional molecular and phenotypic evidence, the name *Candida adriatica* sp. nov. (MycBank no. MB 563572) is proposed for the strains of Group A. Group B differed from the type strains of *Candida ishiwadae*, *Nakazawaea holstii* and *Candida peltata* belonging to *Nakazawaea* clade (Kurtzman & Robnett, 2010) by 14, 17 and 23 D1/D2 substitutions, respectively. For the strains of Group B, the name *Candida molendinolei* sp. nov. (MycBank no. MB 563573) is proposed.

For reliable taxonomic placement of the putative two novel species, additional genes such as EF-1 α and SSU rDNA were determined and concatenated together with D1/D2 LSU rDNA and ITS/5.8S rDNA sequences. In the most-parsimonious tree presented in Fig. 1, the strains assigned to *Candida adriatica* sp. nov. were located in the *Lindnera* clade clustering together with the type strain of *Cyberlindnera japonica* and *Candida* sp. NRRL YB-2097. Furthermore, the strains of *Candida molendinolei* sp. nov. were placed in the *Nakazawaea* clade with the type strain of *Candida peltata* as the closest relative with high statistical support (see Fig. S3).

The physiological characteristics of *Candida adriatica* sp. nov. differed from those of *Cyberlindnera americana*, *Cyberlindnera euphorbiiphila*, *Cyberlindnera japonica* and *Cyberlindnera petersonii* (Table 2) based on a combination of the following properties: inability to assimilate raffinose and melezitose, absence of growth at 37 °C and fermentation of sucrose. The phenotypic characteristics of *Candida molendinolei* sp. nov. were compared with those of its phylogenetically closest relatives, i.e. *Candida ishiwadae*, *N. holstii* and *Candida peltata*, as well as with those of *Candida wickerhamii*, which is the most similar species phenotypically (Table 3). Specifically, *Candida molendinolei* sp. nov. differed from *Candida wickerhamii* in its inability to assimilate galactose and its ability to assimilate DL-lactate and xylitol, and its ability to grow at elevated concentrations of NaCl and at 37 °C. Additionally, it differed from its phylogenetically closest relatives by its inability to assimilate sucrose, galactose, maltose, melezitose, methyl α -D-glucoside, starch, L-sorbose, D-arabinose, erythritol and galactitol.

As presented in Fig. 2, *Candida adriatica* sp. nov. and *Candida molendinolei* sp. nov. reproduced asexually by multipolar budding. No sexual state was observed in any of the crosses between strains of the both species under the tested conditions. Based on the above presented results, we conclude that the isolates from olive oil and its by-products are genetically and phenotypically distinct from all currently recognized yeast species and should be classified as representatives of two novel species of the anamorphic genus *Candida* in the *Lindnera* and *Nakazawaea* clades, respectively.

Although all strains of the two novel yeast species included in our study were isolated from olive oil or from by-products of it, members of the species *Candida molendinolei* sp. nov. do

Fig. 1. Phylogenetic tree showing the placement *Candida adriatica* sp. nov. (Group A) strains in the *Lindnera* clade (Kurtzman & Robnett, 2010) based on the concatenated datasets of SSU rDNA, D1/D2 regions of LSU rDNA and EF-1 α . The GenBank/EMBL/DBJ accession numbers of the SSU rDNA, D1/D2 regions of the LSU rDNA and EF-1 α gene sequences are listed in parentheses after each strain number. One of four most parsimonious trees (tree length 1820, CI=0.5593, RI=0.5885) is presented. The sequences not determined in this study were obtained from GenBank. Bootstrap percentages from 1000 replicates are shown. *Ogataea minuta* was used as the outgroup. Bar, number of nucleotide substitutions.

not seem to only inhabit the olive ecosystem according to very recently deposited D1/D2 and ITS sequences of strain CLIB 1308 isolated from cider in France (GenBank accession nos HE574647 and HE574642). One strain of each novel species was recovered from spoiled olive oil. Strains of *Candida adriatica* exhibited lipolytic activity, whereas this property was not detected in strains of *Candida molendinolei*. The possible effect of these novel yeast species on the quality of olive oil needs further study.

Latin diagnosis of *Candida adriatica* Čadež, Cardinali, Ciafardini et Péter sp. nov.

In medio liquido cum glucoso et peptono et extracto levidino post dies 3 ad 25 °C cellulae gemmantes ovoideae (3.0–5.0 × 2.0–3.5 µm), singulae, binae vel racemis conexas. Post dies decem sedimentum formatur. Cultura, in agar cum glucoso et peptono

et extracto levidino post dies 7 in 25 °C exhibet colonias rotundas cum nitido margine et albo colore. In agar farinae Zea mays post dies 5 in 25 °C, mycelium et pseudomycelium non formantur. Ascospores non possunt videri. Glucosum et sucrosus (lente) fermentantur. Glucosum, D-xylosum, sucrosus, maltosum, α , α -trehalosum (aliquando lente), methyl α -D-glucosidum, cellobiosum, salicinum, arbutinum, glycerolum, D-mannitolum, xylitolum, D-glucitolum, D-mannitolum, glucono- δ -lactonum, D-gluconatum, acidum DL-lacticum, acidum succinicum, acidum citricum, ethanolum et propanum-1, 2-diolum (lente) assimilantur. D-Galactosum, L-sorbosum, D-glucosaminum, N-acetyl-D-glucosaminum, D-ribosum, L-arabinosum, D-arabinosum, L-rhamnosum, melibiosum, lactosum, raffinose, melezitose, inulinum, amyllum, erythritolum, ribitolum, L-arabinitolum, galactitolum, inositolum, 2-keto-D-gluconatum, D-glucuronatum, D-galacturonatum, methanolum, butane-2,3-diolum et hexadecanum non assimilantur. Kalium

Table 2. Physiological characteristics that differentiate *Candida adriatica* sp. nov. (Group A) from related species (Kurtzman, 2011a)

Species: 1, Group A; 2, *Cyberlindnera americana*; 3, *Cyberlindnera euphorbiiphila*; 4, *Cyberlindnera japonica*; 5, *Cyberlindnera petersonii*. +, Positive; –, negative; s, slow.

Characteristic	1	2	3	4	5
Sucrose fermentation	s	–	–	–	+
Assimilation of:					
Raffinose	–	–	–	–	+
Melezitose	–	+	+	+	+
Growth at 37 °C	–	–	+	+	+

nitricum, *natrium nitrosum*, *ethylaminum* et *L-lysinum* assimilantur at non *cadaverinum*, *creatinum*, *creatininum*, *D-glucosaminum* nec *imidasolum*. *Vitamina externa crescentiae necessaria sunt*. *Maxima temperatura crescentiae* 35 °C. *Materia amyloidea iodophila non formatur*. *Urea non finditur*. *Diazonium caeruleum B non respondet*. *In medio cum 0.01 % cycloheximido et 10 % NaCl (lente et variabile) crescit neque cum 50 % glucoso vel 16 % NaCl*. *Proteinae hydrolisantur, lipides hydrolysis mutabilis est*.

Table 3. Physiological characteristics that differentiate *Candida molendinolei* sp. nov. (Group B) from closely related species (Kurtzman, 2011b; Lachance *et al.*, 2011)

Species: 1, Group B; 2, *Nakazawaea holstii*; 3, *Candida peltata*; 4, *Candida ishiwadae*; 5, *Candida wickerhamii*. +, Positive; –, negative; s, slow; v, variable; w, weak; ND, no data.

Characteristic	1	2	3	4	5
Trehalose fermentation	s	+	–	s	v
Assimilation of:					
Sucrose	–	+	+	+	–
Galactose	–	+	+	v	+
Maltose	–	+	+	+	–
Melezitose	–	+	+	v	–
Methyl- α -D-glucoside	–	+	+	v	–
Soluble starch	–	+	+	+	–
L-Sorbose	–	+	+	s	–
L-Arabinose	+	+	+	s	w
D-Arabinose	–	+	+	v	v
Erythritol	–	v	v	+	–
Galactitol	–	v	v	v	–
DL-Lactate	+	–	–	v	–
Xylitol	+	ND	+	v	–
Nitrate	+	+	–	+	+
Growth in/at:					
10 % NaCl	+	+ / w	–	+	–
37 °C	+ / w	v	+	–	–

Fig. 2. Vegetative cells of (a) *Candida adriatica* sp. nov. ZIM 2334^T and (b) *Candida molendinolei* sp. nov. DBVPG 5508^T grown in YPG broth for 3 days at 25 °C. Bars, 10 µm.

Typus ZIM 2334^T (=CBS 12504^T=NCAIM Y.02001^T) *ex sedimento olei olivae in Norico isolatus, preservatus in collectione* Industrial Collection of Microorganisms (ZIM), *Noricum*.

Description of *Candida adriatica* Čadež, Cardinali, Ciafardini & Péter sp. nov.

Candida adriatica (adri.a'ti.ca. L. fem adj. *adriatica* from *Mare Adriaticum*, referring to the origin of the isolates).

In YM broth after 3 days at 25 °C, the cells are ovoid (3.0–5.0 × 2.0–3.5 µm), occurring singly, in pairs or in clusters. Budding is multipolar. Sediment is present. On YM agar after 7 days at 25 °C, the streak culture is butyrous and glossy to dull white in colour with sharp edges. Pseudomycelium or true mycelium are not formed. Formation of ascospores is not observed. Glucose and sucrose (slow) are fermented. Glucose, D-xylose, sucrose, maltose, α,α -trehalose (variable), methyl α -D-glucoside, cellobiose, salicin, arbutin, glycerol, xylitol, D-glucitol, D-mannitol, D-glucono- δ -lactone, D-gluconate, DL-lactate, succinate, citrate, ethanol and propane-1,2-diol (slow) are assimilated; D-galactose, L-sorbose, D-glucosamine, *N*-acetyl-D-glucosamine, D-ribose, L-arabinose, D-arabinose, L-rhamnose, melibiose, lactose, raffinose,

melezitose, inulin, starch, erythritol, ribitol, L-arabinitol, galactitol, inositol, 2-keto-D-gluconate, D-glucuronate, D-galacturonate, methanol, butane-2,3-diol and hexadecane are not assimilated. Potassium nitrate, sodium nitrite, ethylamine and lysine are assimilated; cadaverine, creatine, creatinine, glucosamine and imidazole are not assimilated as nitrogen sources. Growth in vitamin-free medium is absent. Maximum growth temperature is 35 °C. Starch-like compounds are not produced. Urease activity and the diazonium blue B reaction are negative. No growth occurs with 0.01 % cycloheximide, on 50 % (w/w) glucose or with 1 % acetic acid. Growth in 10 % NaCl is slow and variable. Proteinase activity is positive; lipase activity is variable. The MycoBank no. is MB 563572.

The type strain, ZIM 2334^T (=CBS 12504^T=NCAIM Y.02001^T), was isolated from sediment of extra virgin olive oil collected in Koper, Slovenia.

Latin diagnosis of *Candida molendinolei* Čadež, Turchetti et Péter sp. nov.

In extracto malti post dies tres ad 25 °C cellulae vegetativae subglobosae, ovoidae aut ellipsoidae (1.5–4.0 × 2.5–5.0 µm), singulae aut binae gemmationis causa sunt. Gemmatio multipolaris. Cellulae gemmantes 50 % usque ad 75 % omnium. Sedimentum formatur. In agar malti post dies tres ad 25 °C cultura est butyrosa vel molles in textura, nitidae, glabrae, albae in colore, planae, cum margine integra. Asci non formatur. Glucosum et trehalosum (aliquando lente) fermentantur. Glucosum, D-glucosaminum (aliquando lente), N-acetylglucosaminum (aliquando lente), D-ribosum (aliquando lente), D-xylosum (aliquando lente), L-arabinosum, L-rhamnosum, α,α-trehalosum, cellobiosum, salicinum, arbutinum, glycerolum, ribitolum, xylitolum, L-arabinitolum (lente), D-glucitolum, D-mannitolum, D-glucono-δ-lactonum (aliquando lente), acidum DL-lacticum, acidum succinicum, acidum citricum et ethanolum assimilantur. Galactosum, L-sorbosum, D-arabinosum, sucrosum, maltosum, methyl α-D-glucosidum, melibiosum, lactosum, raffinose, melezitosum, inulinum, amylum, erythritolum, galactitolum, inositolum, 2-keto-D-gluconatum, D-gluconatum, D-glucuronatum, D-galacturonatum, methanolum et hexadecanum non assimilantur. Propanum-1,2-diolum et butanum-2,3-diolum non assimilantur aut assimilantur lente. Kalium nitricum, natrium nitrosum, ethylaminum, L-lysinum et cadaverinum assimilantur neque creatinum, creatininum, D-glucosaminum nec imidazolium. Vitamina externa crescentiae necessaria sunt. Maxima temperatura crescentiae 37 °C (exiguae). Materia amyloidea iodophila non formatur. Urea non finditur. Diazonium caeruleum B non respondet. In medio cum 0.01 % cycloheximido et 10 % NaCl crescit neque cum 50 % glucosio vel 16 % NaCl.

Typus DBVPG 5508^T (=CBS 12508^T=NCAIM Y.02000^T) *isolatus ex aqua tractationis olivarum et in Italia, preservatus est in collectione zymotica* DBVPG Industrial Yeasts Collection, Perugia, Italia.

Description of *Candida molendinolei* Čadež, Turchetti & Péter sp. nov.

Candida molendinolei (mo.len.din.o'le.i. L. n. *molendinum* -i a milling place, mill-house; L. n. *oleum* -i oil; N.L. gen. n. *molendinolei* referring to an oil-mill house, the place from which the isolates were obtained).

In malt extract broth after 3 days at 25 °C, the cells are subglobose to ovoid or ellipsoid (1.5–4.0 × 2.5–5.0 µm), occurring singly or in pairs. Budding is multipolar. Budding cells occur at a level of 50–75 % of the total number of cells. Sediment is present. On 3 % MEA after 3 days at 25 °C, the steak culture is butyrous to viscous, glossy, dull white in colour and flat or slightly raised at the centre with an entire margin. On corn meal agar, neither pseudohyphae nor septate hyphae are formed. Ascospore formation is not observed. Glucose and α,α-trehalose (slow) are fermented. Glucose, D-glucosamine (slow), N-acetyl-D-glucosamine (slow), D-ribose (slow), D-xylose (slow), L-arabinose, L-rhamnose, α, α-trehalose, cellobiose, salicin, arbutin, glycerol, ribitol, xylitol, L-arabinitol (slow), D-glucitol, D-mannitol, D-glucono-δ-lactone (slow), DL-lactate, succinate, citrate and ethanol are assimilated; galactose, sorbose, D-arabinose, sucrose, maltose, methyl α-D-glucoside, melibiose, lactose, raffinose, melezitose, inulin, starch, erythritol, galactitol, inositol, 2-keto-D-gluconate, D-gluconate, D-glucuronate, D-galacturonate, methanol and hexadecane are not assimilated; propane-1,2-diol and butane-2,3-diol are not assimilated or are assimilated with slow growth. Potassium nitrate, sodium nitrite, ethylamine, lysine and cadaverine are assimilated, but creatine, creatinine, glucosamine and imidazole are not assimilated as nitrogen sources. Growth in vitamin-free medium is absent. Maximum growth temperature is 37 °C, although weak growth is observed. Starch-like compounds are not produced. Urease activity and the diazonium blue B reaction are negative. Growth occurs with 0.01 % cycloheximide and with 10 % NaCl, but no growth was observed in 50 % (w/w) glucose, 1 % acetic acid or 16 % NaCl. The MycoBank no. is MB 563573.

The type strain, DBVPG 5508^T (=CBS 12508^T=NCAIM Y.02000^T), was isolated from olive vegetation water in Italy.

Acknowledgements

We acknowledge Vasilij Valenčič from the Institute for Ecology, Olive Oil and Control, LABS LLC, Slovenia for olive oil samples and Vesna Režič-Dereani for collecting yeast isolates from the olive environment. We also thank Dr Livio Antonielli (DBA) and Biagio Zullo (DAPES) for useful and critical discussions. The work was partly funded by the Slovenian Research Agency (P4-0116 and MRIC-UL ZIM, IP-0510).

References

- Bleve, G., Lezzi, C., Chiriatti, M. A., D'Ostuni, I., Tristezza, M., Di Venere, D., Sergio, L., Mita, G. & Grieco, F. (2011). Selection of non-conventional yeasts and their use in immobilized form for the bioremediation of olive oil mill wastewaters. *Bioresour Technol* **102**, 982–989.

- Cadez, N., Poot, G. A., Raspor, P. & Smith, M. T. (2003). *Hanseniaspora meyeri* sp. nov., *Hanseniaspora clermontiae* sp. nov., *Hanseniaspora lachancei* sp. nov. and *Hanseniaspora opuntiae* sp. nov., novel apiculate yeast species. *Int J Syst Evol Microbiol* **53**, 1671–1680.
- Cadez, N., Raspor, P. & Smith, M. T. (2006). Phylogenetic placement of *Hanseniaspora-Kloeckera* species using multigene sequence analysis with taxonomic implications: descriptions of *Hanseniaspora pseudo-guilliermondii* sp. nov. and *Hanseniaspora occidentalis* var. *citrica* var. nov. *Int J Syst Evol Microbiol* **56**, 1157–1165.
- Ciafardini, G. & Zullo, B. A. (2002). Microbiological activity in stored olive oil. *Int J Food Microbiol* **75**, 111–118.
- Ciafardini, G., Zullo, B. A. & Iride, A. (2006a). Lipase production by yeasts from extra virgin olive oil. *Food Microbiol* **23**, 60–67.
- Ciafardini, G., Zullo, B. A., Cioccia, G. & Iride, A. (2006b). Lipolytic activity of *Williopsis californica* and *Saccharomyces cerevisiae* in extra virgin olive oil. *Int J Food Microbiol* **107**, 27–32.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Koidis, A., Triantafyllou, E. & Boskou, D. (2008). Endogenous microflora in turbid virgin olive oils and the physicochemical characteristics of these oils. *Eur J Lipid Sci Technol* **110**, 164–171.
- Kurtzman, C. P. (2011a). *Lindnera* Kurtzman, Robnett & Basehoar-Powers (2008). In *The Yeasts, a Taxonomic Study*, 5th edn, vol. 2, pp. 521–543. Edited by C. P. Kurtzman, J. W. Fell & T. Boekhout. Amsterdam: Elsevier.
- Kurtzman, C. P. (2011b). *Nakazawaea* Y. Yamada, Maeda & Mikata (1994). In *The Yeasts, a Taxonomic Study*, 5th edn, vol. 2, pp. 637–639. Edited by C. P. Kurtzman, J. W. Fell & T. Boekhout. Amsterdam: Elsevier.
- Kurtzman, C. P. (2011c). Phylogeny of the ascomycetous yeasts and the renaming of *Pichia anomala* to *Wickerhamomyces anomalus*. *Antonie van Leeuwenhoek* **99**, 13–23.
- Kurtzman, C. P. & Robnett, C. J. (2003). Phylogenetic relationships among yeasts of the ‘*Saccharomyces* complex’ determined from multigene sequence analyses. *FEMS Yeast Res* **3**, 417–432.
- Kurtzman, C. P. & Robnett, C. J. (2010). Systematics of methanol assimilating yeasts and neighboring taxa from multigene sequence analysis and the proposal of *Peterozyma* gen. nov., a new member of the *Saccharomycetales*. *FEMS Yeast Res* **10**, 353–361.
- Kurtzman, C. P., Robnett, C. J. & Basehoar-Powers, E. (2008). Phylogenetic relationships among species of *Pichia*, *Issatchenkia* and *Williopsis* determined from multigene sequence analysis, and the proposal of *Barnettozyma* gen. nov., *Lindnera* gen. nov. and *Wickerhamomyces* gen. nov. *FEMS Yeast Res* **8**, 939–954.
- Kurtzman, C. P., Fell, J. W., Boekhout, T. & Robert, V. (2011). Methods for isolation, phenotypic characterization and maintenance of yeasts. In *The Yeasts, a Taxonomic Study*, 5th edn, vol. 1, pp. 87–110. Edited by C. P. Kurtzman, J. W. Fell & T. Boekhout. Amsterdam: Elsevier.
- Lachance, M.-A., Boekhout, T., Scorzetti, G., Fell, J. W. & Kurtzman, C. P. (2011). *Candida* Berkhout (1923). In *The Yeasts, a Taxonomic Study*, 5th edn, vol. 2, pp. 987–1278. Edited by C. P. Kurtzman, J. W. Fell & T. Boekhout. Amsterdam: Elsevier.
- Minter, D. M. (2009). *Cyberlindnera*, a replacement name for *Lindnera* Kurtzman *et al.*, nom. illegit. *Mycotaxon* **110**, 473–476.
- Phaff, H. J., Blue, J., Hagler, A. N. & Kurtzman, C. P. (1997). *Dipodascus starmeri* sp. nov., a new species of yeast occurring in cactus necroses. *Int J Syst Bacteriol* **47**, 307–312.
- Romo-Sánchez, S., Alves-Baffi, M., Arévalo-Villena, M., Ubeda-Iranzo, J. & Briones-Pérez, A. (2010). Yeast biodiversity from oleic ecosystems: study of their biotechnological properties. *Food Microbiol* **27**, 487–492.
- Servili, M., Selvaggini, R., Esposto, S., Taticchi, A., Montedoro, G. & Morozzi, G. (2004). Health and sensory properties of virgin olive oil hydrophilic phenols: agronomic and technological aspects of production that affect their occurrence in the oil. *J Chromatogr A* **1054**, 113–127.
- Servili, M., Rizzello, C. G., Taticchi, A., Esposto, S., Urbani, S., Mazzacane, F., Di Maio, I., Selvaggini, R., Gobbetti, M. & Di Cagno, R. (2011). Functional milk beverage fortified with phenolic compounds extracted from olive vegetation water, and fermented with functional lactic acid bacteria. *Int J Food Microbiol* **147**, 45–52.
- Swofford, D. L. (2002). PAUP*: phylogenetic analysis using parsimony (and other methods), version 4. Sunderland, MA: Sinauer Associates.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.
- Vichi, S., Romero, A., Tous, J. & Caixach, J. (2011). The activity of healthy olive microbiota during virgin olive oil extraction influences oil chemical composition. *J Agric Food Chem* **59**, 4705–4714.
- White, T. J., Bruns, T., Lee, S. & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols: A Guide to Methods and Applications*, pp. 315–322. Edited by N. Innis, D. Gelfand, J. Sninsky & T. White. London: Academic Press.
- Zullo, B. A., Cioccia, G. & Ciafardini, G. (2010). Distribution of dimorphic yeast species in commercial extra virgin olive oil. *Food Microbiol* **27**, 1035–1042.