Antonie van Leeuwenhoek (2014) 105:593–601
DOI 10.1007/s10482-014-0114-7

ORIGINAL PAPER

Leucosporidium escuderoi f.a., sp. nov., a basidiomycetous yeast associated with an Antarctic marine sponge

Federico Laich · Renato Chávez · Inmaculada Vaca

Received: 17 June 2013 / Accepted: 6 January 2014 / Published online: 17 January 2014

Abstract A basidiomycetous yeast, strain E2A-C3-II, was isolated from a marine sponge (*Hymeniacidon* sp.) collected at a depth of 6 m in Fildes Bay, King George Island, Antarctica. The phylogenetic analysis revealed that the yeast isolated is related to *Leucosporidium drummii*, *Leucosporidiella muscorum* and to the *Leucosporidium scottii* group, including *Leucosporidiella creatinivora* and *Leucosporidiella yakutica*. The analysis of the nucleotide differences and the genetic distances of the D1/D2 domain of the LSU rDNA gene and 5.8S ITS regions support that strain E2A-C3-II represents a new species. The novel species can be distinguished from *L. drummii* by its ability to assimilate L-sorbose, L-rhamnose, lactose and ribitol. The maximum temperature for growth was 25 °C. On the basis of morphological, biochemical and physiological characterization, and phylogenetic and nucleotide analysis, a novel basidiomycetous yeast species, *Leucosporidium escuderoi* f.a., sp. nov., is proposed. The type strain is E2A-C3-II^T (=CBS 12734^T =CECT 13080^T). The Mycobank (http://www.mycobank.org) accession number is MB 804654. The nucleotide sequences of D1/D2 domain of the LSU rDNA gene and 5.8S-ITS regions obtained in this work have been deposited in Genbank under the Accession numbers JN181009 and JN197600, respectively.

Keywords *Leucosporidium* · New yeast species · Marine sponge · Antarctica

Electronic supplementary material The online version of this article (doi:10.1007/s10482-014-0114-7) contains supplementary material, which is available to authorized users.

F. Laich
Instituto Canario de Investigaciones Agrarias, Ctra. Boquerón s/n. Valle de Guerra, Santa Cruz de Tenerife, Spain

F. Laich
Departamento Central de Investigación, Universidad Laica "Eloy Alfaro" de Manabí, Av. Circunvalación y Calle 12, Manta, Ecuador

R. Chávez
Departamento de Biología, Facultad de Química y Biología, Universidad de Santiago de Chile (USACH), Alameda 3363, 9170022 Estación Central, Santiago, Chile

I. Vaca (✉)
Departamento de Química, Facultad de Ciencias, Universidad de Chile, Las Palmeras 3425, Ñuñoa, Santiago, Chile
e-mail: inmavaca@uchile.cl

Introduction

It has been known for many years that marine sponges, including those from the maritime Antarctica, provide important habitat for several prokaryotic and eukaryotic microorganisms. Comprehensive information about the association of archaea, diatoms, dinoflagelates, filamentous fungi and bacteria with sponges can be found in the current literature

(Webster et al. 2004; Hentschel et al. 2006; Taylor et al. 2007a; Olson and Kellog 2010; Webster and Taylor 2012). However, the association between yeasts and marine sponges has been overlooked. Recently, the ascomycetous yeasts *Debaryomyces hansenii* and *Metschnikowia australis*, and the basidiomycetous yeasts *Bullera pseudoalba*, *Cryptococcus laurentii*, *Rhodotorula mucilaginosa*, *Rhodotorula pinicola, Cystofilobasidium infirmominiatum* and *Leucosporidiella creatinivora* were isolated from Antarctic marine sponges (Duarte et al. 2013; Vaca et al. 2013). In addition, an unidentified yeast belonging to the genus *Leucosporidium* (namely strain E2A-C3-II) was also isolated from a sample of Antarctic sponge (Vaca et al. 2013).

The yeasts of the genera *Leucosporidium* (teleomorph) and *Leucosporidiella* (anamorph) are classified in the order Leucosporidiales (Microbotryomycetes, Pucciniomycotina; Sampaio et al. 2003, 2004; Sampaio 2011a, b), with the exception of *Leucosporidium fasciculatum*, which has not been included, to date, in any order of the Microbotryomycetes (Turchetti et al. 2011). Most of the yeast species of these genera are psychrotolerant or psychrophilic organisms, and they have been isolated from rather cold environments around the world, including terrestrial and marine Antarctic ecosystems (Di Menna 1960, 1966; Fell et al. 1969; Goto et al. 1969; Carrasco et al. 2012; Duarte et al. 2013; Vaca et al. 2013).

In the present paper, the description of a novel psychrotolerant species from the genus *Leucosporidium*, isolated from an Antarctic marine sponge, is proposed. Although the novel species was described based on the asexual form (anamorph), we have used the teleomorphic name (*Leucosporidium*) according to the suggestion of Kurtzman et al. (2011a) and in conformity with Article 59 of the International Code of Nomenclature for algae, fungi and plants, which states that only one name must be used for a fungus with no more dual nomenclature for anamorphs and teleomorphs (McNeill et al. 2012). In order to highlight the fact that the sexual stage of the species has not been observed, and according to the suggestion of some yeast taxonomists (e.g. Lachance 2012; Péter 2012), we have included the use of *forma asexualis* (f.a., refers to asexual form) which will be discussed below. To our knowledge, this is the first description of a new species of yeast isolated from a marine sponge from any geographical location.

Materials and methods

Sponge sampling and yeast isolation

The sponges sampling and the isolation of the strain E2A-C3-II have already been described (Vaca et al. 2013). Briefly, fragments of marine sponges (*Hymeniacidon* sp.) were collected from a small rocky reef located about 300 meters off the coast of Fildes Bay (62°12′0″S 58°57′51″W), King George Island, Antarctica. The fragments were collected by hand six meters deep using scuba diving and were transferred directly to a sterilized plastic bag. The samples were transported to the laboratory facilities at "Professor Julio Escudero" Base located by Fildes Bay. Pieces of approximately 1 cm^3 of the inner tissues from the sponge samples were excised under sterile conditions with a scalpel and forceps, and directly spread onto potato dextrose agar (PDA, Difco) medium, containing benzyl penicillin and streptomycin (100 µg/ml each). The PDA plates were incubated at 20 °C for 7 days. Subcultivations of the yeast were performed on YPD agar (5 g/l yeast extract, 3 g/l peptone, 20 g/l glucose, 15 g/l agar) at 20 °C for 3 days. The yeast was subsequently preserved in 30 % (w/v) glycerol at −80 °C.

Morphological, physiological and biochemical characterization of yeast isolate

The morphological characterization of colonies was carried out on 5 % malt extract agar (ME5 %) and YM agar (3 g/l yeast extract, 3 g/l malt extract, 5 g/l peptone, 10 g/l glucose, 15 g/l agar) at 18 and 25 °C for 7 days. For microscopy, the cultures were grown on ME5 % and YM broth at 18 and 25 °C for 2 days and observed under Nomarski differential interference contrast (DIC) microscope. A CCD camera was used to record images. Formation of hyphae was induced in cultures after prolonged incubation (eight months) on ME5 % agar, YM agar, corn meal agar (CMA, Oxoid), MYP agar (7 g/l malt extract, 0.5 g/l yeast extract, 2.5 g/l soytone, 15 g/l agar), SG agar (2 g/l soytone, 2 g/l glucose, 15 g/l agar) and hay-infusion agar (50 g/l finely hashed hay, 15 g/l agar) at 4, 18 and 25 °C. The formation of hyphae was regularly observed (every 15 days). For nuclei observation, the Giemsa staining protocol described by Sampaio et al. (2001) was used.

Physiological and biochemical tests of the yeast were carried out as described by Yarrow (1998) and

Kurtzman et al. (2011b). The carbon assimilation and fermentation tests, and nitrogen compounds assimilation tests were performed in triplicate in liquid medium at 18 and 25 °C, and the results were recorded after 1, 2 and 3 weeks. The effect of temperature was examined between 4 and 35 °C (at 5 °C intervals) on YPD and GPY agar (5 g/l yeast extract, 5 g/l peptone, 40 g/l glucose, 15 g/l agar) during 15 days.

DNA sequencing, phylogenetic analysis and estimation of genetic distances

Total DNA was extracted by the CTAB method described by Kurtzman and Robnett (1998). Nucleotide sequence of the D1/D2 domain of the LSU rDNA gene was amplified and sequenced using primers NL1 and NL4 (Kurtzman and Robnett 1998), while the ITS1-5.8S-ITS2 region was amplified and sequenced using primers ITS1 and ITS4 (White et al. 1990). The sequence data assembly and editing were performed using ChromasPro version 1.5. Comparisons with sequences from GenBank were done using BLASTN (Altschul et al. 1997). For phylogenetic analysis, sequences were retrieved either from the NCBI GenBank database (http://www.ncbi.nlm.nih.gov/genbank/) or CBS Collections database (http://www.cbs.knaw.nl/collections/Biolomics.aspx?Table=Yeasts%202011). Sequences were aligned using the multiple alignment program MUSCLE (Edgar 2004). The phylogenetic trees were constructed using the Maximum-likelihood (ML) analysis with the MEGA version 5 program (Tamura et al. 2011), using the general time reversible (GTR) model of nucleotide substitution type with a gamma distribution with invariable sites (G + I).The starting tree for the analysis was generated using NJ/BioNJ and nearest-neighbour interchange (NNI) was used for tree searching. The robustness of branches was assessed by bootstrap analysis of 1,000 replicates.

To estimate genetic distances, the number of base substitutions per site between pairwise alignments was calculated with MEGA version 5, using the Kimura two parameter model (Kimura 1980).

Results and discussion

Vaca et al. (2013) found yeasts in some Antarctic sponge samples, including a strain identified as E2A-C3-II belonging to a novel yeast species from the order Leucosporidiales (Microbotryomycetes, Pucciniomycotina).

The biochemical analysis showed that the strain E2A-C3-II^T, *Leucosporidiella muscorum* and *Leucosporidium golubevii* displayed similar physiological characteristics and can be differentiated by their ability to assimilate D-arabinose, L-arabinose or arbutin (Table 1). On the other hand, strain E2A-C3-II^T can be distinguished from *Leucosporidium drummii* by its ability to assimilate L-sorbose, L-rhamnose, lactose and ribitol (Table 1). The teleomorphic stage (teliospores or basidia) was not observed after the incubation on different culture media and at different temperatures during eight months. Consistent with this observation, dikaryotic stage was not detected with Giemsa staining (data not shown).

No mating experiments were performed because only one strain belonging to the novel species was isolated. Therefore, it will be important to demonstrate if sexual cycle does exist in strain E2A-C3-II^T when more isolates of this species are available. In accordance, we have adopted the use of the suffix "f.a." (for *forma asexualis*, asexual form) in this paper, as suggested by Lachance (2012) and Péter (2012). This nomenclature emphasizes that the description of the novel species is based on its asexual form (anamorph) and highlights the importance to reveal the sexual cycle of strain E2A-C3-II^T in the future. Recently, other researchers have used the same suffix in similar cases (e.g. Groenewald and Smith 2013; Lachance and Kurtzman 2013).

The phylogenetic analysis based on the combined sequences of the ITS region and LSU rDNA gene (D1/D2 domains) shows that strain E2A-C3-II^T clustered with low ML bootstrap support (52 %) with *Leucosporidium drummii* CBS 11562^T, *Leucosporidiella muscorum* CBS 6921^T and *Leucosporidium scottii* group, including *Leucosporidiella creatinivora* and *Leucosporidiella yakutica* (Fig. 1). The analysis based on 5.8S-ITS rDNA sequences alone showed a similar result with higher bootstrap support (90 %) (Online Resource 1). The topology of these trees was coincident with the topology previously published by Yurkov et al. (2012a). In the case of the analysis based on the D1/D2 sequences alone, the novel species clustered (with a low support value) with a group formed by *Leucosporidium drummii* CBS 11562^T, *Leucosporidium drummii* strain K75b, and two

Table 1 Comparison of phenotypic properties of *Leucosporidium escuderoi* f.a. sp. nov. with others recognized species belonging to the order Leucosporidiales

Characteristic	*L. escuderoi*[a]	*L. drummii*[b]	*L. muscorum*[c]	*L. golubevii*[d]	*L. fragaria*[e]	*L. scottii*[f]	*L. yakutica*[g]	*L. creatinivora*[h]	*L. fellii*[i]	*M. intermedium*[j]
Assimilation of										
D-Galactose	d	w/d	d	d/+	+/d	+/d	d	–	–	−/w/d
L-Sorbose	+	–	+	+	+	+	+	+	+	+/d
D-Glucosamine	w	+	d	+	+	+	d	d	+	±
D-Xylose	+	+	+	+	+	+	+	+/w	+	±/d
L-Arabinose	–	–	−/v	+	+	d	–	–	–	–
D-Arabinose	–	–	d	d	+/w/d	d	d	d	+	−/d
L-Rhamnose	–	+	–	–	–	+	+	∓	+	±
Arbutin	–	nd	+	–	+	+	+	+	nd	+
Lactose	w	–	d	+	+	−/v	d	−/w	–	−/w/d
Raffinose	+	+	+	+	+	+	+	+/w	–	–
Ribitol	d	–	d	+	+/w/d	d	d	d	+	±/d
Growth with:										
Cycloheximide0.01 %	+	+	∓	d	–	d	d	d	+	±
Cycloheximide0.1 %	–	nd	–	–	–	–	–	–	+	–

"+" positive, "−" negative, *w* weak, *v* variable, *d* delayed growth, *s* slow growth, *nd* not determined

[a] Data from this study (strain E2A-C3-II^T)

[b] Data from Yurkov et al. (2012a)

[c] Data from CBS database (strain 6921^T) and Sampaio (2011a)

[d] Data from Sampaio et al. (2003)

[e] Data from CBS database(strain CBS 6254^T) and Sampaio (2011a)

[f] Data from CBS database (strain CBS 5930^T) and Sampaio (2011b)

[g] Data from CBS database (strain CBS 8621^T)

[h] Data from CBS database (strainCBS 8620^T) and Sampaio (2011a)

[i] Data from CBS database (strain CBS 7287^T)

[j] Data from CBS database (strains CBS 7226^T, 6522, 6523 and 7281)

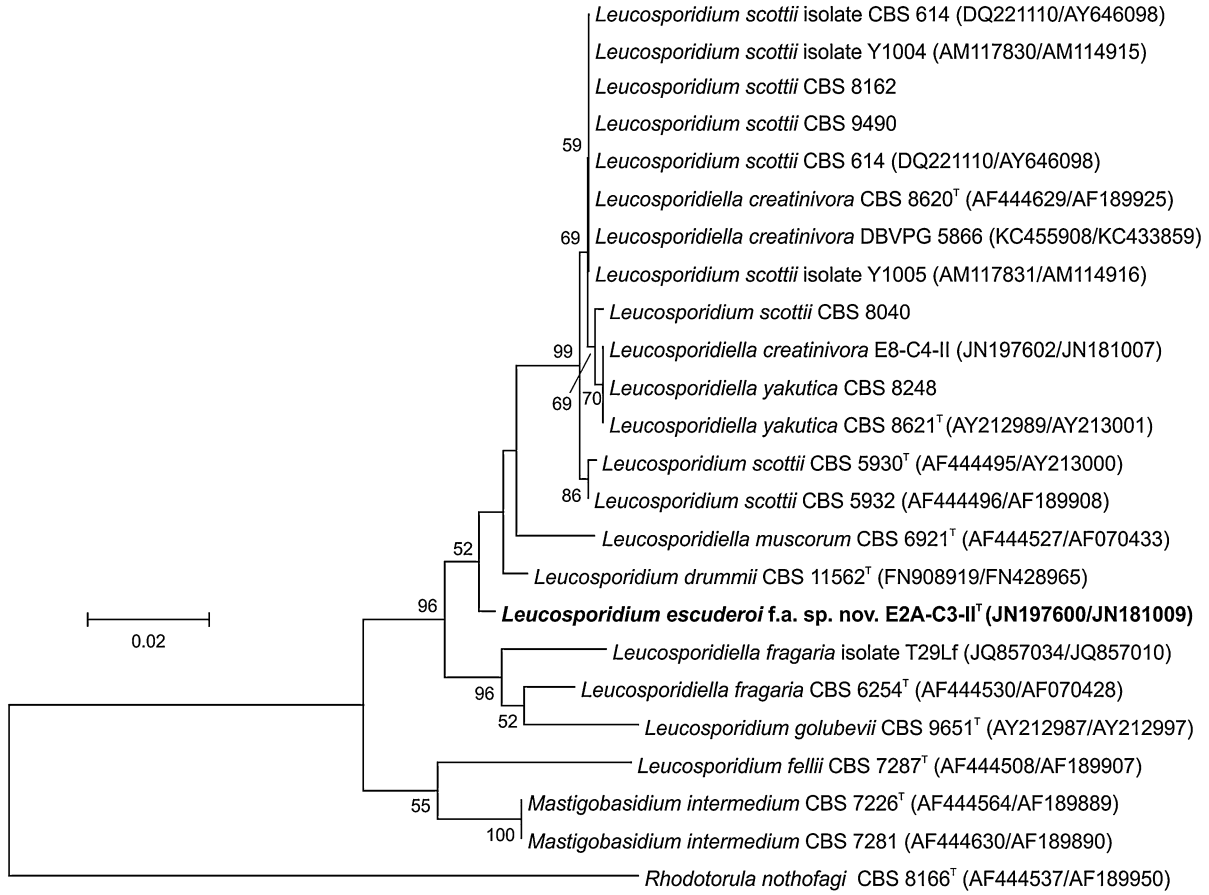

Fig. 1 Maximum-likelihood phylogenetic tree of *Leucosporidium escuderoi* f.a. sp. nov. E2-AC3-II^T and related species of the order Leucosporidiales (Sampaio et al. 2003), based on the 5.8-ITS region and the D1/D2 domain of the LSU rDNA gene. Bootstrap values (>50 %) based on 1,000 replicates are given on each node. GenBank accession numbers of each sequence are shown in *parenthesis*. Sequences without accession numbers were retrieved from the CBS database. *Rhodotorula nothofagi* CBS 8166^T (Microbotryomycetes, Pucciniomycotina) was used as outgroup. *Bar*, number of substitutions per site

unidentified Leucosporidiales sp.strains MS-1 and MS-3 (Online Resource 2). The simultaneous presence in the same clade of *Leucosporidium* species for which sexual state was observed, *Leucosporidium* species f.a., and *Leucosporidiella* species (asexual form) (Fig. 1) suggest the need of a more comprehensive phylogenetic study of all the clade to standardize the species nomenclature.

It is interesting to note that the majority of the strains phylogenetically related to strain E2A-C3-II^T (Fig. 1; Online Resources 1 and 2) have been obtained from soils or soil-related substrates from cold and temperate habitats. *Leucosporidium drummii* CBS 11562^T was isolated from grassland soil in the Biosphere Reserve Schorfheide-Chorin (Germany) (Yurkov et al. 2012a, 2012b) and according to the GenBank database, *Leucosporidium drummii* strain K75b was isolated from sand in the White Sea (Russia). *Leucosporidiella fragaria* (strain T29Lf), *Leucosporidiella creatinivora* (strain T13Lc) and some *Leucosporidium scottii* strains have been isolated from soil samples from Antarctica (Di Menna 1960, 1966; Carrasco et al. 2012; Vero et al. 2013). Leucosporidiales sp. strains MS-1 and MS-3 were isolated from permafrost soils samples in Smith Lake (Alaska) (Panikov and Sizova 2006) and according to the GenBank database, *Leucosporidium* sp. 1 RB-2011 was isolated from root cultures in Albert Mountain, Nantahala National Forest (North Carolina, USA). In the cases of uncultured strains, clone MATC I24 was obtained from ectomycorrhizal root tips of *Betula nana* in Toolik Lake (Alaska) (Deslippe et al. 2012), and clone A 13

Table 2 Pairwise comparison of genetic distance between the D1/D2 domains (upper diagonal) and between the 5.8S-ITS rDNA sequences (lower diagonal) from *Leucosporidium escuderoi* sp. nov. and the closely related type strains[a]

	L. escuderoi	*L. drummii*	*L. muscorum*	*L. golubevii*	*L. fragaria*
L. escuderoi		0.0081	0.0148	0.0198	0.0148
L. drummii	0.0149		0.0165	0.0181	0.0131
L. muscorum	0.020	0.020		0.0182	0.0131
L. golubevii	0.0391	0.0478	0.0582		0.0048
L. fragaria	0.0388	0.0404	0.0613	0.0333	

[a] According to Yurkov et al.(2012a), species delimitation between *L. scottii*, *L. creatinivora* and *L. yakutica* is questionable, so these type strains were not included in the analysis

OTU60 was obtained from humic horizon soil in the Bonanza Creek Long Term Ecological Research (Alaska) (Taylor et al. 2007b). Different to these soil-related yeasts, *Leucosporidiella creatinivora* (strain E8-C4-II) and the novel species E2A-C3-II^T were isolated from Antarctic marine sponges (Vaca et al. 2013). Considering that sponges collect nutrient particles by filtering water, it is reasonable to hypothesize that these macroinvertebrates may also trap (and keep) yeasts that have terrestrial origin.

Previous studies have suggested that nucleotide differences in the D1/D2 domain of the LSU rDNA sequences are useful as a mean of delimiting basidiomycetous yeast species (Fell et al. 2000). In the case of the D1/D2 domain from species closely related with strain E2A-C3-II^T, the differences between the type strains ranged between 3 nucleotide differences (genetic distance of 4.8 nt per 1,000 bp) and 11 nucleotide differences (genetic distance of 18.2 nt per 1,000 bp) (Table 2). Likewise, strain E2A-C3-II^T, showed between 5 and 12 nucleotides differences in comparison to other type strains representing, in terms of genetic distance, between 8.1 and 19.8 nt per 1,000 bp (Table 2). Therefore, we think that sequence- and genetic distance-based delimitation, using the D1/D2 domain, support that strain E2A-C3-II^T represents a new species.

In the case of 5.8S-ITS rDNA sequences, differences between type strains of species closely related with strain E2A-C3-II^T ranged between 14 nucleotide differences (genetic distance of 20 nt per 1,000 bp) and 31 nucleotide differences (genetic distance of 61.3 nt per 1,000 bp) (Table 2). However, when the 5.8S-ITS rDNA sequences from strain E2A-C3-II^T and *Leucosporidium drummii* CBS 11562^T were compared, a lower number of nucleotide differences were observed (9 nucleotide differences; genetic distance of 14.9 nt per 1,000 bp; Table 2). Taking in account this result, we performed a detailed comparison between the D1/D2 domain and 5.8S-ITS rDNA sequences from strain E2A-C3-II^T and *Leucosporidium drummii* strains available in databases (see Online Resources 1 and 2 for details of accession numbers of these sequences). We did not detect any D1/D2 or ITS sequences matching the new yeast species (Online Resources 3 and 4). On the contrary, the D1/D2 domain and 5.8S-ITS rDNA sequences are divergent between strain E2A-C3-II^T and the *Leucosporidium drummii* strains (Online Resources 3 and 4). Therefore, the combined analysis of the two gene regions allows proper differentiation of *Leucosporidium drummii* and the new yeast species.

The description of new species of yeasts based on one strain is viewed differently among current taxonomists and it is commonly accepted that descriptions based on single strain have limitations (Kurtzman 2010; Lachance 2011). Thus, when possible, species descriptions should be based on multiple strains. Antarctica, specially the submarine environment, is difficult to access. In addition, in the Antarctic submarine environment, yeasts seem to be sparse making it difficult to recover multiple strains of the same species. This is supported by reports where the obtainment of a large number of cultivable yeasts from aquatic Antarctic environments is very difficult (for recent examples see Carrasco et al. 2012; Vaca et al. 2013). Thus, the diversity of marine yeasts from Antarctica is largely unknown. The yeast species here described was isolated from an Antarctic marine sponge, a very uncommon environment.

Based on the analysis of phenotypic characteristics, the phylogenetic placement, the nucleotide differences and the genetic distances between sequences, the strain E2A-C3-II^T represents a novel species of the

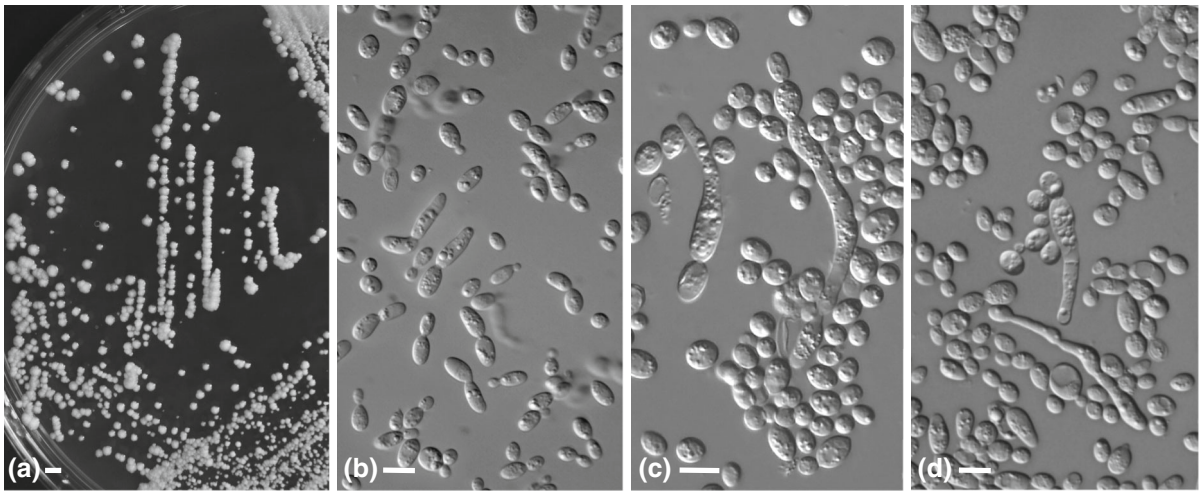

Fig. 2 Morphology of *Leucosporidium escuderoi* f.a. sp. nov. E2A-C3-II^T (=CBS 12734^T =CECT 13080^T). **a** Colonies on ME5 % agar after 7 days at 25 °C. **b-d** Budding cells after 2 days in YM broth (**b**), after 7 days on SG agar (**c**), and after 10 days on MYP agar at 25 °C (**d**). Microscopic characteristics were examined under differential interference contrast (Nomarski) optical microscope. *Bars*, 2 mm (**a**) and 10 μm (**b-d**)

genus *Leucosporidium* isolated from an Antarctic marine sponge, for which the name *Leucosporidium escuderoi* f.a. sp. nov., is proposed.

Description of *Leucosporidium escuderoi* Laich, Chávez & Vaca f.a., sp. nov.

Leucosporidium escuderoi f.a. (es.cu.de'roi N.L. gen. masc. n. *escuderoi* of Escudero, referring to Professor Julio Escudero, an outstanding Chilean international jurist specialized in Antarctic issues, whose name was also used to name the permanent scientific Chilean base in Antarctica where the type strain was isolated; f.a. forma asexualis).

After 7 days on ME5 % agar at 25 °C, colonies are circular with undulate margin (0.98–1.73 mm in diameter), cream colored, pulvinate, butyrous, with smooth and matt surface (Fig. 2a). After 2 days of growth in ME5 % and YM broth at 25 °C, cells are ovoid or usually ellipsoidal (3.8–6.8 × 4.5–10 μm in ME5 % and 3.9–6.8 × 4.8–10.7 μm in YM). In both media the reproduction occurs singly or in pairs by polar budding (Fig. 2b). Pseudohyphae were observed in MYP agar, corn meal agar and SG agar after 15 days at 18 and 25 °C. Pseudohyphae are elongated cylindrical cells or short chains of ovoid and cylindrical cells (Fig. 2c, d). Neither true mycelium nor teliospores were observed. Fermentation ability is negative. It assimilates D-glucose, D-galactose (delayed), L-sorbose, D-glucosamine (weak), D-ribose (weak), D-xylose, sucrose, maltose, α,α-trehalose, methylα-D-glucoside, cellobiose, salicin, lactose (weak), raffinose, melezitose, glycerol, ribitol (delayed), xylitol (delayed), L-arabinitol (delayed), D-glucitol, D-mannitol, D-glucono-δ-lactone, 2-keto-D-gluconate, 5-keto-D-gluconate, D-gluconate, D-glucuronate, D-galacturonate, DL-lactate (delayed), succinate, citrate, propane 1,2 diol (delayed), quinic acid, palatinose, L-malic acid (delayed), tween 80 and ethanol. No growth occurs on L-arabinose, D-arabinose, L-rhamnose, arbutin, melibiose, inulin, starch, erythritol, galactitol, *myo*-inositol, butane 2,3 diol, D-glucarate, D-galactonate and methanol. Nitrogen compound assimilation tests are positive for nitrate, nitrite, ethylamine, L-lysine, cadaverine and creatinine (delayed), but negative for creatine, glucosamine, imidazole and D-tryptophan. It grows on vitamin-free medium or in the presence of cycloheximide (0.01 %). No growth occurs on cycloheximide (0.1 %), acetic acid (1 %) and D-glucose (50 and 60 %). Growth on 10 % NaCl plus 5 % glucose is weak but on 16 % NaCl is negative. Urease hydrolysis and Diazonium blue B reactions are positive. No starch-like substance is produced. Acid production is negative. The maximum temperature for growth is 25 °C.

The type strain of *Leucosporidium escuderoi* is E2A-C3-II^T (=CBS 12734^T =CECT 13080^T), which was isolated from a marine sponge (*Hymeniacidon* Bowerbank, 1858) collected at a depth of six meters in Fildes Bay, King George Island, Chilean Antarctica,

in 2009. It has been deposited in Colección Española de Cultivos Tipo (CECT), Valencia, Spain, as strain CECT 13080^T and Central bureau voor Schimmelcultures (CBS), Utrecht, the Netherlands, as strain CBS 12734^T. The Mycobank accession number is MB 804654, and the nucleotide sequences of D1/D2 domain of the LSU rDNA gene and 5.8S-ITS regions have been deposited in Genbank under the accession numbers JN181009 and JN197600, respectively.

Acknowledgments The authors are grateful to two anonymous reviewers for their valuable suggestions on the manuscript. F. Laich is supported by the "Recursos y Tecnologías Agrarias in coordination with the Comunidades Autónomas del Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica 2004–2007″ program, financed with the involvement of the European Social Fund. R. Chávez was supported by Instituto Antártico Chileno (INACH, Grant G_06–10) and DICYT-USACH. I. Vaca was supported by Grant Fondecyt 11090192 and PBCT PDA 13.

Conflict of interest The authors declare that they have no conflict of interest.

References

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25:3389–3402

Carrasco M, Rozas JM, Barahona S, Alcaíno J, Cifuentes V, Baeza M (2012) Diversity and extracellular enzymatic activities of yeasts isolated from King George Island, the sub-Antarctic region. BMC Microbiol 12:251

Deslippe JR, Hartmann M, Simard SW, Mohn WW (2012) Long-term warming alters the composition of Arctic soil microbial communities. FEMS Microbiol Ecol 82:303–315

Di Menna ME (1960) Yeasts from Antarctica. J Gen Microbiol 23:295–300

Di Menna ME (1966) Yeasts in Antarctic soils. Antonie Van Leeuwenhoek 32:29–38

Duarte AWF, Dayo-Owoyemi I, Nobre FS, Pagnocca FC, Chaud LCS, Pessoa A, Felipe MGA, Sette LD (2013) Taxonomic assessment and enzymes production by yeasts isolated from marine and terrestrial Antarctic samples. Extremophiles 17:1023–1035

Edgar R (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32:1792–1797

Fell JW, Statzell AC, Hunter IL, Phaff HJ (1969) *Leucosporidium* gen. n., the heterobasidiomycetous stage of several yeasts of the genus *Candida*. Antonie Van Leeuwenhoek 35:433–462

Fell JW, Boekhout T, Fonseca A, Scorzetti G, Statzell-Tallman A (2000) Biodiversity and systematics of basidiomycetous yeasts as determined by large-subunit rDNA D1/D2 domain sequence analysis. Int J Syst Evol Microbiol 50:1351–1371

Goto S, Sugiyama J, Lizuka H (1969) A taxonomic study of Antarctic yeasts. Mycologia 61:748–774

Groenewald M, Smith MT (2013) The teleomorph state of *Candida deformans* Langeron, Guerra and description of *Yarrowia yakushimensis* comb. nov. Antonie Van Leeuwenhoek 103:1023–1028

Hentschel U, Usher KM, Taylor MW (2006) Marine sponges as microbial fermenters. FEMS Microbiol Ecol 55:167–177

Kimura M (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16:111–120

Kurtzman CP (2010) Description of new yeast species—is one strain enough? Bull BISMiS 1:17–24

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

Kurtzman C, Fell JW, Boekhout T (2011a) Definition, classification and nomenclature of the yeasts. In: Kurtzman CP, Fell JW, Boekhout T (eds) The yeasts, a taxonomic study, vol 1, 5th edn. Elsevier, New York, pp 3–5

Kurtzman C, Fell JW, Boekhout T, Robert V (2011b) Methods for isolation, phenotypic characterization and maintenance of yeasts. In: Kurtzman CP, Fell JW, Boekhout T (eds) The yeasts, a taxonomic study, vol 1, 5th edn. Elsevier, New York, pp 87–110

Lachance MA (2011) Microbial species descriptions: the importance of multiple strains. Yeast Newsletter website. http://www.uwo.ca/biology/YeastNewsletter/PDFs/Lachance—Importance of multiple strains—IUMS 2011.pdf. Accessed 5 August 2013

Lachance MA (2012) In defense of yeast sexual life cycles: the *forma asexualis*: an informal proposal. Yeast Newsl 61:24–25

Lachance M-A, Kurtzman CP (2013) The yeast genus *Tortispora* gen. nov., description of *Tortispora ganteri* sp. nov., *Tortispora mauiana* f.a., sp. nov., *Tortispora agaves* f.a., sp. nov., *Tortispora sangerardonensis* f.a., sp. nov., *Tortispora cuajiniquilana* f.a., sp. nov., *Tortispora starmeri* f.a., sp. nov. and *Tortispora phaffii* f.a., sp. nov., reassignment of *Candida caseinolytica* to *Tortispora caseinolytica* f.a., comb. nov., emendation of *Botryozyma*, and assignment of *Botryozyma*, *Tortispora* gen. nov. and *Trigonopsis* to the family Trigonopsidaceae fam. nov. Int J Syst Evol Microbiol 63:3104–3114

McNeill J, Barrie FR, Buck WR et al (2012) International Code of Nomenclature for algae, fungi, and plants (Melbourne Code). Regnum Veg 154. A.R.G. Gantner Verlag, Koenigstein

Olson JB, Kellogg CA (2010) Microbial ecology of corals, sponges, and algae in mesophotic coral environments. FEMS Microbiol Ecol 73:17–30

Panikov NS, Sizova MV (2006) Growth kinetics of microorganisms isolated from Alaskan soil and permafrost in solid media frozen down to −35 °C. FEMS Microbiol Ecol 59:500–512

Péter G (2012) In support of the *forma asexualis*. Yeast Newsl 61:25–26

Sampaio JP (2011a) Leucosporidiella Sampaio (2003). In: Kurtzman CP, Fell JW, Boekhout T (eds) The yeasts, a taxonomic study, vol 3, 5th edn. Elsevier, New York, pp 1801–1806

Sampaio JP (2011b) Leucosporidium Fell, Statzell, Hunter and Phaff (1969). In: Kurtzman CP, Fell JW, Boekhout T (eds) The yeasts, a taxonomic study, vol 3, 5th edn. Elsevier, New York, pp 1485–1494

Sampaio JP, Gadanho M, Santos S, Duarte FL, Pais C, Fonseca A, Fell JW (2001) Polyphasic taxonomy of the basidiomycetous yeast genus *Rhodosporidium*: *Rhodosporidium kratochvilovae* and related anamorphic species. Int J Syst Evol Microbiol 51:687–697

Sampaio JP, Gadanho M, Bauer R, Weiß M (2003) Taxonomic studies in the Microbotryomycetidae: *Leucosporidium golubevii* sp. nov., *Leucosporidiella* gen. nov. and the new orders Leucosporidiales and Sporidiobolales. Mycol Prog 2:53–68

Sampaio JP, Golubev WI, Fell JW, Gadanho M, Golubev NW (2004) *Curvibasidium cygneicollum* gen. nov., sp. nov. and *Curvibasidium pallidicorallinum* sp. nov., novel taxa in the Microbotryomycetidae (Urediniomycetes), and their relationship with *Rhodotorula fujisanensis* and *Rhodotorula nothofagi*. Int J Syst Evol Microbiol 54:1401–1407

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28:2731–2739

Taylor MW, Radax R, Steger D, Wagner M (2007a) Sponge-associated microorganisms: evolution, ecology, and biotechnological potential. Microbiol Mol Biol Rev 71:295–347

Taylor DL, Herriott IC, Long J, O'Neill K (2007b) TOPO TA is A-OK: a test of phylogenetic bias in fungal environmental clone library construction. Environ Microbiol 9:1329–1334

Turchetti B, Thomas Hall SR, Connell LB, Branda E, Buzzini P, Theelen B, Müller WH, Boekhout T (2011) Psychrophilic yeasts from Antarctica and European glaciers: description of *Glaciozyma* gen. nov., *Glaciozyma martinii* sp. nov. and *Glaciozyma watsonii* sp. nov. Extremophiles 15:573–586

Vaca I, Faúndez C, Maza F, Paillavil B, Hernández V, Acosta F, Levicán G, Martínez C, Chávez R (2013) Cultivable psychrotolerant yeasts associated with Antarctic marine sponges. World J Microbiol Biotechnol 29:183–189

Vero S, Garmendia G, González MB, Bentancur O, Wisniewski M (2013) Evaluation of yeasts obtained from Antarctic soil samples as biocontrol agents for the management of post-harvest diseases of apple (*Malus* × *domestica*). FEMS Yeast Res 13:189–199

Webster NS, Taylor MW (2012) Marine sponges and their microbial symbionts: love and other relationships. Environ Microbiol 14:335–346

Webster NS, Negri AP, Munro MMHG, Battershill CN (2004) Diverse microbial communities inhabit Antarctic sponges. Environ Microbiol 6:288–300

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR Protocols: a guide to methods and applications. Academic Press, San Diego, pp 315–322

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

Yurkov AM, Schäfer AM, Begerow D (2012a) *Leucosporidium drummii* sp. nov., a member of the Microbotryomycetes isolated from soil. Int J Sys Evol Microbiol 62:728–734

Yurkov AM, Kemler M, Begerow D (2012b) Assessment of yeast diversity in soils under different management regimes. Fungal Ecol 5:24–35