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Short communication

Spencermartinsiella japonica f.a., sp. nov., a novel yeast species isolated from biofilm in a reverse osmosis system

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ABSTRACT

Novel *Spencermartinsiella* strains, JCM 35526^{T} and 261-2C, were isolated from biofilm formed on a reverse osmosis membrane in the phosphate recovery system of a semiconductor factory. Morphological, biochemical, physiological, and chemotaxonomic analyses as well as sequence analysis of the concatenated internal transcribed spacer region and D1/D2 domains of the large subunit of the rRNA gene confirmed that strains JCM 35526^{T} and 261-2C, were distinct from all currently known *Spencermartinsiella* species. The holotype and isotype strains of the new species, which is named *Spencermartinsiella japonica*, are JCM 35526^{T} and MUCL 58310^{I}, respectively.

Keywords: acidic conditions, industrial environment, phylogeny, taxonomy

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The family *Trichomonascaceae*, order *Dipodascales*, currently includes 12 recognized genera: *Crinitomyces*, *Deakozyma*, *Diddensiella*, *Groenewaldozyma*, *Limtongella*, *Saprochaete*, *Starmerella*, *Sugiyamaella*, *Trichomonascus* (asexual morph *Blastbotys*), *Wickerhamiella*, *Zygoascus*, and *Spencermartinsiella* (Groenewald et al., 2023). To date, five species of *Spencermartinsiella* have been described, with all known strains isolated from rotting wood (Dlauchy et al., 2012; Q.C. Guo et al., 2012; X. Guo et al., 2024; Morais et al., 2016; Péter et al., 2011).

This study describes two *Spencermartinsiella* strains (strain JCM 35526^{T}= isolate 243-a22 and isolate 261-2C) isolated from biofilm present on a reverse osmosis (RO) membrane in the phosphate recovery system of a semiconductor factory. The formation of biofilms causes several problems, including pipe contamination, metal corrosion, and membrane fouling in water systems (Mattila-Sandholm & Wirtanen, 1992). Biofilms are three-dimensional aggregates that form when microorganisms attach to the surface of a material and produce an extracellular matrix. They are composed of multiple colonized yeast cells containing a matrix of extracellular polymeric secretions consisting of polysaccharides, proteins, lipids, nucleic acids, and other components that serve as a barrier to protect the cells (Di Pippo et al., 2018). In this study, we describe two strains belonging to a new species (strains JCM 35526^{T} and 261-2C), which were isolated from a biofilm under acidic conditions (pH 2–3), and is proposed the name *Spencermartinsiella japonica* f.a. sp. nov.

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The strain JCM 35526^{T} was isolated on Mar 2020 from a small biofilm-contaminated circulating water obtained from the RO membrane module, which presented at least three different species of yeast. The other strain 261-2C was subsequently isolated on Aug 2021 from a suspension using a small sample of biofilm-contaminated RO membrane. To isolate strain 261-2C, a 20×20 mm piece of membrane was cut out and placed in sterile physiological saline solution (0.85% NaCl) and sonicated for 5 min. Strains JCM 35526^{T} and 261-2C were both isolated by plating the suspensions on Difco™ potato dextrose agar (PDA) (BD; NJ, USA) at pH 5.6, and stock cultures were frozen at −80 °C in 20% glycerol (v/v) until use. The two strains were collected at different time points and from different membrane modules, but both were isolated from the same RO system in the same semiconductor factory located in Mie Prefecture, Japan.

Most of the morphological, physiological, and biochemical characteristics were examined according to the standard methods (Kurtzman et al., 2011). The assimilation of nitrogen compounds was investigated on solid media with starved inocula (Nakase & Suzuki, 1986). Genomic DNA was extracted from yeast cells or mycelia according to the protocol described in Makimura et al. (1994).

The DNA fragment covering the D1/D2 domain of the 26S rRNA gene (LSU D1/D2) and the internal transcribed spacer region, including the 5.8S rRNA gene (ITS) was amplified and sequenced as described in White et al. (1990). The PCR primers ITS5 and NL4 were used to amplify the ITS and the LSU D1/D2 (O'Donnell, 1993). Pairwise comparisons of the sequences with those in the GenBank database were conducted using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) (Altschul et al., 1990, 1997) and align-

ments were then carried out in CLUSTAL X (Thompson et al., 1997). Molecular phylogenetic analysis was performed as described in Bai et al. (2002). The evolutionary history was inferred using the neighbor-joining method (Saitou & Nei, 1987), and the optimal tree obtained, which had a total branch length of 0.63392195, was selected. The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to each branch (Felsenstein, 1985). The phylogenetic tree was drawn to scale, with branch lengths expressed in the same units as those of the evolutionary distances used to infer the tree. Evolutionary distances were computed based on the Tamura−Nei model (Tamura & Nei, 1993) and were expressed as the number of base substitutions per site. This analysis comprises two sets of nucleotide sequences, each consisting of seven sequences from the ITS and LSU regions. These sequences include our two strains as well as five other known *Spencermartinsiella* species. All positions containing gaps and missing data were eliminated, resulting in a total of 969 positions in the final dataset. The alignment datasets and trees have been deposited in TreeBASE under the accession number S31399. Phylogenetical analyses were conducted using MEGA7 (Kumar et al., 2016). Reference sequences were retrieved from GenBank under the accession numbers indicated in the tree (Fig. 1). The sequences of *Diddensiella santjacobensis* (C. Ramírez & A.E. González) G. Péter, Dlauchy & Kurtzman and *Middelhovenomyces tepae* (Grinb.) Kurtzman & Robnett were used as outgroups.

The sequence similarities of the LSU D1/D2 and the ITS were compared among known *Spencermartinsiella* species (Table 1). The results showed that the sequences in strain JCM 35526^{T} differed by 4−20 and 46−56 nucleotide substitutions (mismatch), respectively, from those in related *Spencermartinsiella* species. No nucleotide substitutions were found between strains JCM 35526^{T} and 261-2C in the LSU D1/D2. Strain JCM 35526^{T} differed from strain 261-2C by only one substitution in the ITS (Table 1), but both strains were clustered together with a high degree of confidence based on the concatenated sequence of LSU D1/D2 and the ITS (Fig. 1). The two

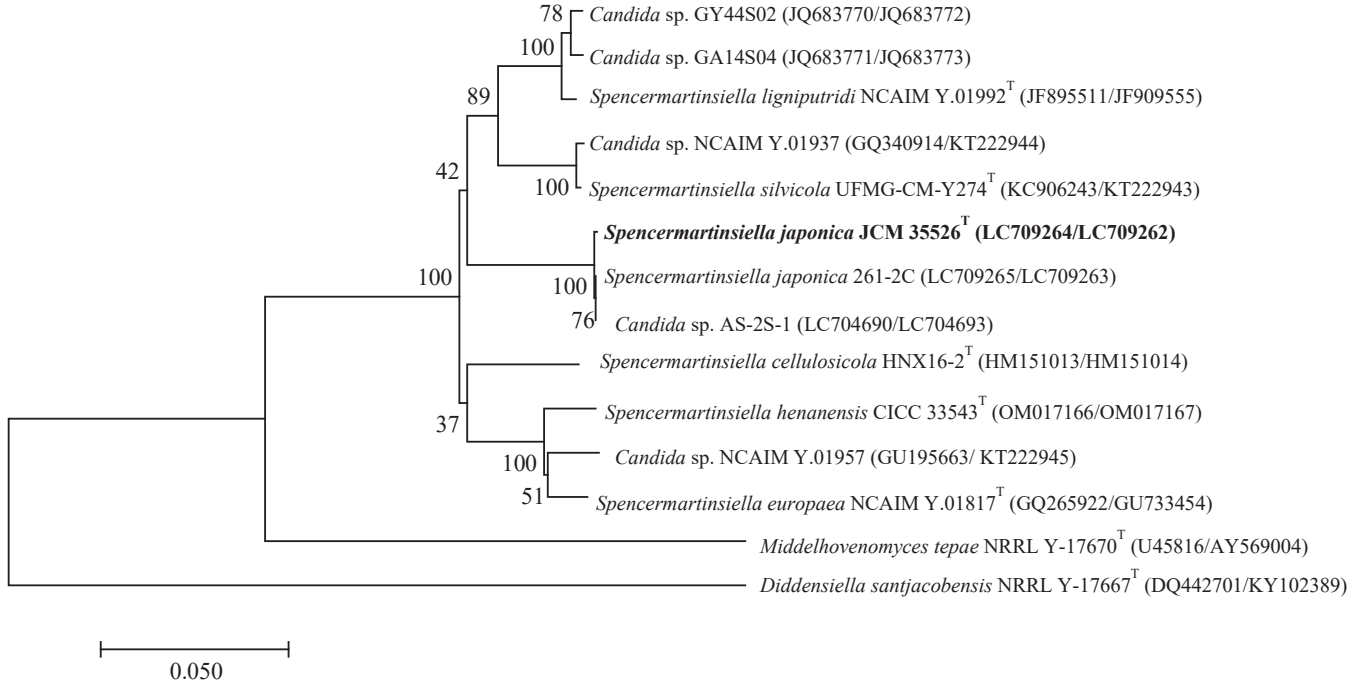

Fig. 1 – The neighbor-joining tree constructed with concatenated sequences of the ITS and LSU D1/D2 domains, showing the phylogenetic position of *Spencermartinsiella japonica* JCM 35526^{T} f.a., sp. nov. within the genus *Spencermartinsiella* and related species. The sequences of *Diddensiella santjacobensis* and *Middelhovenomyces tepae* were used as outgroups. Accession numbers are provided in brackets. *Bar*: 0.05 substitutions per nucleotide position.

Table 1. Number of nucleotide substitutions (mismatches) in the LSU D1/D2 and the ITS gene region sequence among the species most closely related to *Spencermartinsiella japonica* JCM 35526^{T} f.a., sp. nov.

		Number of nucleotide substitutions of the LSU D1/D2 domains						
	strains[a]	1	2	3	4	5	6	7
Number of nucleotide substitutions of the ITS region	1	-	0	4	19	13	13	20
	2	1	-	4	19	13	13	20
	3	53	53	-	16	15	17	17
	4	56	58	50	-	11	11	3
	5	46	45	39	26	-	9	12
	6	50	48	36	44	34	-	11
	7	53	55	40	33	44	45	-

Each value indicates the nucleotide substitutions (nt) between the indicated species pairs. All positions containing gaps and missing data were eliminated.

[a] 1, *S. japonica* f.a., sp. nov. JCM 35526^{T} (LC709264/LC709262); 2, *S. japonica* 261-2C (LC709265/LC709263); 3, *S. cellulosicola* HNX 16-2^{T} (HM151013/HM151014); 4, *S. europaea* NCAIM Y. 01817^{T} (GQ265922/GU733454); 5, *S. ligniputridi* NCAIM Y.01992^{T} (JF895511/JF909555); 6, *S. silvicola* UFMG-CM-Y274^{T} (KC906243/KT222943); 7, *S. henanensis* CICC 33543^{T} (OM017166/OM017167)

strains were also similar in terms of their morphological, phylogenetic, and physiological characteristics, which indicated that they are conspecific. They differed from their phylogenetically closest relative, *Spencermartinsiella cellulosicola* (F.Y. Bai & X.Y. Guo) C.G. Morais, C.A. Lara, E. Oliveira, G. Péter, Dlauchy & C.A. Rosa, in terms of their assimilation of D-arabinose, L-arabinose, cadaverine hydrochloride, glycerol, hexadecane, lactose, and methyl-α-glucoside. Molecular and phylogenetic comparisons demonstrated that strains JCM 35526^{T} and 261-2C belong to a novel ascomycetous yeast species. Pseudohyphae were present in JCM 35526^{T} but hardly any septate hyphae formed on PDA during incubation for 5 d at 25 °C (Fig. 2). Septate hyphae in this strain were observed in rare occasions on V8 juice agar medium after incubation for 10 d at 25 °C (Fig. 3). Ascospore formation was not observed either single or mixed cultures with other cell strains including isolate 261-2C. To date, all species of the genus *Spencermartinsiella* have been isolated from rotten wood samples (Dlauchy et al., 2012; Q.C. Guo et al., 2012; X. Guo et al., 2024; Morais et al., 2016; Péter et al., 2011). In contrast, the novel species described in this study was obtained from an industrial environment.

Based on the analyses of morphological, biochemical, phylogenetical, and chemotaxonomic analysis, it was assigned to genus *Spencermartinsiella* according to the current yeast taxonomy (Dlauchy et al., 2012; Q.C. Guo et al., 2012; X. Guo et al., 2024; Kurtzman et al., 2011; Morais et al., 2016; Péter et al., 2011). We propose the name *Spencermartinsiella japonica* f.a., sp. nov. for the species is described as follows.

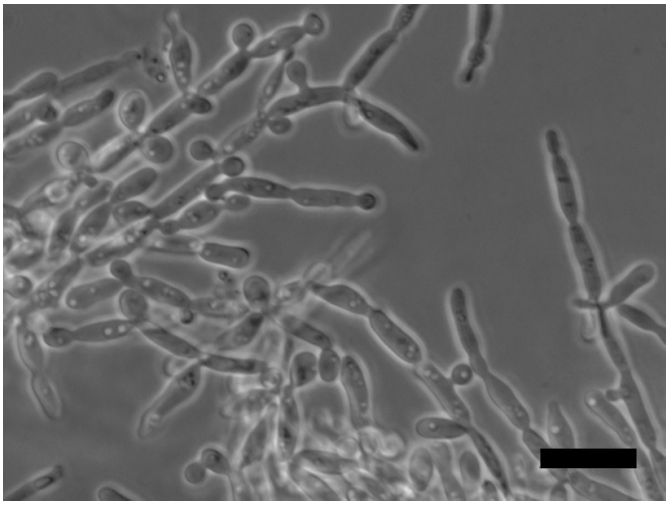

Fig. 2 – Pseudohyphae of *Spencermartinsiella japonica* JCM 35526^{T} f.a., sp. nov. after incubation on potato dextrose agar for 5 d at 25 °C. *Bar*: 10 μm.

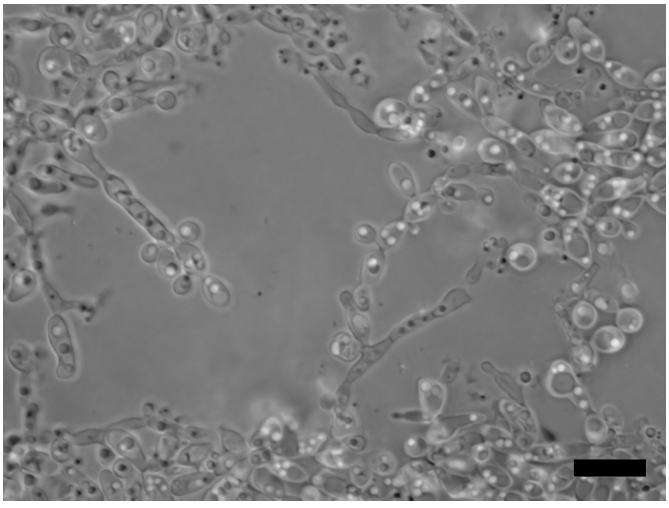

Fig. 3 – Septate hyphae and blastoconidia of *Spencermartinsiella japonica* JCM 35526^{T} f.a., sp. nov. after incubation on V8 juice agar for 10 d at 25 °C. *Bar*: 10 μm.

Spencermartinsiella japonica Doi, Mishima & Ikeda f.a., sp. nov. Figs. 2, 3

Mycobank no.: MB 851072.

Type: JAPAN, Mie Prefecture, obtained from biofilm present on a reverse osmosis (RO) membrane in the phosphate recovery system of a semiconductor factory in Mar 2020, isolated by H. Doi and A. Mishima. The holotype strain JCM 35526^{T} (isotype MUCL 58310^{I}) was preserved in a metabolically inactive state by lyophilization in the Microbe Division, Japan Collection of Microorganisms (JCM), RIKEN Bioresource Research Center, Ibaraki, Japan. The lyophilized metabolically inactive isotype MUCL 58310^{I} was preserved in the BCCM/MUCL Agro-food and Environmental Fungi Collection, Belgium.

Gene sequences from the holotype: LC709264 (LSU D1/D2), and LC709262 (ITS).

Etymology: *Japonica* refers to Japan as the location where the species was originally isolated.

After incubation for 5 d at 25 °C on PDA, cells are spherical to elongated spindle-shaped (1.1–2.1 × 2.0–13.6 μm), occurring singly or in pairs (Fig. 2). In slide culture on PDA, potato carrot agar, yeast malt extract agar, corn meal agar and V8 juice agar, septate hyphae and blastoconidia are present, but septate hyphae rarely form on V8 juice agar after more than 10 d at 25 °C (Fig. 3). Budding is multilateral (Fig. 2). After 5 d of growth on PDA at 25 °C, the streak culture is soft, white in color, glistening, raise with a smooth surface, and has an entire margin. Ascospores are not observed after 3 wk cultivation at 25 °C on PDA, potato carrot agar, yeast malt extract agar, corn meal agar, and V8 juice agar. Fermentation is absent. *N*-acetyl-D-glucosamine, D-arabinose (variable), L-arabinose, cellobiose, erythritol, ethanol (variable), galactitol, galactose, D-glucitol (variable), D-glucosamine, glucose, inulin, maltose, D-mannitol (variable), melibiose, melezitose, methyl-α-glucoside, raffinose, ribitol, L-rhamnose (variable), D-ribose (variable), salicin, L-sorbose, sucrose, trehalose (variable), xylitol, and D-xylose are assimilated, but gluconolactone, glycerol, *myo*-inositol, lactose, methanol, and soluble starch are not. Citrate and succinate are assimilated, but D-gluconate, glucuronate calcium salt, glucuronate potassium salt, 2-keto-D-gluconate, and DL-lactate are not. Hexadecane is assimilated. Ammonium sulfate, cadaverine hydrochloride, creatinine (variable), ethylamine hydrochloride (variable), L-lysine (variable), and L-proline (variable) are assimilated, but potassium nitrate, sodium nitrate, sodium nitrite, creatinine, and imidazole are not assimilated as a sole source of nitrogen. Growth is observed in vitamin-free, 10% NaCl medium and 50% glucose (variable) but is negative in the presence of 1% acetate. Starch-like compounds and acids are not produced. Diazonium blue B reaction is negative. Urease and gelatinase activity also negative. Growth well under acidic conditions (pH2–6). The maximum growth temperature is 36 °C.

Disclosure

The authors declare that there are no conflicts of interest. This study received no specific grant from any funding agency.

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