**Allodekkera sacchari** gen. nov., sp. nov., a yeast species in the Saccharomycetales isolated from a sugar factory

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**Abstract**

Three yeast isolates, G5-5(5), G5-9(3) and G5-9(4), were obtained from the sugar cane juice and waste from sugar production plant (Korach Industry Co., Ltd) in Korach province, Thailand. They were found to belong to the same species based on DNA sequence identity of the small subunit ribosomal RNA gene (SSU) and the D1/D2 region of the large subunit rRNA gene (LSU D1/D2). A BLAST search of the GenBank database revealed they had 93% nucleotide sequence identity to *Dekkera bruxellensis* for the SSU (1742 bp), but their LSU D1/D2 sequence (572 bp) showed less than 90% identity to all available sequences in the database. Phylogenetic analyses with neighbour-joining and maximum-parsimony methods using the aligned LSU D1/D2 and SSU sequences (a total of 2072 positions after removal of gaps) inferred that the three isolates were separated from all known taxa in the Saccharomycetales, and that the neighbouring taxa were species of *Dekkera/Brettanomyces*. Physiological and biochemical characters revealed distinct differences between the three isolates and *Dekkera/Brettanomyces* species, including the ability to assimilate several carbon sources and inability to ferment glucose. Thus, isolates G5-5(5), G5-9(3) and G5-9(4) should be assigned to a novel taxon, for which the name *Allodekkera sacchari* gen. nov., sp. nov. is proposed. The type strain of the type species is G5-5(5) (*=CBS 14167†=JCM 18455†=TISTR 5950†), with MycoBank number MB815477 (for the genus) and MB817751 (for the species). Two additional strains of the species are G5-9 (3) (*=JCM 18456) and G5-9(4) (*=JCM 18457).

Thailand is a hotspot of yeast bioresources because of its variety of climates and ecological systems, with many novel micro-organisms, including yeasts, having been discovered. These novel yeasts have included species from the genera *Metschnikowia*, *Occultifur*, *Ogataea*, *Pseudoyzyma*, *Wickerhamiella*, *Wickerhamomyces*, *Zygosaccharomyces* and *Candida* within the *Ambrosiozyma*, *Ogataea* and *Yamadazyma* clades [1–11]. Some of these novel yeast species were isolated from the phylloplane of sugar cane, one of the most popular and widely planted crops in Thailand [5, 6, 8, 10, 11].

Sugar production in Thailand is economically very important, as evidenced by the fact that it is the world’s fourth largest producer of sugar cane with a production volume of up to 98 million tons per year [12]. Most of the waste after sugar cane juice extraction is left unused, but it is a promising source of potential biomass energy, such as that from bioethanol production. We attempted to isolate ethanol-producing yeasts at a relatively high temperature (40°C) from sugar cane juice and the waste sediment left after the juice extraction from 10 factories in Thailand, since any such yeast may potentially be of value to utilize the waste from sugar production for bioethanol production. As a result, the isolation of the ethanol-producing *Pichia kudriavzevii* strain G1-4(1) and *Torulaspora globosa* strain G1-12(3) was reported [13]. At another of these 10 factories, we obtained six yeast isolates that could grow at 40°C, classified as two *Saccharomyces cerevisiae* strains, one *Pichia kudriavzevii* strain and three non-ethanol-producing undescribed yeast isolates. In this study, we demonstrate that...

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**Keywords:** *Allodekkera sacchari*; ascomycetous yeasts; sugar production plant.

**Abbreviations:** ITS, internal transcribed spacer; LSU D1/D2, D1/D2 domain of the large subunit ribosomal RNA gene; MP, maximum-parsimony; NJ, neighbour-joining; SSU, small subunit ribosomal RNA gene.

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The GenBank/EMBL/DDBJ accession numbers for the LSU D1/D2 sequence of the *A. sacchari* isolates G5-5(5), G5-9(3) and G5-9(4) are AB898502, AB898503 and AB898504, respectively, while those for the SSU sequence are AB915874, AB915875 and AB915876, respectively. The GenBank/EMBL/DDBJ accession number for the ITS region including 5.8S ribosomal RNA gene sequence of strain G5-5(5) is LC172229.

One supplementary figure is available with the online Supplementary Material.
these three strains are members of the same species and are placed in an unaffiliated Saccharomycetales (Saccharomycetes, Ascomycota) clade near Dekkera/Brettanomyces. The isolation and physiological characteristics of these three isolates are also reported.

Sugar cane juice and wastes were collected in March 2010 from 10 factories of Korach Industry Co., Ltd, Thailand (15° 07' 31.1" N 102° 26' 52.3" E). The materials collected from the factories were enriched for yeast as specified below. One gram of waste sediment or 1 ml sugar cane juice was added into 5 ml selection medium [1 % (w/v) glucose, 0.3 % (w/v) yeast extract, 0.3 % (w/v) peptone, 3.0 % (v/v) ethanol and 0.01 % (w/v) chloramphenicol, pH 4.5] in a test tube of 150 mm [14], and incubated at 40°C under an oxygen-limited condition, using the Candle jar method, for 7 days [13]. The liquid was then streaked on a high-sugar (glucose-rich) YPD agar plate [10 % (w/v) glucose, 0.3 % (w/v) yeast extract, 0.3 % (w/v) peptone and 2.0 % (w/v) agar, pH 4.5] and incubated under the same condition for 7 days with isolation of single colonies. Pure cultures were kept on YPD agar slants at 4°C.

Morphological, physiological and biochemical characteristics were examined as described in The Yeasts, A Taxonomic Study 5th ed. [15], and were compared with those published for related species. Colony morphology on YM agar slants was examined after incubation for one month at 25°C. Ascospore formation was tested using individual cultures grown on each of 5% malt extract, corn meal, YM, PDA, McElroy’s acetate and Gorodkowa agars or as cocultures of each combination of the three isolates on YM agar slants at 17 and 25°C. The cultures were examined under a light microscope each week for the first month and then monthly for the next four months. Assimilation of nitrogen sources was examined on agar media. Growth tests at various temperatures were performed in YM broth using metal block baths. The major ubiquinone present in isolate G5-5(5)T was determined by reversed-phase thin-layer chromatography in comparison to the standard ubiquinones of Q-8 and Q-9 [16], prepared from Candida rhagii JCM 9839T and Candida sojae JCM 1644T, respectively.

Genomic DNA was extracted according to Maniatis et al. [17] and then the LSU D1/D2, SSU, and ITS fragments were amplified by PCR using the following primers: F63 (5′-GCA TATCAAATAGCGGAAGGAAG-3′) and LR3 (5′-GGG TCCGTGTTCAAGACG-3′) for the LSU D1/D2 fragment [18]; NS1 (forward, 5′-GTAGCTCATATGCTGTTC-3′) and NS2 (reverse, 5′-TCCGAGGTTCACCTACCGGA-3′) for the SSU fragment [19]; and ITS5 (forward, 5′-GGAAGTAAAGTGTAACAAGG-3′) and ITS4 (reverse, 5′-TCCCTCGGTATTGATGC-3′) for the ITS fragment [20]. PCR for the ITS region was performed with the following modified program: an initial denaturation at 94°C for 3 min, followed by 40 cycles of 30 s at 94°C, 30 s at 55°C and 30 s at 72°C, and a final extension of 5 min at 72°C. The purified PCR products were directly sequenced by ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) according to the manufacturer’s instruction on each strand using the primers F63 and LR3 for the LSU D1/D2 fragment; NS1, NS2 (reverse, 5′-GGCTGCTGCGAACCATG-3′), NS7 (forward, 5′-GGAGGATAAACACACCTGTCG-3′) and NS8 for the SSU fragment; and ITS5, ITS2 (reverse, 5′-GCTGGTCTTCTGATGC-3′), ITS3 (forward, 5′-GATCCTGAAAGAAGCAGGC-3′) and ITS4 for the ITS fragment. The ITS sequence was determined only for G5-5(5)T as a reference information. The sequences obtained were compared to those available in the International Nucleotide Sequence Database (INSDB) by BLASTn search. The sequences were aligned with related species using CLUSTAL X version 1.8 software [21]. Phylogenetic tree reconstruction using the neighbour-joining (NJ) and maximum-parsimony (MP) methods, and evolutionary analyses were performed using MEGA6 software [22]. Evolutionary distances were calculated using Kimura’s 2-parameter method [23], where gaps were completely deleted. The MP analysis used the Tree-Bisection-Regrafting (TBR) algorithm [24]. All positions containing gaps and missing data were eliminated.

The yeast isolate G5-5(5)T (=CBS 14167T=JCM 18455T=TISTR 5950T) was obtained from the sugar cane juice from a sugar cane press-processing machine, while isolates G5-9(3) (=JCM 18456) and G5-9(4) (=JCM 18457) were obtained from the sugar cane waste sediments.

The DNA sequences of the LSU D1/D2 (572 bp) and SSU (1742 bp) fragments of isolates G5-5(5)T, G5-9(3) and G5-9 (4) were found to be identical among the three isolates, making them likely to be the same species. A BLASTn search of the GenBank database revealed the isolates had 93% nucleotide sequence identity to Dekkera bruxellensis for the SSU (1742 bp), but their LSU D1/D2 sequence (572 bp) showed less than 90% identity to all available sequences in the database. Phylogenetic analysis using the SSU plus LSU D1/D2 sequence of isolates G5-5(5)T, G5-9(3) and G5-9(4) together with 54 species in the order Saccharomycetales revealed that the three novel isolates clustered with Dekkera/Brettanomyces species rather than with Ogataea in both the NJ and MP trees (Fig. S1, available in the online Supplementary Material). Previous research reported that Dekkera bruxellensis was associated with the genera Pichia, Ogataea, Ambrosiozyma, Kregervanrija and Saturnispora based on phylogenetic analyses using five gene sequences [25]. The authors, however, expressed doubt about the association due to the placement of D. bruxellensis on a long branch. Our phylogenetic analyses supported the association of Pichia, Kregervanrija and Saturnispora with Dekkera/Brettanomyces (Fig. S1). Next, MP trees were reconstructed using the SSU plus LSU D1/D2 sequences of the three isolates [G5-5(5)T, G5-9(3) and G5-9(4)], and species from the genera Dekkera/Brettanomyces, Pichia, Kregervanrija and Saturnispora as well as their related species. Again, isolates G5-5(5)T, G5-9(3) and G5-9(4) clustered with but were separate from the Dekkera/Brettanomyces species clade (Fig. 1). It has previously been reported that 40–50 base differences in the LSU D1/D2 sequence were most frequently detected when
sequence similarities were compared among genera in the Saccharomycetales [26]. Thus, isolates G5-5(5), G5-9(3) and G5-9(4) are likely to represent a novel taxon at the genus level, of which the placement appeared to be near to the Dekkera/Brettanomyces species clade. The major ubiquinone of isolate G5-5(5) was Q-9, which would also support the association with Dekkera/Brettanomyces species.

The cell morphologies of isolates G5-5(5), G5-9(3) and G5-9(4) resembled each other. Cells were elongate and rarely spherical, being 1.0–2.0 μm wide and 3.0–35.0 μm long (Fig. 2). The streak culture was raised, cream to white, smooth, dull and butyrous. The margin was entire or fringed with filaments. Pseudomyecia bearing bastaconidia abundantly developed under the coverslip in all three isolates (Fig. 2). These morphological features were similar to those of Dekkera/Brettanomyces species.

Dekkera/Brettanomyces species normally grow slowly and are short-lived, whereas isolates G5-5(5), G5-9(3) and G5-9(4) grew quite fast at 25–42 °C. Dekkera anomala and D. bruxellensis cells directly transform into asci, producing one to four ascospores that are hat-shaped or spherical with tangential brims [27]. In contrast, no ascospore formation was detected in G5-5(5), G5-9(3) and G5-9(4) when cultured singly on several different media (5% malt extract, corn meal, YM, PDA, McClary’s agar) or when cocultured (crossed) in any combination of the three isolates on YM agar slants.

The differential physiological characters between the three isolates and Dekkera/Brettanomyces species are summarized in Table 1. The three isolates were distinguished from Dekkera/Brettanomyces species by their ability to assimilate several...
carbon sources and by their inability to ferment D-glucose (Table 1).

As evidenced by the phylogenetic and phenotypic data presented above, the three strains should be classified as representatives of a novel taxon in the Saccharomycetales, for which the name *Allodekkerasacchari* gen. nov., sp. nov. is here proposed.

**DESCRIPTION OF ALLODEKKERA GEN. NOV.**

*JUTAKANOKE, ENDOH, TAKASHIMA, OHKUMA, TANASUPAWAT AND AKARACHARANYA*

*Allodekkerasacchari*(Al.lo.dek’ke.ri. Gr. adj. *allo* different; N.L. fem. n. *Dekkera* a yeast genus; N.L. fem. n. *Allodekkerasacchari* other *Dekkera*, referring to the related genus *Dekkera*).

Growth is by budding and from formation of pseudohyphae. No ascosporic state is known. Sugar fermentation is absent. Growth occurs with several carbohydrates, including hexoses, disaccharides, alcohols, sugar alcohols and organic acids. Nitrate may be utilized as a sole source of nitrogen. Phylogenetic analysis resolves this genus from other members of Saccharomycetales.

Phylogenetic placement: Saccharomycetales, Saccharomycotina, Ascomycota. From the analysis presented in Figs 1 and S1, the genus *Allodekkerasacchari* is related to *Dekkera/Brettanomyces*.

The MycoBank number is MB815477.

Type species: *Allodekkerasacchari* Jutakanoke et al. sp. nov.

**DESCRIPTION OF ALLODEKKERA SACCHARI, SP. NOV.**

*JUTAKANOKE, ENDOH, TAKASHIMA, OHKUMA, TANASUPAWAT AND AKARACHARANYA*

*Allodekkerasacchari*(sac’cha.ri. L. gen. n. *sacchari* of sugar).

After growth for 3 days in YM broth at 25 °C, cells are elongate (1.0–2.0 µm wide and 3.0–35.0 µm long, rarely spherical and reproduce by budding. Abundant pseudomycelia bearing blastoconidia develop under the coverslip in Dalmat plate culture on corn meal agar after 7 days at 25 °C. An ascosporic state is not known. Streak culture on YM agar after 7 days at 25 °C is raised, cream to white, smooth, dull and butyrous. The margin is entire or apparently fringed with filaments which are microscopically observed like Fig. 2(c). D-Glucose, sucrose, maltose, trehalose (slow or latent), melezitose (slow), ethanol, glycerol (weak or slow), ribitol (latent), D-mannitol, D-glucitol (may be latent), methyl α-D-glucoside (may be slow), DL-lactic acid (weak or slow), succinic acid, citric acid, xyitol (slow or may be negative) and N-acetyl-D-glucosamine are assimilated as a sole source of carbon. D-Galactose, L-sorbose, cellobiose, lactose, melibiose, raffinose, inulin, soluble starch, D-xyllose, D-arabinose, L-arabinose, D-ribose, L-rhamnose, *meso*-erythritol, galactitol, methanol, salicin, glucono-δ-lactone, 2-keto-D-gluconic acid, 5-keto-D-gluconic acid, *myo*-inositol, D-glucuronic acid, D-galacturonic acid, D-gluconic acid, D-glucoronolactone, L-arabinitol, quinic acid, saccharic acid, propylene glycol, butane-2,3-diol, xylo-oligosaccharide, and butyrous. The margin is entire or apparently fringed with filaments which are microscopically observed like Fig. 2(c). D-Glucose, sucrose, maltose, trehalose (slow or latent), melezitose (slow), ethanol, glycerol (weak or slow), ribitol (latent), D-mannitol, D-glucitol (may be latent), methyl α-D-glucoside (may be slow), DL-lactic acid (weak or slow), succinic acid, citric acid, xyitol (slow or may be negative) and N-acetyl-D-glucosamine are assimilated as a sole source of carbon. D-Galactose, L-sorbose, cellobiose, lactose, melibiose, raffinose, inulin, soluble starch, D-xyllose, D-arabinose, L-arabinose, D-ribose, L-rhamnose, *meso*-erythritol, galactitol, methanol, salicin, glucono-δ-lactone, 2-keto-D-gluconic acid, 5-keto-D-gluconic acid, *myo*-inositol, D-glucuronic acid, D-galacturonic acid, D-gluconic acid, D-glucoronolactone, L-arabinitol, quinic acid, saccharic acid, propylene glycol, butane-2,3-diol, xylo-oligosaccharide, and butyrous. The margin is entire or apparently fringed with filaments which are microscopically observed like Fig. 2(c). D-Glucose, sucrose, maltose, trehalose (slow or latent), melezitose (slow), ethanol, glycerol (weak or slow), ribitol (latent), D-mannitol, D-glucitol (may be latent), methyl α-D-glucoside (may be slow), DL-lactic acid (weak or slow), succinic acid, citric acid, xyitol (slow or may be negative) and N-acetyl-D-glucosamine are assimilated as a sole source of carbon. D-Galactose, L-sorbose, cellobiose, lactose, melibiose, raffinose, inulin, soluble starch, D-xyllose, D-arabinose, L-arabinose, D-ribose, L-rhamnose, *meso*-erythritol, galactitol, methanol, salicin, glucono-δ-lactone, 2-keto-D-glucionic acid, 5-keto-D-glucoric acid, *myo*-inositol, D-glucuronic acid, D-galacturonic acid, D-gluconic acid, D-glucoronolactone, L-arabinitol, quinic acid, saccharic acid, propylene glycol, butane-2,3-diol, xylo-oligosaccharide,
n-decane and n-hexadecane are not assimilated as a sole source of carbon. Nitrate, nitrite (weak), ethylamine, L-lysine, cadaverine and D-glucosamine (weak) are assimilated as a sole source of nitrogen, while creatine, creatinine, imidazole and D-tryptophan are not. Growth occurs at 42 °C, but not at 45 °C. Gelatin is not liquefied. Arbutin is not split. Acid production from glucose is negative. Urea hydrolysis and starch formation are negative. Growth does not occur in the presence of 0.01% (w/v) cycloheximide, 10% (w/v) NaCl, 1% (v/v) acetic acid or 50% (w/v) glucose. No growth occurs in vitamin-free medium. Diazonium Blue B reaction is negative. The major ubiquinone is Q-9. Fermentation is absent.

The type strain G5-5(5) (=CBS 14167T =JCM 18455T =TISTR 5950T) was isolated from sugar cane juice collected from a sugar cane press-processing machine in Korach Sugar Company, Ltd., Korach province, Thailand. The ex-type culture of JCM 18455T is preserved by lyophilization in the Microbe Division/Japan Collection of Microorganisms (JCM), RIKEN BioResource Center, Tsukuba, Japan.

The MycoBank number is MB817751.

Two additional strains of the species are G5-9(3) (=JCM 18456) and G5-9(4) (=JCM 18457).

Yeast isolation was performed from sugar cane juice and waste collected from 10 sugar production factories in Thailand. However, we could not find any other isolate of *Allodekker a sacchari*. This may indicate that isolation of *Allodekker a sacchari* from sugar production-related sources was accidental. Thus, the ecology of the species is unknown. *Allodekker a sacchari* is currently unique in terms of its phylogenetic position, so the finding of a related species is necessary to disclose the genus and higher taxonomic delineation around *Allodekker a sacchari* and the Dekkera/Brettanomyces species.

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**Conflicts of interest**
The authors declare that there are no conflicts of interest.

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