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Isolation and characterization of three unrecorded yeast species in the order *Tremellales* from soil, wildflowers, and strawberries in Korea

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The purpose of this study was to isolate and identify wild yeasts from soil in Busan, wildflowers in Okcheon-gun, and strawberries in Jeju, Republic of Korea. Among the isolated strains, three were unrecorded in the Republic of Korea. To identify wild yeast strains, pairwise sequence comparisons of D1/D2 region of the 26S rRNA gene sequence were done using Basic Local Alignment Search Tool (BLAST). The cell morphologies were observed by phase contrast microscope and assimilation test are done using API 20C AUX kit. All strains were assigned to the phylum *Basidiomycota*. The two unrecorded yeast strains, BS-6 and PFL-3, belong to the genus *Hannaella* (family *Bulleribasidiaceae*, order *Tremellales*, class *Tremellomycetes*). Strain PST-43 belongs to the genus *Papiliotrema* (family *Rhynchogastremataceae*, order *Tremellales*, class *Tremellomycetes*). All three strains had oval shaped and polar budding cells. This research showed the morphological and biochemical properties of the three unreported yeast species in Korea.

Keywords: 26s rRNA, *Tremellales*, unrecorded yeasts

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Introduction

The genus *Hannaella* is a basidiomycetous yeast in the family *Bulleribasidiaceae* (order *Tremellales*, class *Tremellomycetes*, phylum *Basidiomycota*). *Hannaella* species were isolated from Leaf of *Parthenocissus* sp., air and sediments from the deep-sea floor of Suruga Bay, Japan (Nagahama *et al*., 2003; Wang and Bai, 2008; Li *et al*., 2020). The genus *Hannaella* comprises twelve distinct species and has *Hannaella sinensis* as a type species (https://www.mycobank.org). The genus *Hannaella* belongs to the family *Bulleribasidiaceae*, and the typical characteristics associated with the family *Bulleribasidiaceae* include pseudohyphae and true hyphae maybe present, ballistoconidia present or not. If formed, they are rotationally symmetric, turbinate, flabelliform, globose or subglobese. Budding cells present and cells are oval, clamp connections are absent. Colonies are cream to yellowish, mucoid, smooth and shiny. Additionally, fermentation usually absent and diazonium blue B and urease reaction are positive. The major CoQ system is CoQ-10 (Wang and Bai, 2008).

The genus *Papiliotrema* is a basidiomycetous yeast in the family *Rhynchogastremataceae* (order *Tremellales*, class *Tremellomycetes*, phylum *Basidiomycota*). *Papiliotrema* species were isolated from flower of *Miconia* sp., water sample in Brazil, and bromeliad leaves (Machado Pagani *et al*., 2016). The genus *Papiliotrema* comprises

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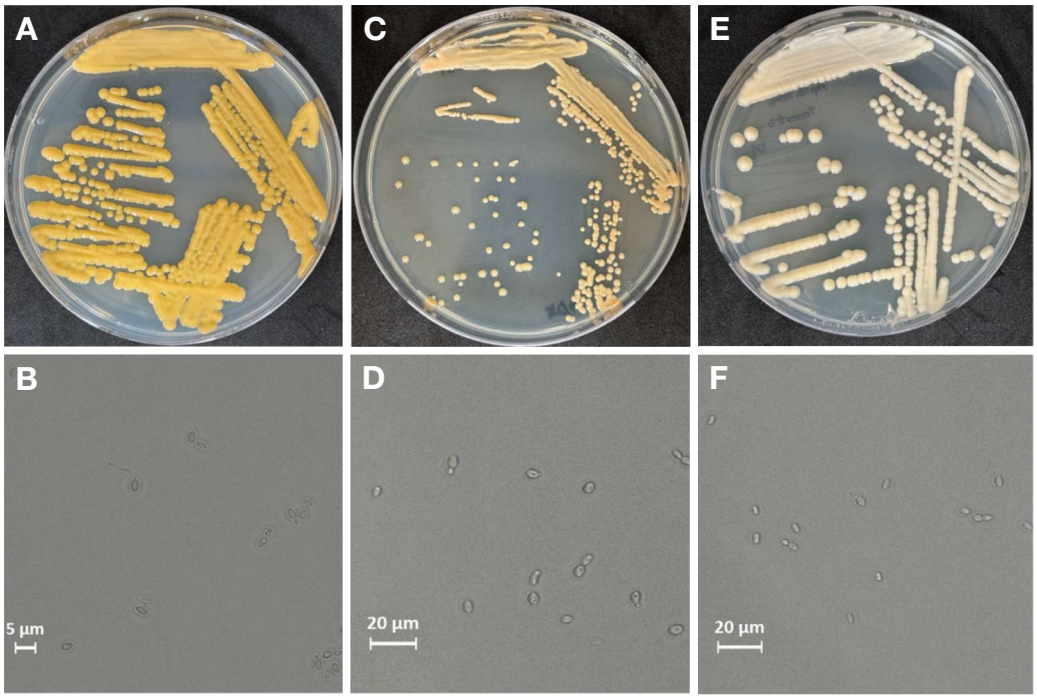


Fig. 1. Morphology from cells from the unrecorded strain *H. pagnoccae* BS-6 incubated at 25°C, and strains *H. sinensis* PFL-3 and P. *baii* PST-43 incubated at 10°C. All strains were grown after 3 days on YPD agar. The colonies of *H. pagnoccae* BS-6 (A), *H. sinensis* PFL-3 (B), and *P. baii* PST-43 (C). The budding cells of *H. pagnoccae* BS-6 (C), *H. sinensis* PFL-3 (D), and *P. baii* PST-43 (F). Bars: 5 μm (B) and 20 μm (D, F).

twelve distinct species and has *Papiliotrema bandonii* as a type species (https://www.mycobank.org). The genus *Papiliotrema* belongs to the family *Rhynchogastremataceae*, and the typical characteristics associated with the family *Rhynchogastremataceae* include pseudohyphae and true hyphae maybe present, basidiocarps maybe present. If present, minute, ca. 0.5 mm in diameter. Colonies are pale to brownish yellow. Budding cells present, clamp connections and haustorial branches on dikaryotic hyphae present. Ballistoconidia can be formed. Sexual reproduction observed in some species. Additionally, fermentation absent and nitrate usually not utilized. The major CoQ system is CoQ-10 (Liu *et al*., 2015).

In this study, wild yeasts were isolated from soil in Busan, wildflowers in Okcheon-gun, and strawberries in Jeju, Republic of Korea. As a result of isolation and identification, a number of strains were found to be previously reported species, and three strains were identified as unrecorded in Korea. These unrecorded strains were assigned to the genera *Hannaella* and *Papiliotrema*. In this study, their phenotypic characteristics were also examined to support species identification.

Materials and Methods

Samples were collected from three different regions in the Republic of Korea. Strain BS-6 was isolated from a wildflower in Haeundae-gu, Busan (35°10′43.4″N, 129°8′13.6″E) on January 12, 2024. Strain PFL-3 was obtained from a wildflower in Okcheon-gun, Chungcheongbuk-do (36°13′40.2″N, 127°37′14.8″E) on January 19, 2024. Strain PST-43 was isolated from a strawberry in Jeju-si, Jeju-do (33°27′54.8″N, 126°29′09.7″E) on January 10, 2024. The samples were serially diluted in distilled water and the suspension was spread in a Yeast Extract Peptone Dextrose (YPD) agar (Difco, USA) and incubated at 25°C for 3 days. The strains are preserved in a metabolically inactive state at the Korean Agricultural Culture Collection, Korea.

The cell morphologies of strains were done by the LEICA (DM500), using yeast strains incubated in YPD agar for 3 days. Phase contrast microscope images and the colonies of the strains BS-6, PFL-3 and PST-43 are shown in Fig. 1. To characterize the biochemical features, API 20C AUX (bioMérieux) were tested according to the manufacturer's instructions.

The genomic DNA were extracted after incubated on YPD agar for 3 days. The D1/D2 region of the 26S rRNA gene sequence was amplified by PCR using NL1 (5′-GCA TATCAATAAGCGGAGGA AAAG-3′) and NL4 (5′-GGT CCGTGTTTCAAGACGG-3′) primers (Kurtzman and Robnett, 1998).

Pairwise sequence comparisons were made using the Basic Local Alignment Search Tool (BLAST) (Altschul *et*

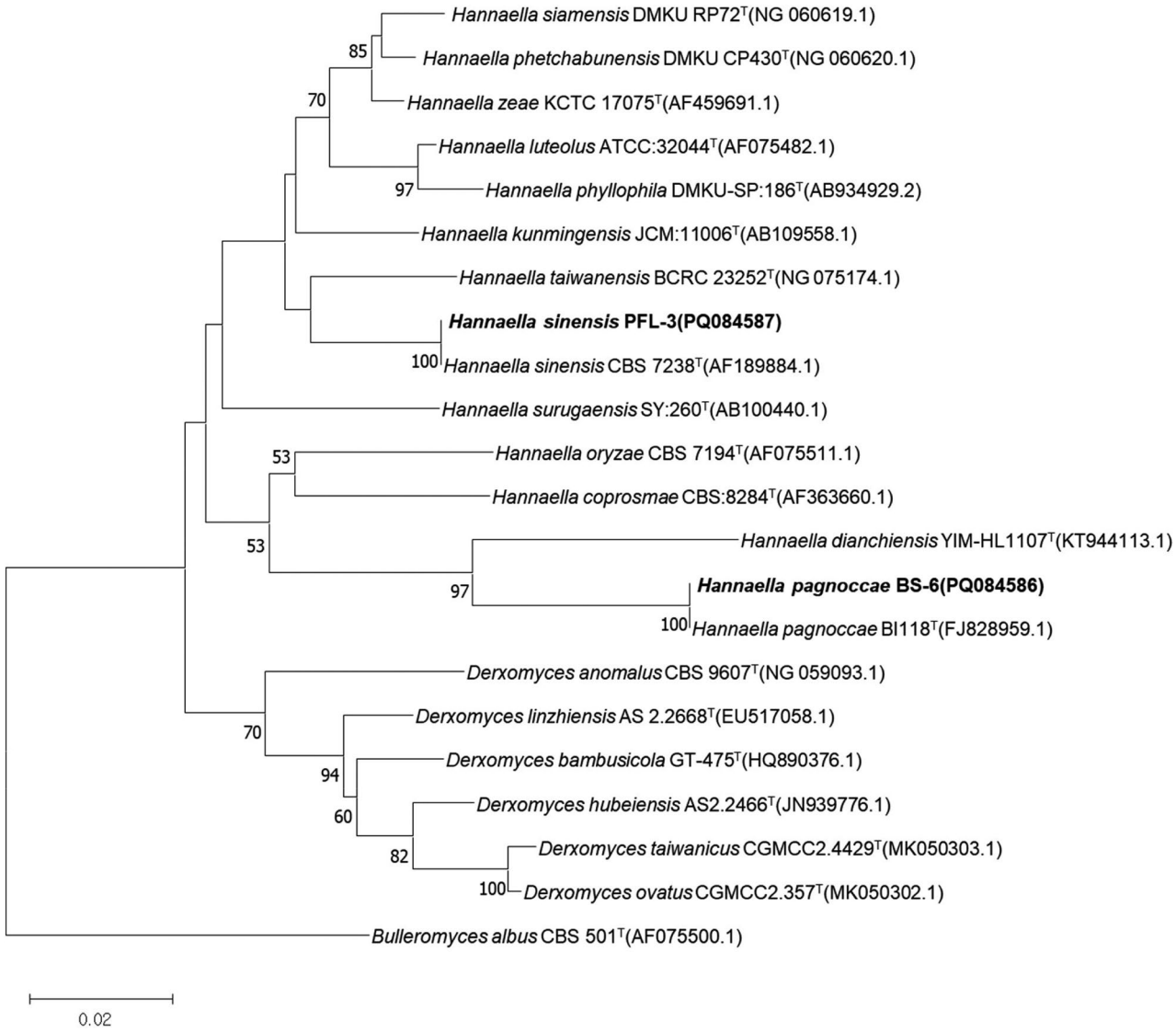


Fig. 2. A Neighbor-joining phylogenetic tree reconstructed from a comparative analysis of 26S rRNA gene sequences showing the relationships of strains BS-6 and PFL-3 with closely related species. Bootstrap values (>50%) based on neighbor-joining methods are shown at the branch nodes. Bar, 0.02 substitutions per nucleotide position.

al., 1997) and to align with the sequences of related species retrieved from GenBank. The MYCOBANK (https://www.mycobank.org/) database identified strain types for each species and close strains gene sequence were obtained from the NCBI (https://www.ncbi.nlm.nih.gov/) for 26s rDNA in a row (Fell *et al*., 2000).

The phylogenetic trees based on the D1/D2 domain of LSU rRNA gene sequence were reconstructed by the neighboring joining algorithm of MEGA 11 program (Tamura *et al*., 2021) The evolutionary distance was calculated using the two-parameter model of Kimura (Kimura, 1983) for neighborhood bond analysis and the confidence level of the class was estimated through bootstrap analysis (1,000 replicates) (Felsenstein, 1985).

Results and Discussion

Ten wild yeasts strains were collected from soil in Busan, wildflowers in Okcheon-gun, and strawberries in Jeju, Republic of Korea. The yeast strains were identified by analyzing D1/D2 domain of 26s rRNA gene sequence similarities that were calculated by the NCBI BLAST. Result of identification based on the D1/D2 domain of 26s rRNA gene sequence, three yeast strains were identified as domestic unrecorded yeast species. The taxonomic composition and identification results are listed in Table 1.

All strains were assigned to the families *Cystofilobasidiaceae* (1 strain), *Rhynchogastremataceae* (4 strains), *Bulleribasidiaceae* (2 strains), *Bulleraceae* (2 strains) and

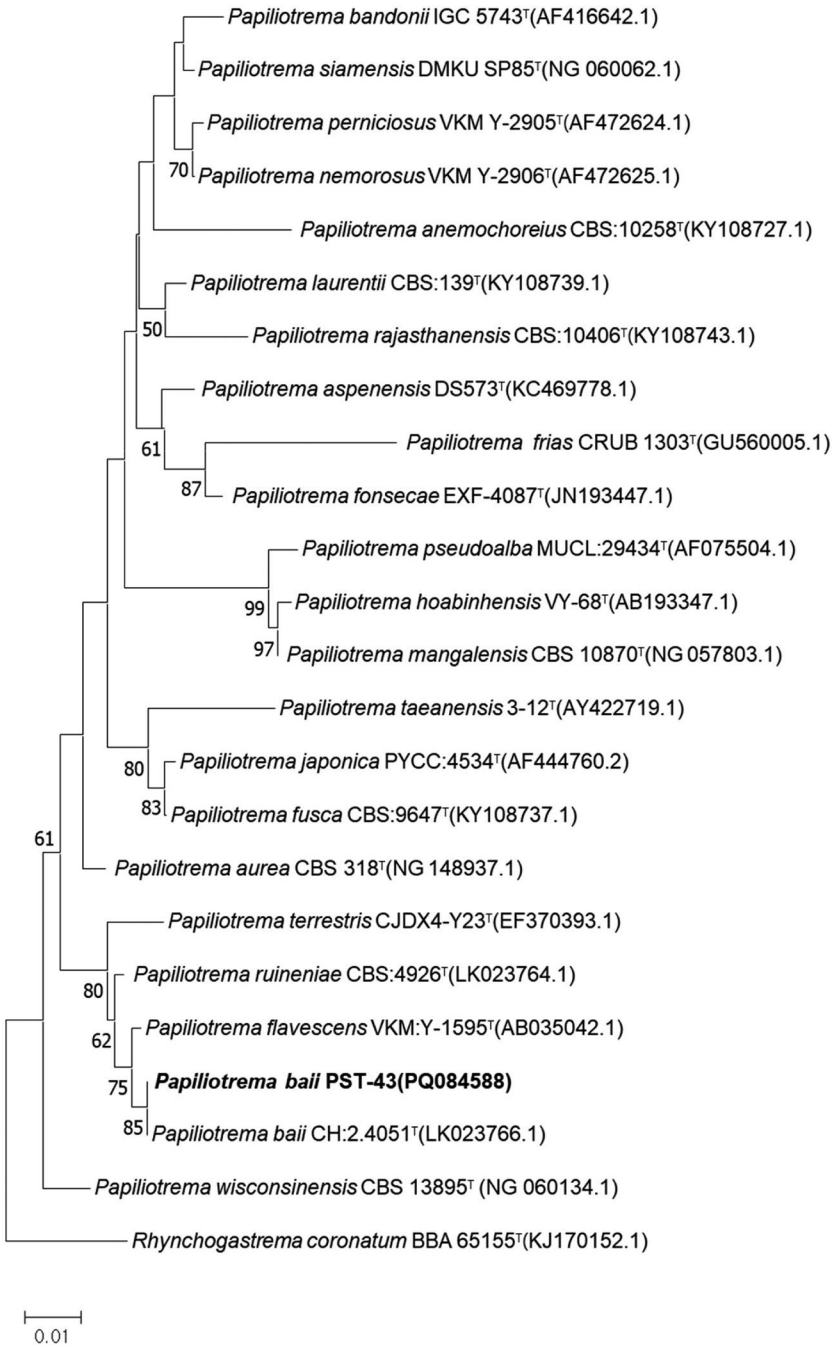


Fig. 3. A Neighbor-joining phylogenetic tree reconstructed from a comparative analysis of 26S rRNA gene sequences showing the relationships of strain PST-43 with closely related species. Bootstrap values (>50%) based on neighbor-joining methods are shown at the branch nodes. Bar, 0.01 substitutions per nucleotide position.

Filobasidiaceae (1 strain) of the phylum *Basidiomycota*. The unrecorded yeast strains BS-6 and PFL-3 belong to the phylum *Basidiomycota* and the genus *Hannaella*, strain PST-43 belong to the phylum *Basidiomycota* and the genus *Papiliotrema*. The phylogenetic tree shows that the isolated strains are closely related to the *Hannaella* and *Papiliotrema* species with the highest 26S rRNA gene sequence similarity (Figs. 2 and 3) and thus supports close relationships, respectively.

Description of *Hannaella pagnoccae* BS-6

Colonies are convex, smooth, and yellow colored after 3 days of incubation on YPD agar at 25°C. In the API

Table 1. Yeasts isolated strains from soil in Republic of Korea.

Phylum	Class	Order	Family	Strain ID	Most closely related species	26S rRNA similarity	Source	Record in Korea
		Cystofilobasidiales	*Cystofilobasidiaceae*	MST-12	*Cystofilobasidium infirmominiatum*	617/618 (99.84%)	Strawberry	Reported
				PST-15	*Papiliotrema flavescens*	564/566 (99.65%)	Strawberry	Reported
				PST-43	*Papiliotrema baii*	554/554 (100.00%)	Strawberry	Unreported
			Rhynchogastremataceae	BS-3	*Papiliotrema horticola*	522/524 (99.61%)	Soil	Reported
		Tremellales		BS-5	*Papiliotrema horticola*	522/524 (99.61%)	Soil	Reported
Basidiomycota	*Tremellomycetes*			BS-6	*Hannaella pagnoccae*	511/511 (100.00%)	Soil	Unreported
			Bulleribasidiaceae	PFL-3	*Hannaella sinensis*	616/616 (100.00%)	Wildflowers	Unreported
				PST-25	*Bullera alba*	607/609 (99.67%)	Strawberry	Reported
			Bulleraceae	PST-35	*Bullera alba*	609/612 (99.67%)	Strawberry	Reported
		Filobasidiales						
			Filobasidiaceae	MFL-3	*Naganishia liquefaciens*	604/604 (100.00%)	Wildflowers	Reported

All strains were cultured under 25°C for 3 days.

Table 2. Phenotypic characteristics that differentiate *H. pagnoccae* BS-6, *H. sinensis* PFL-3 and related species and strain.

	1	2	3	4
Growth on				
Temp (°C)	10−30	10−30	20−25	19−25
Assimilation of				
Sucrose	+	+	+	+
Raffinose	+	+	+	+
Galactose	+	+	+	+
Trehalose	+	+	+	+
Maltose	+	+	+	+
Melezitose	+	+	+	+
Methyl-α-D-glucoside	−	−	+	+
Cellobiose	w	+	+	+
D-Xylose	+	+	+	+
L-Arabinose	+	+	+	+
Glycerol	−	−	v	+
N-Acetyl-D-Glucosamine	−	w	−	ND
2-Keto-D-Gluconate	+	+	ND	+
Adonitol	+	w	+	v
Xylitol	w	w	+	+
Inositol	−	−	+	+
D-Sorbitol	+	+	−	ND
D-Lactose (bovine origin)	−	−	v	−

Strains: 1, *H. pagnoccae* BS-6; 2, *H. sinensis* PFL-3; 3, *H. pagnoccae* BI118^{T}; 4, *H. sinensis* CBS 7238^{T}.
+, positive; w, weakly positive; −, negative; v, variable; ND, No Data.

20C AUX test, strain BS-6 is positive for glucose, calcium 2-keto-gluconate, L-arabinose, D-xylose, adonitol, D-galactose, D-sorbitol, D-maltose, D-saccharose, D-trehalose, D-melezitose, D-raffinose; weak positive for xylitol, D-cellobiose, but negative glycerol, inositiol, methyl-α-D-glucopyranoside, *N*-acetyl-glucosamine, D-lactose (Table 2).

Strain BS-6 (KCTC 37293) was isolated from soil collected in Busan, Republic of Korea.

Description of *Hannaella sinensis* PFL-3

Colonies are convex, smooth, and cream yellow colored after 3 days of incubation on YPD agar at 25°C. In the API 20C AUX test, strain PFL-3 is positive for glucose, calcium 2-keto-gluconate, L-arabinose, D-xylose, D-galactose, D-sorbitol, D-cellobiose, D-maltose, D-saccharose, D-trehalose, D-melezitose, D-raffinose; weak positive for adonitol, xylitol, *N*-acetyl-glucosamine but, negative for glycerol, inositol, methyl-α-D-glucopyranoside, D-lactose (Table 2).

Strain PFL-3 (KCTC 37302) was isolated from wildflowers collected in Okcheon-gun, Chungcheongbuk-do, Republic of Korea.

Table 3. Phenotypic characteristics that differentiate *P. baii* PST-43 and related species and strain.

	1	2	3
Growth on			
Temp (°C)	10−30	25−30	19−25
Assimilation of			
Sucrose	+	+	+
Raffinose	+	+	+
Galactose	+	+	+
Trehalose	+	+	+
Maltose	+	+	+
Melezitose	+	+	+
Methyl-α-D-glucoside	−	+	v
Cellobiose	+	+	+
D-Xylose	+	+	+
L-Arabinose	+	+	+
Glycerol	+	+	v
N-Acetyl-D-Glucosamine	−	ND	−
2-Keto-D-Gluconate	+	+	+
Adonitol	+	+	+
Xylitol	+	+	+
Inositol	+	+	+
D-Sorbitol	+	v	v
D-Lactose (bovine origin)	+	+	+

Strains: 1, *P. baii* PST-43; 2, *P. baii* PYCC 6532^T; 3, *P. flavescens* CBS 942^T.
+, positive; −, negative; v, variable; ND, No Data.

Description of *Papiliotrema baii* PST-43

Colonies are convex, smooth, and cream colored after 3 days of incubation on YPD agar at 25°C. In the API 20C AUX test, strain PST-43 is positive for glucose, glycerol, calcium 2-keto-gluconate, L-arabinose, D-xylose, adonitol, xylitol, D-galactose, inositol, D-sorbitol, D-cellobiose, D-lactose, D-maltose, D-saccharose, D-trehalose, D-melezitose, D-raffinose. But, negative for methyl-α-D-glucopyranoside, *N*-acetyl-glucosamine (Table 3).

Strain PST-43 (KCTC 37303) was isolated from strawberry collected in Jeju, Republic of Korea.

Conflicts of Interest

The authors of this paper have no affiliation with any interests and are solely responsible for the paper.

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