RESEARCH NOTE

Mycobiology 40(4) : 255-257 (2012)
http://dx.doi.org/10.5941/MYCO.2012.40.4.255

pISSN 1229-8093
eISSN 2092-9323

Two New Species of *Cryptococcus* sp. and *Candida* sp. from Wild Flowers in Korea

Jin-Hong Min[1], Min-Gu Kang[1], Jin-Ju Ryu[1], Hyang-Burm Lee[2], Chang-Mu Kim[3], Ha-Kun Kim[1] and Jong-Soo Lee[1]*

[1]*Department of Biomedicinal Science and Biotechnology, Paichai University, Daejeon 302-735, Korea*
[2]*Division of Applied Bioscience and Biotechnology, Chonnam National University, Gwangju 500-757, Korea*
[3]*National Institute of Biological Resources, Korea (Environmental Research Complex), Incheon 404-708, Korea*

(Received November 19, 2012. Revised November 26, 2012. Accepted December 3, 2012)

Among 80 types of yeast isolated from wild flowers in Daejeon, Korea, two species that have not yet been identified by phylogenetic analysis of the internal transcribed spacer-2 (ITS2) genes and 26S rDNA sequences were identified as *Candida* sp. 44-C-1 and *Cryptococcus* sp. 9-D-1. Neither of the newly identified species formed ascospores, while *Candida* sp. 44-C-1 formed pseudomycelium and *Cryptococcus* sp. 9-D-1 did not.

KEYWORDS : 26S rDNA sequence, *Candida* sp. 44-C-1, *Cryptococcus* sp. 9-D-1, Internal transcribed spacer 2, Phylogenetic analysis

Several species of yeast belonging to the GRAS strain have long been used in the preparation of alcoholic beverages and foods such as bread and soy sauce. Since yeast have been reported to produce bioactive compounds with effects such as anti-hypertension [1, 2], anti-dementia [3], and anti-angiogenesis [4-9], the use of yeast for the production bioactive agents in alternative drugs and functional foods has been investigated.

Despite many studies of various yeasts isolated from fermented foods including alcohol fermentation products [10-13], little is known about yeasts present on wild flowers and fruits in the natural environment in Korea.

In a previous study [14], we isolated and identified 80 types of yeast from wild flowers in the City of Daejeon, Korea and its vicinity. Among the isolated yeasts, two novel species that had not yet been identified by phylogenetic analyses were recovered. These novel yeasts are characterized in the present study.

Yeasts were isolated and identified as followed. Wild flowers from mountains or fields in and around Daejeon, Korea were collected and suspended in sterilized distilled water. The supernatants were then inoculated in yeast extract-peptone-dextrose agar medium (10 g yeast extract, 20 g peptone, 20 g dextrose, 15 g agar and 1 L of distilled water) containing antibacterial streptomycin (50 μg/mL) and ampicillin (50 μg/mL) and incubated for 2 days at 30°C.

Extraction of the genomic DNA from the isolated yeasts was accomplished using a Genomic DNA prep kit for yeast (Solgent, Daejeon, Korea). Primers ITS1 and ITS4 were used for amplification of the internal transcribed spacer (ITS) region [14, 15]. The D1/D2 region of 26S rRNA was amplified by PCR using NL-1 (5'-GCA TAT CAA TAA GCG GAG GAA AAG-3') and NL-4 (5'-GGT CCG TGT TTC AAG ACG G-3') as the forward and reverse primers, respectively. Amplification reactions were performed in a total volume of 25 μL containing 2.5 μL 10 × PCR buffer, 0.5 μL 10 mM dNTPs, 0.5 μL of each primer at 20 pmol/μL, 0.13 μL Taq DNA polymerase (5 U/μL). PCR was performed using a GeneAmp PCR system 9700 (Applied Biosystem, Foster City, CA, USA) with and initial denaturation stage of 5 min at 95°C, followed by 30 cycles of denaturation for 30 sec at 95°C, annealing for 1 min at 52°C, extension for 1 min at 72°C and a final extension at 72°C for 5 min. Amplification products were analyzed by gel electrophoresis on 1.5% agarose gel using a 1 kb DNA ladder as a marker. A gel extraction kit (Qiagen, Valencia, CA, USA) was used for purification of the amplified PCR products and DNA strands of the amplified DNA fragments were then sequenced with the same PCR primers. Sequences of the amplified gene for each isolated yeast were merged in a single nucleotide alignment. Multiple sequence alignment by Florence Corpet was used for alignment of the resulting sequence and relevant sequences available in the GenBank

*Corresponding author <E-mail : biotech8@pcu.ac.kr>

Table 1. ITS2 region and 26S rDNA sequence analysis of the new yeasts

Isolated no.	Putative species	Related Genbank accession No.	Identity (%)	Remarks
9-D-1[a]	*Cryptococcus* sp. SJ8L05	FJ153175.1	253/254 (99)	ITS2 region
	Cryptococcus sp. KCTC 17072	AF459689.1	612/612 (100)	26S rDNA
44-C-1[b]	*Candida* sp. TrB1-1	AY559447.1	320/323 (99)	ITS2 region
	Candida sp. TrB1-1	AY562397.1	570/573 (99)	26S rDNA

ITS, internal transcribed spacer.
[a]GenBank accession No. KC242237.
[b]GenBank accession No. KC242238.

database maintained by the NCBI with manual adjustment when necessary.

Screening for new species among yeasts isolated from wild flowers. Among 80 yeasts isolated from wild flowers in Daejeon, Korea, 78 were identified based on ITS gene sequence analysis [14, 15]. However, *Candida* sp. 44-C-1 (GenBank accession No. KC242238) and *Cryptococcus* sp. 9-D-1 (GenBank accession No. KC242237) were only identified as *Candida* sp. TrB1-1 (GenBank accession No. AY562397.0) and *Cryptococcus* sp. KCTC 17072 (GenBank accession No. AF459689.1) based on 26S rDNA sequence analysis (Table 1). Furthermore, these organisms were only identified *Candida* sp. TrB1-1 (GenBank accession No. AY559471.1) and *Cryptococcus* sp. SJ8L05 (GenBank accession No. FJ153175.1) based on ITS2 region sequence analysis (Table 1). Therefore, we concluded that these two organisms were novel yeasts. A phylogenetic tree of these new yeasts is shown in Figs. 1 and 2.

Cryptococcus sp. 9-D-1 and *Candida* sp. 44-C-1 were isolated from *Rhododendron mucronulatum* and *Albizia julibrissin* Durazz, respectively, in Daejeon, Korea.

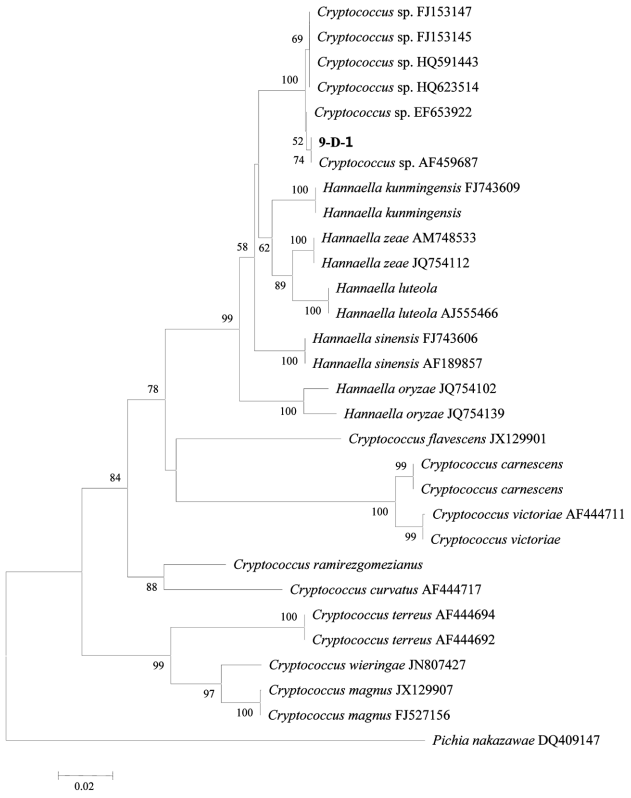

Fig. 1. Neighbor-joining phylogenetic tree based on the D1/D2 region of the 26S rDNA sequence of *Cryptococcus* sp. 9-D-1 isolated from *Rhododendron mucronulatum* in Daejeon, Korea.

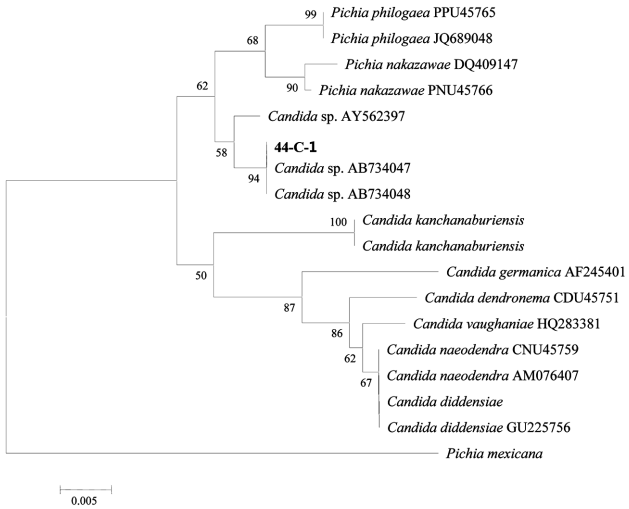

Fig. 2. Neighbor-joining phylogenetic tree based on the D1/D2 region of the 26S rDNA sequence of *Candida* sp. 44-C-1 isolated from *Albizia julibrissin* Durazz in Daejeon, Korea.

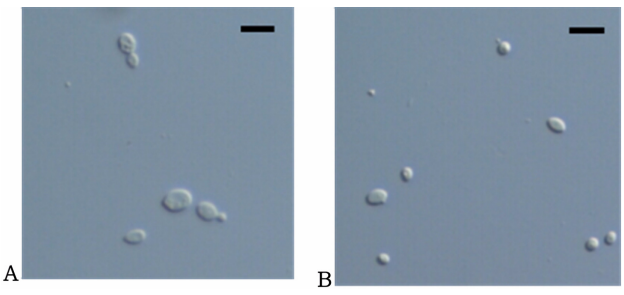

Fig. 3. Microscopic features of newly identified yeasts. A, *Cryptococcus* sp. 9-D-1; B, *Candida* sp. 44-C-1 (scale bars: A, B = 5 μm).

Table 2. Morphological characteristics of yeast recovered from wild flowers of Daejeon, Korea

Characteristics	*Cryptococcus* sp. 9-D-1	*Candida* sp. 44-C-1
Shape	O	E
Vegetalle reproduction	MB	MB
Size (μm)	4.0~5.0 × 3.0~4.5	2.5~4.0 × 3.5~4.0
Ascospore	–	–
Pseudomycelium	–	+

O, oval; E, ellipsoidal; MB, multilateral budding.

Morphological characteristics of new yeasts strains. Taxonomic descriptions and microbiological structures of unrecorded *Candida* sp. 44-C-1 and *Cryptococcus* sp. 9-D-1 are shown in Fig. 3 and Table 2. *Cryptococcus* sp. 9-D-1 was oval and did not form ascospores or pseudomycelium. However, *Candida* sp. 44-C-1 was ellipsoidal, and formed pseudomycelium, but not ascospores.

Acknowledgements

This work was supported by the Korean Indigenous Species Research Project of the National Institute of Biological Resources (NIBR), Ministry of Environment, Republic of Korea.

References

1. Kim JH, Lee DH, Jeong SC, Chung KS, Lee JS. Characterization of antihypertensive angiotensin I-converting enzyme inhibitor from *Saccharomyces cerevisiae*. J Microbiol Biotechnol 2004;14:1318-23.
2. Jeong SC, Kim JH, Kim NM, Lee JS. Production of antihypertensive angiotensin I-converting enzyme inhibitor from *Malassezia pachydermatis* G-14. Mycobiology 2005; 33:142-6.
3. Lee DH, Lee DH, Lee JS. Characterization of a new antidementia β-secretase inhibitory peptide from *Saccharomyces cerevisiae*. Enzyme Microb Technol 2007;42:83-8.
4. Jeong SC, Lee DH, Lee JS. Production and characterization of an anti-angiogenic agent from *Saccharomyces cerevisiae* K-7. J Micriobiol Biotechnol 2006;16:1904-11.
5. Jang IT, Kim YH, Yi SH, Lim SI, Lee JS. Screening of a new fibrinolytic substances-producing yeast. Kor J Mycol 2011;39:227-8.
6. Jang IT, Kim YH, Kang MG, Yi SH, Lim SI, Lee JS. Production of tyrosinase inhibitor from *Saccharomyces cerevisiae*. Kor J Mycol 2012;40:60-4.
7. Lee DH, Lee JS, Yi SH, Lee JS. Production of the acetycholinesterase inhibitor from *Yarrowia lipolytica* S-3. Mycobiology 2008;36:102-5.
8. Lee JS, Hyun KW, Jeong SC, Kim JH, Choi YJ, Miguez CB. Production of ribonucleotides by autolysis of *Pichia anomala* mutant and some physiological activites. Can J Microbiol 2004;50:489-92.
9. Lee JS, Yi SH, Kwon SJ, Ahn C, Yoo JY. Enzyme activities and physiological functionality of yeasts from traditional *Meju*. Korean J Appl Microbiol Biotechnol 1997;25:448-53.
10. Lee JS, Yi SH, Kwon SJ, Ahn C, Yoo JY. Isolation identification and cultural conditions of yeasts from traditional *Meju*. Korean J Appl Microbiol Biotechnol 1997;25:435-41.
11. Lee JS, Yi SH, Kim JH, Yoo JY. Isolation of wild killer yeast from traditional *Meju* and production of killer toxin. Korean J Biotechnol Bioeng 1999;14:434-9.
12. Kim JH, Kim NM, Lee JS. Physiological characteristics and ethanol fermentation of thermotolerant yeast *Saccharomyces cerevisiae* OE-16 from traditional *Meju*. Korean J Food Nutr 1999;12:490-5.
13. Min JH, Kim YH, Kim JH, Choi SY, Lee JS, Kim HK. Comparison of microbial diversity of Korean commercial *Makgeolli* showing high β-glucan content and high antihypertensive activity, respectively. Mycobiology 2012;40: 138-41.
14. Min JH, Hyun SH, Kang MG, Lee HB, Kim CM, Kim HK, Lee JS. Isolation and identification of yeasts from wild flowers of Daejeon city and Chungcheongnam-do in Korea. Kor J Mycol 2012;40:141-4.
15. Kang MG, Hyun SH, Ryu JJ, Min JH, Kim HK, Lee JS. Note on newly isolated yeasts from wild flowers in Daejeon city, Korea. Kor J Mycol 2012;40:174-6.