

Mycological Characteristics of Nine Unrecorded Yeasts from Flowers in the Orchard of Yesan-gun, Chungcheongnam-do and Hanbat Arboretum in Daejeon City, Korea

Sang-Min Han, Se-Hee Hyun, Ja-Won Shin, Ha-Kun Kim and Jong-Soo Lee*

Department of Biomedical Science and Biotechnology, Paichai University, Daejeon 302-735, Korea

ABSTRACT : Six unrecorded yeasts, *Cryptococcus festucosus* 41-3, *Cryptococcus heveanensis* 56-4, *Debaryomyces nepalensis* 95-4, *Issatchenkia occidentalis* 142-1, *Dioszegia zsolitii* 39-1, and *Kwoniella europaea* 47-2 were screened from 108 yeasts isolated from flowers and fruits in orchards of Yesan-gun, Chungcheongnam-do, Korea. The morphological and cultural characteristics of these unrecorded yeasts were investigated. They had various shapes, including ellipsoidal, globose, and oval, and also had budding mode in vegetable reproduction, except *I. occidentalis* 142-1 (fission mode). *K. europaea* 47-2 only formed pseudomycelium. *D. zsolitii* 39-1 did not grow in yeast extract-malt extract medium, potato dextrose medium, and vitamin-free medium. *C. festucosus* 41-3 grew well in 5% NaCl-containing yeast extract-peptone-dextrose medium and had a growth pH range of 7.0~10.0. Three unrecorded yeasts *Ogataea polymorpha* HB45-1, *Rhodotomula hinnulla* HB62-2, and *Cryptococcus rajasthanensis* HB80-4 were screened from 51 yeasts isolated from flowers in Hanbat arboretum in Daejeon city, Korea. They were globose in shape and did not form pseudomycelium. In addition, *O. polymorpha* HB45-1 and *C. rajasthanensis* HB80-4 had budding mode in vegetable reproduction. All of them grew well in vitamin-free medium and *C. rajasthanensis* HB80-4 also grew in 50% glucose and 5% NaCl-containing YPD medium.

KEYWORDS : Mycological characteristics, Unrecorded yeasts, Orchard and arboretum

Until now, almost all yeasts have been isolated and reported from Korean fermented foods and their raw materials such as traditional alcohol beverages and nuruks, soy sauces and soy pastes and its koji, meju etc. [1-4].

Recently, we isolated various yeasts from wild flowers in cities [5, 6], mountains [7], and coastal areas [8], and islands including Jeju-island [9], Ulleungdo and Yokjido [10], Korea. We also screened and characterized unrecorded yeasts [11, 12].

In a previous paper, we reported on yeast diversities of

flowers and fruits in some orchards [13] of Yesan-gun and Hanbat arboretum [14] of Daejeon city, Korea. This study describes the microbiological characteristics of the unrecorded yeasts screened from the above mentioned orchards and arboretum.

Characteristics of the unrecorded yeasts from orchard flowers of Yesan-gun, Chungcheongnam-do, Korea

We screened the unrecorded yeasts by searching Keris, PubMed and other fungal taxonomy databases [11] and investigated their morphological and cultural characteristics using a previously described method [11, 12].

Six unrecorded yeasts, *Cryptococcus festucosus* 41-3 and *Cryptococcus heveanensis*, 56-4, *Debaryomyces nepalensis* 95-4, *Issatchensis occidentalis* 142-1, *Dioszegia zsolitii* 39-1, and *Kwoniella europaea* 47-2 were screened from 108 yeasts from flowers and fruits in three orchards of Yesan-gun, Korea. The morphological and cultural characteristics of the six unrecorded yeasts are summarized in Table 1. All of the unrecorded yeasts had various shapes, and did not form pseudomycelium, except *K. europaea* 47-2.

All of the unrecorded yeasts, except *D. zsolitii* 39-1 grew

Kor. J. Mycol. 2014 September, 42(3): 231-234
<http://dx.doi.org/10.4489/KJM.2014.42.3.231>
pISSN 0253-651X • eISSN 2383-5249
© The Korean Society of Mycology

*Corresponding author

E-mail: biotech8@pcu.ac.kr

Received August 6, 2014

Revised September 23, 2014

Accepted September 23, 2014

©This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Table 1. Morphological and cultural characteristics of the newly reported yeasts from wild flowers in orchards of Yesan-gun in Chungcheongnam-do, Korea

Strains	<i>Cryptococcus festuosus</i> 41-3	<i>Cryptococcus heveanensis</i> 56-4	<i>Debaryomyces nepalensis</i> 95-4	<i>Dioszegia zsoldii</i> 39-1	<i>Issatchenkia occidentalis</i> 142-1	<i>Kwoniella europaea</i> 47-2
Isolation Source (Name of flowers)	<i>Prunus tomentosa</i>	<i>Prunus salicina</i> L.	<i>Diospyros kaki</i>	<i>Prunus salicina</i> L.	<i>Prunus persica</i>	<i>Prunus persica</i>
Morphological characteristics						
Shape	E ¹⁾	G	G ²⁾	G	O ³⁾	O
Vegetable reproduction	B	B	B	B	F ⁴⁾	B ⁵⁾
Size (μm)	0.2×0.8	1.4×1.4	1.3×1.3	2.1×2.1	1.2×1.8	1.6×1.8
Ascospore	-	-	+	+	+	+
Pseudomycelium	-	-	-	-	-	+
Cultural characteristics						
Growth on YM ⁴⁾ medium	+++	+++	+++	-	+++	++
YPD medium	++	+++	+++	+++	+++	+++
PD medium	+	-	+	-	++	+
Vitamin-free medium	-	-	++	-	++	++
50% Glucose YP medium	+	-	-	-	-	-
5%(20%) NaCl-YPD	++ (-)	- (-)	- (-)	- (-)	- (-)	+
Colony color in YPD medium	C ⁷⁾	C	C	O ⁸⁾	W ⁹⁾	C
Growth range in temp./pH	20~37°C /pH 7~10	20~30°C /pH 4~7	20~37°C /pH 4~8	25~37°C /pH 7~8	20~37°C /pH 4~8	20~30°C /pH 4~7

¹⁾E, Ellipsoidal ²⁾G, Globose ³⁾O, Oval ⁴⁾F, Fission ⁵⁾B, Budding ⁶⁾YM, Yeast extract-malt extract ⁷⁾C, Cream ⁸⁾O, Orange ⁹⁾W, White.

Table 2. Morphological and cultural characteristics of the newly reported yeasts from wild flowers in Hanbat arboretum of Daejeon city, Korea

Strains	<i>Cryptococcus rajasthanensis</i> HB80-4	<i>Ogataea polymorpha</i> HB45-1	<i>Rhodotorula hinmulea</i> HB62-2
Isolation source (Name of Flowers)	Unknown flower	<i>Elaeagnus umbellata</i> T.	Unknown flower
Morphological characteristics			
Shape	G ¹⁾	G	G
Vegetable reproduction	B	B ²⁾	F ³⁾
Size (μm)	1.3×1.3	0.9×0.9	1.2×1.3
Ascospore	-	+	-
Pseudomycelium	-	-	-
Cultural characteristics			
Growth on YM ⁴⁾ medium	+++	++	+++
YPD medium	+++	+++	+++
PD medium	++	++	++
Vitamin-free medium	++	++	++
50% Glucose YP medium	+	-	-
5%(20%) NaCl-YPD	++ (-)	++ (-)	- (-)
Colony color in YPD medium	C ⁵⁾	C	C
Growth range in temp./pH	20~30°C / pH 4~7	20~30°C / pH 7~8	20~30°C / pH 4~10

¹⁾G, Globose ²⁾B, Budding ³⁾F, Fission ⁴⁾YM, Yeast extract-malt extract ⁵⁾C, Cream

well in yeast extract-peptone-dextrose (YPD), yeast extract-malt extract (YM), and potato-dextrose (PD) broth.

D. nepalensis 95-4, *I. occidentalis* 142-1, and *K. europaea* 47-2 grew in vitamin-free media. In particular, *C. festu-*

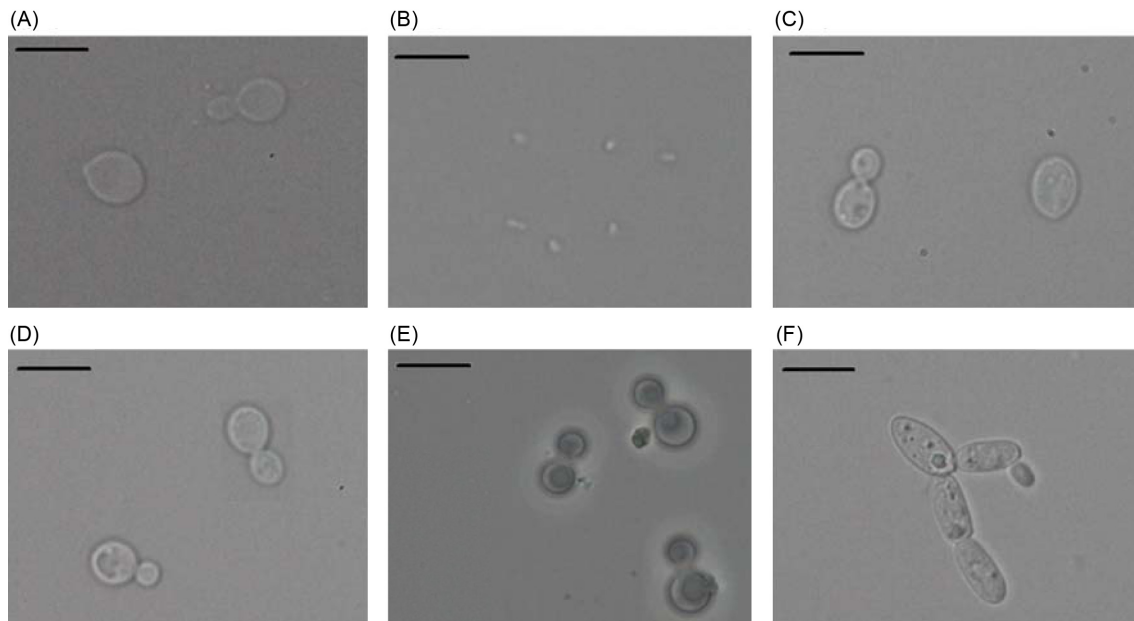


Fig. 1. Morphological characterization of six isolates of yeasts (Scale bar = 2 μ m). A; *Dioszegia zsolzii* 39-1, B; *Cryptococcus festuosus* 41-3, C; *Kwoniella europaea* 47-2, D; *Cryptococcus heveanensis* 56-4, E; *Debaryomyces nepalensis* 95-4, F; *Issatchenkia occidentalis* 142-1.

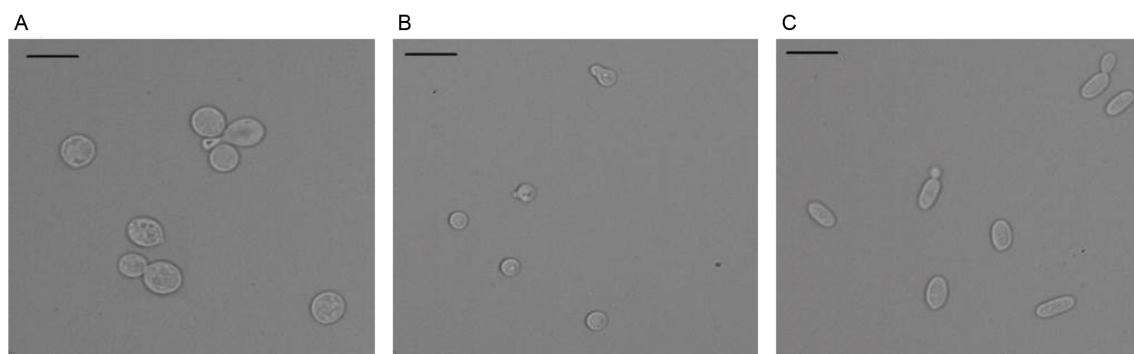


Fig. 2. Morphological characterization of three isolates of yeasts (Scale bar = 2 μ m). A; *Cryptococcus rajasthanensis* HB80-4, B; *Ogataea polymorpha* HB45-1, C; *Rhodotorula hinnulea* HB62-2.

cosus 41-3 grew well in 5%-NaCl containing YPD medium and was also an alkalophile, growing in the range of pH 7.0~10.0.

Bai *et al.* [15] first reported on a new ballistoconidium-forming yeast, *Dioszegia zsolzii*, from various plant leaves collected in Yunnan, China. *Cryptococcus festuosus* was also reported as a new hymenomycetous yeast in the holtermannia clade [16], and Guerreiro *et al.* [17] reported on *Kwoniella europaea*, basidiomycetes yeast, which have a tetrapolar mating system. *Cryptococcus heveanensis* [18] was known as a sister species to the pathogenic *Cryptococcus* species and *Issatchenkia occidentalis* [19] was first isolated from the esophagus of a leukemic pa-

tient. Ethanol production of *Debaryomyces nepalensis* [20] was also reported. However, their taxonomic characteristics or application in industry have not yet been studied in detail.

Characteristics of the unrecorded yeasts from flowers in Hanbat arboretum of Daejeon city, Korea

Ogataea polymorpha HB45-1, *Rhodotorula hinnulea* HB 62-2, and *Cryptococcus rajasthanensis* HB80-4 were screened as unrecorded yeasts from 51 yeasts from flowers and fruits in Hanbat arboretum, Daejeon city, Korea. All of them were globose in shape and did not have pseudomycelium. Three unrecorded yeasts grew well in YPD,

YM, and PD media and vitamin-free medium. *C. rajasthanensis* HB80-4 was sugar-tolerant, growing in 50% glucose-containing YPD medium and was also a halo-tolerant yeast with *O. polymorpha* HB45-1, grew in 5% NaCl-containing YPD medium.

Ogataea polymorpha is closely related to *Ogataea parapolyomorpha*, the ascosporic state of *Candida parapolyomorpha* [21] and *Cryptococcus rajasthanensis*, an anamorphic yeast, is also related to *C. laurentii*, isolated from Rajasthan, India [22].

ACKNOWLEDGEMENTS

This study was funded by the project on survey and excavation of Korean indigenous species of NIBR under the Ministry of Environment, Republic of Korea.

REFERENCES

1. Lee JS, Yi SH, Kwon SJ, Ahn C, Yoo JY. Enzyme activities and physiological functionality of yeasts from traditional Meju. *Kor J Appl Microbiol Biotechnol* 1997;25:448-53.
2. Kim JH, Kim NM, Lee JS. Physiological characteristics and ethanol fermentation of thermotolerant yeast *Saccharomyces cerevisiae* OE-16 from traditional meju. *Kor J Food Nutr* 1999; 12:490-5.
3. Jeong SC, Lee DH, Lee JS. Production and characterization of an anti-angiogenic agent from *Saccharomyces cerevisiae* K-7. *J Microbiol Biotechnol* 2006;16:1904-11.
4. Lee JS, Choi YJ, Kwon SJ, Yoo JY, Chung DH. Screening and characterization of osmotolerant and gas-producing yeasts from traditional Doenjang and Kochujang. *Food Biotechnol* 1996;5:54-8.
5. 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.
6. 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.
7. Min JH, Ryu JJ, Kim HK, Lee JS. Isolation and identification of yeasts from wild flowers in Gyejoksan, Oseosan and Beakamsan of Korea. *Kor J Mycol* 2013;41:47-51.
8. Min JH, Lee HB, Lee JS, Kim HK. Identification of yeasts isolated from wild flowers collected in coast areas of Korea based on the 26S rDNA sequences. *Kor J Mycol* 2013;41:185-91.
9. Hyun SH, Mun HY, Lee HB, Kim HK, Lee JS. Isolation of yeasts from wild flowers in Gyeonggi-do and Jeju island of Korea and production of anti-gout xanthine oxidase inhibitor. *J Microbiol Biotechnol* 2013;41:389-90.
10. Hyun SH, Min JH, Kim AR, Kim HK, Lee JS. Isolation and diversity of yeasts from wild flowers in Ulleung do and Yokjido, Korea. *Kor J Mycol* 2014;42:28-33.
11. Hyun SH, Lee HB, Kim CM, Lee JS. New records of yeasts from wild flowers in coast near areas and inland areas, Korea. *Kor J Mycol* 2013;41:74-80.
12. Hyun SH, Lee HB, Lee JS. Characteristics of unrecorded yeasts, *Rhodospiridium fluviale*, *Rhodospiridium paludigenum*, *Candida* sp. 80-J-3 and *Kluyveromyces thermotolerans* isolated from wild flowers in Korea. *Kor J Mycol* 2014;41:181-4.
13. Hyun SH, Lee JG, Park WJ, Kim HK, Lee JS. Isolation and diversity of yeasts from fruits and flowers of orchard in Sinam-myeon of Yesan-gun, Chungcheongnam-do, Korea. *Kor J Mycol* 2014;42:21-7.
14. Hyun SH, Min JH, Kim SA, Lee JS, Kim HK. Yeasts associated with fruits and blossoms collected from Hanbat arboretum, Daejeon, Korea. *Kor J Mycol* 2014;42:178-82.
15. Bai FY, Takashima M, Nakase T. *Dioszegia zsolttii* sp. nov., a new ballistoconidium-forming yeast species with two varieties. *J. Gen. Appl. Microbiol* 2002;48:17-23.
16. Golubev W, Sampaio J, Alves L, Golubev N. *Cryptococcus festuosus* sp. nov. a new hymenomycetous yeast in the Holtermannia clade. *Can J Microbiol* 2004;50:1001-6.
17. Guerreiro MA, Springer DJ, Rodrigues JA, Rusche LN, Findley K, Heitman J, Fonseca A. Molecular and genetic evidence for a tetra polar mating system in the basidiomycetous yeast *Kwoniella mangrovensis* and two sibling species. *Eukaryot Cell* 2013;12:746-60.
18. Metin B, Findley K, Heltman J. The mating type locus(mat) and sexual reproduction of *Cryptococcus heveanensis*: insights into the evolution of sex and sex-determining chromosomal regions in fungi. *PLoS Genet* 2010;6:1-19.
19. Sahand I, Moragues MD, Alhambra A, Palacio A, Quindos G, Ponton J. Isolation of *Issatchenkia occidentalis* from the esophagus of a leukemic patient. *Rev Iberoam Micol* 2006; 23:235-7.
20. Kumdam H, Murthy S, Gummadi S. Production of ethanol and arabitol by *Debaryomyces nepalensis*: influence of process parameters. *AMB Express* 2013;3:1-12.
21. Kurtzman C. A new methanol assimilating yeast, *Ogataea parapolyomorpha*, the ascosporic state of *Candida parapolyomorpha*. *Antonie van Leeuwenhoek* 2011;100:455-62.
22. Saluja P, Prasad GS. *Cryptococcus rajasthanensis* sp. nov., an anamorphic yeast species related to *Cryptococcus laurentii*, isolated from Rajasthan, India. *Int J Syst Evol Microbiol* 2007;57:414-8.