

Research Article



CrossMark

Open Access

동해 연안해역에서 분리한 해양효모의 분자계통학적 해석

진일석, 김용환, 윤원갑, 박년호, 김종식*

Phylogeny of Marine Yeasts Isolated from Coastal Seawater in the East Sea of Korea

Il-Seok Chin, Yong-Hwan Kim, Won-Kap Yun, Nyun-Ho Park and Jong-Shik Kim* (Gyeongbuk Institute for Marine Bio-Industry, Department of Research and Development, Uljin 36315, Korea)

Received: 15 June 2017 / Revised: 19 June 2017 / Accepted: 22 June 2017

Copyright © 2017 The Korean Society of Environmental Agriculture

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.

ORCID

Jong-Shik Kim

<http://orcid.org/0000-002-8181-1707>

Il-Seok Chin

<http://orcid.org/0000-0002-6230-171x>

Abstract

BACKGROUND: Yeasts are used in a variety of industries. However, most industries are biased toward *Saccharomyces cerevisiae*; so we sought to explore non-conventional yeasts (NCY). This study aimed to isolate yeasts from seawater collected from the East Sea of Korea and to analyze the NCY.

METHODS AND RESULTS: We first collected seawater and performed pure isolation using four kinds of medium (GPY, DOB + CSM, DG18, and SCG). In total, 314 strains and 17 genera were isolated by ITS sequencing, including *Aureobasidium pullulans* (236 strains), *Cryptococcus* (19 strains), *Cystobasidium* (18 strains), and *Rhodotorula* (9 strains). Upon in-depth analysis, *A. pullulans*, the most dominant genus (236 strains), was divided into Group II (147 strains), Unknown I (8 strains), and Unknown II (49 strains).

CONCLUSION: In this study, a total of 314 strains were isolated from seawater; many of these yeasts have been found and reported in seawater previously. In-depth analysis of *A. pullulans*, showed the dominance of Group I (21 strains) and Group II (147 strains). We also discovered

Unknown I (8 strains) and Unknown II (49 strains), which have not been reported previously.

Key words: Bioethanol, Biosurfactant, Marine yeast, Seawater

서론

해양효모는 다양한 산업에 사용되며, 주로 *Saccharomyces cerevisiae*가 사용된다. 그러나 많은 산업은 *S. cerevisiae*에 편향되어 있어, 비전통적인 해양효모(NCY)를 탐색하고자 하였다. 본 연구는 동해 연안해역에서 채취된 해수로부터 NCY를 분리하고 분석하는 것을 목적으로 하였다. 먼저 해수를 채취하고 GPY, DOB + CSM, DG18, SCG 등 네 가지 배양매체를 사용하여 순수 배양을 수행하였다. 총 314개의 균주와 17개의 속이 ITS 시퀀싱을 통해 분리되었으며, *Aureobasidium pullulans* (236 균주), *Cryptococcus* (19 균주), *Cystobasidium* (18 균주), *Rhodotorula* (9 균주)가 포함되었다. 심층 분석을 통해, 가장 우점 속인 *A. pullulans* (236 균주)는 Group II (147 균주), Unknown I (8 균주), Unknown II (49 균주)로 분류되었다. (Deak 2009).

가 (Kuty and Philip, 2008; Satpute *et al.*, 2010; Zaky *et al.*, 2014).

Yarrowia Candida (Zaky *et al.*, 2014), *Aureobasidium pullulans* (Renshaw *et al.*, 2002).

가 1894 (Kuty and Philip, 2008), *Cryptococcus*, *Rhodotorula*가

*Corresponding author: Jong-Shik Kim

Phone: +82-54-720-3451; Fax: +82-54-780-3469;

E-mail: jskim@gimb.or.kr

Table 1. Chemical characteristics of seawater from East-sea

pH	W.T (°C)	Salinity (%)	Chl-a (ug/l)	SS	DO	COD	T-N	NO2-N	NO3-N	PO4-P	SiO2-Si	Na
8.01	15.4	33.8	0.32	7.69	7.89	1.24	0.56	0	0.42	0.02	0.32	1.12
Mg	Ca	K	Fe	T-P	TOC	Cd	Pb	Cu	Hg	Cr	Cu	B
1253	431	191	1.54	0.06	0.96	0.031	1.422	0.365	ND	1.124	0.234	47.248

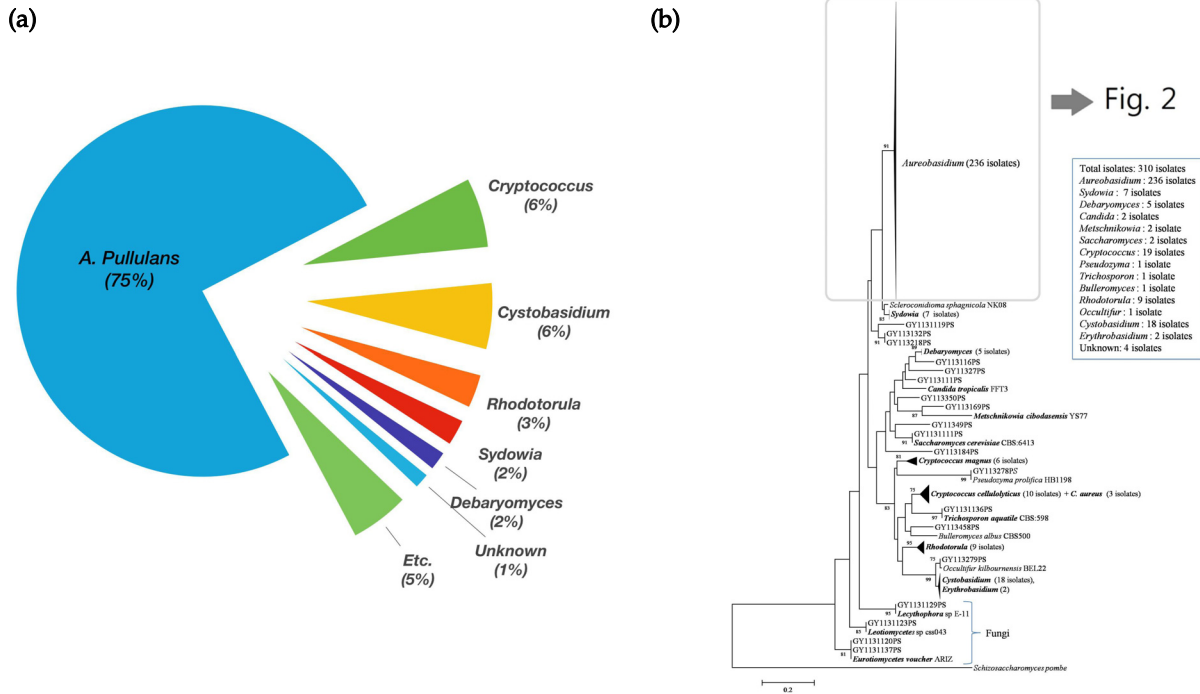


Fig. 1(a). A Pie chart showing the yeast component of East-sea. 1(b). Molecular phylogenetic tree constructed by neighbor-joining method using the sequences of representative yeast isolates from seawater and related yeasts. The numerals represent the confidence levels from 1000 replicate bootstrap samplings (frequencies of less than 75% are not indicated).

7.89 mg/L
I (7.5 mg/L
(COD) 1.02-
1.42 mg/L 1.24 mg/L
II (2 mg/L
-a(chl.-a) 0.15-0.47
mg/L 0.32 mg/L
(SS) 3.17-8.27 mg/L , 6.595 mg/L
(salinity) 33.90-34.03%
9%
(NO₃-N)
0.42 mg/L, (NO₂-N) 0.002 mg/L,
(T-N) 0.56 mg/L, (PO₄-P) 0.024 mg/L,
(T-P) 0.06 mg/L, (SiO₂-Si) 0.321 mg/L
(Na)
9,456 mg/L, (Mg) 1,253 mg/L,
(Ca) 431 mg/L, (K) 191 mg/L
(Fe) 1.54 mg/L
(B) 47.248 mg/L, (Cr)
1.124 mg/L, (Cu) 0.365
mg/L, (Pb) 1.422 mg/L, (Hg)
(Table 1).
해양호모 분포
ITS
310
. *A. pullulans* (236 , 75%), *Cryptococcus* (19
, 6%), *Cystobasidium* (18 , 6%), *Rhodotorula* (9
, 3%), *Sydowia* (7 , 2%), *Debaryomyces* (4
, 2%), *Metschnikowia* (2 , 1%), *Saccharomyces* (2 , 1%), *Pseudozyma* (1 , 0.3%), *Trichosporon* (1 , 0.3%), *Bulleromyces* (1 , 0.3%), *Rhodotorula* (9 , 3%), *Oocultifur* (1 , 0.3%), *Cystobasidium* (18 , 6%), *Erythrobasidium* (2 , 0.6%), *Unknown* (4 , 1.3%)

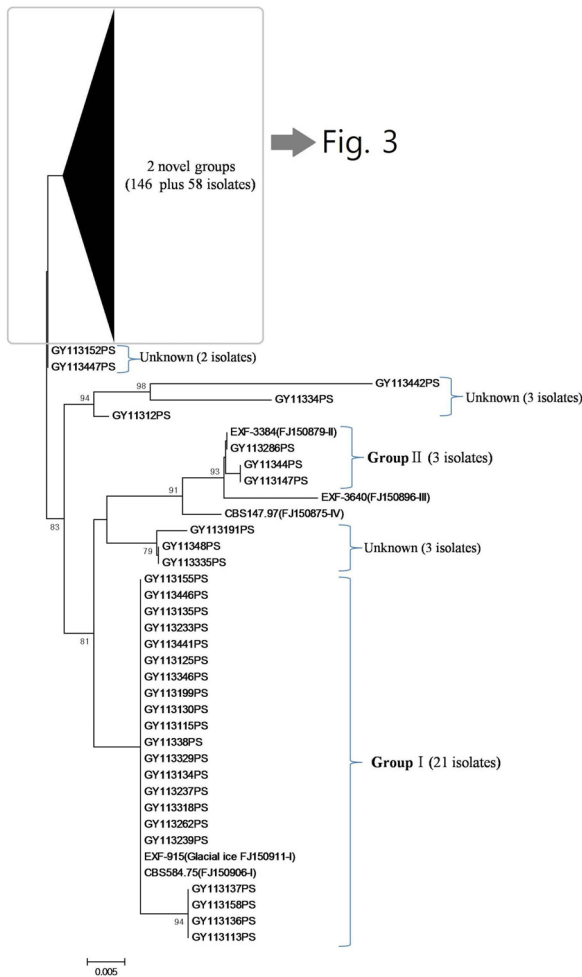


Fig. 2. Molecular phylogenetic tree constructed by neighbor-joining method using the sequences of representative yeast isolates from seawater and related yeasts.

Candida (2), *Erythrobasidium* (2), *Trichosporon* (1), *Pseudozyma* (1), *Bulleromyces* (1), *Occultifur* (1), *Unknown* (4)

. 가 , *A. pullulans*가 75% 236 .

GPY 119 , DOB 76 , DG18 60 , SCG 59
GPY 가 가 (Fig. 1(a), (b)).

236 *A. pullulans* (deep analysis) . Group I (21) .

Group II (3), Unknown(8)
146 58 2

(novel groups) (Fig. 2).
2

Group II(146), Unknown I (8),
Unknown II (49), UnknownIII(1)

(Fig. 3).

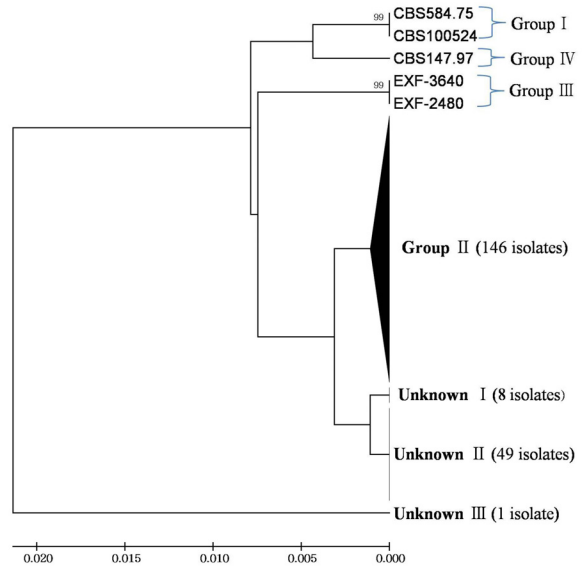


Fig. 3. Molecular phylogenetic tree constructed by UPGMA method using the sequences of representative yeast isolates from seawater and related yeasts.

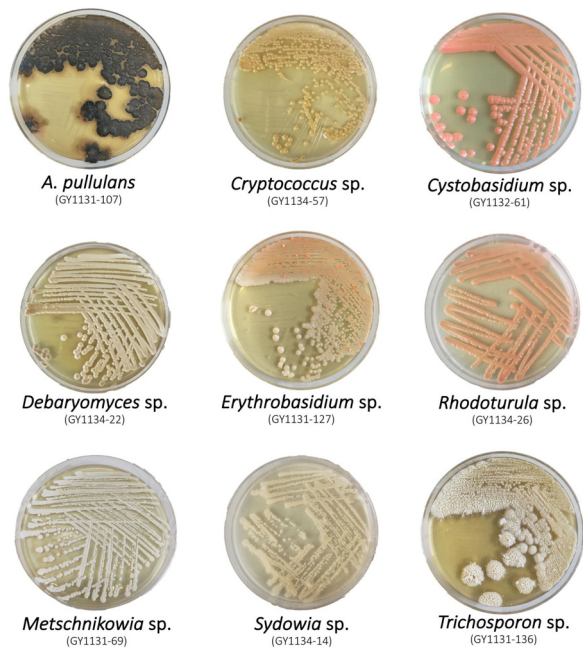


Fig. 4. Various yeasts isolates cultured on GPY growth medium from East-sea.

해수에서 분리한 효모의 형태학적 관찰
. 가
A. pullulans 가
(球形) 가
, 가
(filamentous) . *Cryptococcus* sp., *Debaryomyces*

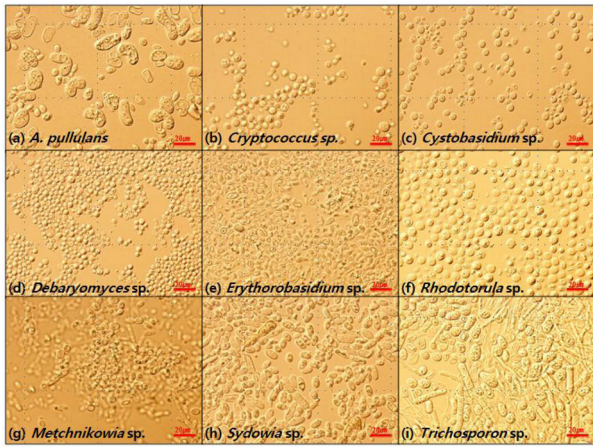


Fig. 5. Morphological observation with a microscope (Nikon Eclipse 80i, Tokyo, Japan).

sp., *Metschnikowia* sp. 가
 가 . *Cryptococcus* sp. , *Debaryomyces*
 sp. *Metschnikowia* sp. . *Erythrobasidium*
 sp., *Cystobasidium* sp., *Rhodotorula* sp.
 가
 , 가 가 . *Sydowia* sp. 가 가
 가
Trichosporon sp.
 가
 (Fig. 4), Fig. 5

고 찰

TOC
 0.76-1.09 mg/L 가 가
 가
 (DOM; Dissolved Organic Matter)
 ,
 humic
 (Kim *et al.*, 2002 and,
 Imai *et al.*, 2002).

(NO₃⁻)
 (NO₂⁻)
 (T-N)
 , 0.43-0.62 mg/L
 (TN)
 (TP) . (PO₄-P)

가
 (PO₄) 가 (T-P)
 0.02-0.06 mg/L 가
 가
 Unknown 가 A.
pullulans
 가 (Kim *et al.*, 2016b).
*A. pullulans*가
 가
 가
 Table 2 30
 (Kutty and Philip, 2008)

Sydowia, *Pseudozyma*, *Bulleromyces*, *Occultifur*,
Cystobasidium, *Erythrobasidium* 6 , 30
 (Table 2).

가 가 가 가
 , Table 1

Acknowledgements

This study was supported by a grant from the National Research Foundation of Korea (NRF), funded by Korean government MSIP (Ministry of Science, ICT and Future Planning) (No. NRF-2014R1A2A1A11052888).

References

Bhat, J., & Kachwalla, N. (1955). Marine yeasts off the Indian coast. Proceedings of the Indian Academy of Sciences. Section B, Plant Sciences, 41(1), 9-15.
 Deak, T. (2009). Ecology and biodiversity of yeasts with potential value in biotechnology. Yeast biotechnology: diversity and applications (eds. Satyanarayana, T., and Kunze, G.), pp. 151-168. Springer Science+Business

- Media B.V., Dordrecht.
- Fell, J. W., Ahearn, D. G., Meyers, S. P., & Roth, F. J. (1960). Isolation of yeasts from Biscayne bay, Florida and adjacent benthic areas. *Limnology and Oceanography*, 5(4), 366-371.
- Fell, J. W., & Hunter, I. L. (1968). Isolation of heterothallic yeast strains of *Metschnikowia Kamienski* and their mating reactions with *Chlamydozoma Wickerham* spp. *Antonie Van Leeuwenhoek*, 34(1), 365-376.
- Imai, A., Fukushima, T., Matsushige, K., Kim, Y. H., & Choi, K. (2002). Characterization of dissolved organic matter in effluents from wastewater treatment plants, *Water Research*, 36(4), 859-870.
- Kim, J. S., & Kim, D. S. (2015) Yeasts in the flowers of wild Fleabane [*Erigeron annuus* (L.) Pers]. *Korean Journal of Environmental Agriculture*, 34(3), 254-259.
- Kim, J. S., Kim, D. S., Jeon, S. M., & Ko, S. H. (2016a). Yeasts associated with roots of the endemic plant *Mankyua chejuense*. *Korean Journal of Environmental Agriculture*, 35(2), 137-142.
- Kim, J. S., Lee, I. K., Kim, D. W., & Yun, B. S. (2016b). Aureosurfactin and 3-deoxyaureosurfactin, novel biosurfactants produced by *Aureobasidium pullulans* L3-GPY. *The Journal of Antibiotics*. 69, 759-761.
- Kim, M. C., Kang, C. G., Park, H. Y., Lee, D. S., Kim, Y. S., & Lee, W. J. (2006). Isotopic evidence of marine yeast to artificial culture of *Moina macrocopa*. *The Korean Journal of Microbiology*, 42(2), 111-115.
- Kim, Y. H., Lee, S. H., Imai, A., & Matsushige, K. (2002). Characterization of dissolved organic matter in a shallow eutrophic lake and inflowing water. *Environmental Engineering Research*, 7(2), 93-101.
- Kohlmeyer, J., & Kohlmeyer, E. (1979). *Marine mycology: the higher fungi*, pp.1-704. Academic Press, New York, USA.
- Kutty, S., & Philip, R. (2008). Marine yeasts - a review. *Yeast*, 25(7), 465-483.
- Liu, J., Liu, Z., Chi, Z., Liang, Zhang, L., & Zhang, D. (2009). Intraspecific diversity of *Aureobasidium pullulans* strains from different marine environments. *Journal of Ocean University Of China*, 8(3), 241-246.
- Munn, C. B. (2004). Marine eukaryotic microbes. In *Marine microbiology-ecology and its applications*. pp. 135-136. Garland Science, London and New York.
- Oswal, N., Sarma, P. M., & Zinjarde, S. S. (2002). Palm oil mill effluent treatment by a tropical marine yeast. *Bioresource Technology*, 85(1), 35-37.
- Paula, C. R., Purcho, A., & Gambale, W. (1983). Yeasts from beaches in the southern area of Sao Paulo state Baixada Santista, Brazil. *Revista de Microbiologia*, 14(2), 136-143.
- Pitt, J. I., & Miller, M. W. (1970). The parasexual cycle in yeasts of the genus *Metschnikowia*. *Mycologia*, 62(3), 462-473.
- Renshaw, J. C., Robson, G. D., Trinci, A. P. J., Wiebe, M. G., Livens, F. R., Collison, D., & Taylor, R. J. (2002). Fungal siderophores: structures, functions and applications. *Mycological Research*, 106(10), 1123-1142.
- Rhishipal, R., & Philip, R. (1998). Selection of marine yeasts for the generation of single cell protein from prawn-shell waste. *Bioresource Technology*, 65(3), 255-256.
- Saitou, N., & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, 4(4), 406-425.
- Satpute, S.K., Banat, I.M., Dhakephalkar, P.K., Banpurkar, A. G., & Chopade, B. A. (2010). Biosurfactants, bioemulsifiers and exopolysaccharides from marine microorganisms. *Biotechnology Advances*. 28, 436-450.
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., & Kumar, S. (2011). MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, 28(10), 2731-2739.
- Yamasato, K., Goto, S., Ohwada, K., Okuno, D., Araki, H., & Iizuka, H. (1974). Yeast from the Pacific Ocean. *Journal of General and Applied Microbiology*, 20(5), 289-307.
- Zaky, A., Tucker, G., Daw, Z., & Du, C. (2014). Marine yeast isolation and industrial application. *FEMS Yeast Research*, 14(6), 813-825.