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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

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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

효모는 다양한 산업에 사용되며, 주로 *Saccharomyces cerevisiae*가 사용된다. 그러나 많은 산업은 *S. cerevisiae*에 편향되어 있어, 비전통적 해양 효모(NCY)의 다양성을 탐구하는 것이 필요하다. 본 연구는 동해 연안 해역에서 수집된 해수에서 NCY를 분리하고 분석하는 것을 목표로 하였다. 먼저, GPY, DOB + CSM, DG18, SCG 등 네 가지 배양 매체를 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, *A. pullulans*는 가장 우점 속(236 균주)으로, Group II(147 균주), Unknown I(8 균주), Unknown II(49 균주)로 분류되었다.

본 연구에서, 총 314개의 균주가 해수에서 분리되었으며, 많은 균주가 이전에 해수에서 보고된 바 있다. *A. pullulans*의 심층 분석은 Group I(21 균주)과 Group II(147 균주)의 우점을 보여주었다. 또한, 이전에 보고되지 않았던 Unknown I(8 균주)과 Unknown II(49 균주)도 발견되었다. 이 연구는 동해 연안 해역의 해양 효모 다양성을 탐구하고, 바이오에탄올, 바이오서팩탄트 등 다양한 산업에 활용 가능한 균주를 발굴하는 데 기여할 것으로 기대된다.

본 연구는 동해 연안 해역에서 수집된 해수에서 NCY를 분리하고 분석하는 것을 목표로 하였다. 먼저, GPY, DOB + CSM, DG18, SCG 등 네 가지 배양 매체를 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, *A. pullulans*는 가장 우점 속(236 균주)으로, Group II(147 균주), Unknown I(8 균주), Unknown II(49 균주)로 분류되었다. 이 연구는 동해 연안 해역의 해양 효모 다양성을 탐구하고, 바이오에탄올, 바이오서팩탄트 등 다양한 산업에 활용 가능한 균주를 발굴하는 데 기여할 것으로 기대된다.

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가 (Yamasato *et al.*, 1974),
 Biscayne *Candida tropicalis*
*Rhodotorula rubra*가, *Candida*가
 (Fell *et al.*, 1960). Mumbai *Yarrowia*
*lipolytica*가
 (Oswal *et al.*, 2002).
 (obligate) (facultative)
 가 가 (Kohlmeyer and
 Kohlmeyer, 1979).
 가
 1 가
 1 10
 177 26
 (Kohlmeyer and Kohlmeyer, 1979).
 가 . *Candida*, *Debaryomyces*,
Kluyveromyces *Ascomycetes*
 , *Cryptococcus*, *Rhodosporeidium*, *Rhodotorula*
Basidiomycetes
 (Munn, 2004).
Metchnikowia,
Kluyveromyces, *Rhodosporeidium*, *Candida*, *Cryptococcus*,
Rhodotorula, *Torulopsis* ,
 (*Candida*,
Cryptococcus, *Debaryomyces*, *Pichia*, *Hansenula*,
Rhodotorula, *Saccharomyces*, *Trichosporon*)
 (Kutty and Philip, 2008).
 (Sub-Antarctic)
 19% (4°C) 20°C
 43%
 (4°C)
 20°C (Grant Reports,
 1994).
 가
 (Kim *et al.*, 2006),

재료 및 방법

해양수질분석

2016 12

(N37.059 , E129.428),
 (Niskin
 Sampler, USA) (Go-Flow, USA)
 , CTD (Sea
 Bird SBE 911plus, USA)
 1L HNO3 4°C
 1.2 μm (1822-047, Whatman, Maidstone,
 UK)
 (Varian 720-ES
 ICP, Australia) (Bran Lubbe ACCS
 V, Japan)
효모의 순수분리
 50 L
 (XX1504700, Millipore, Schwalbach, Germany)
 (1822-047, Whatman, Maidstone, UK)
 (GAST LR37697,
 Gast Manufacturing, Inc., Benton Harbor, MI, USA)

(Kim *et al.*, 2016). 가
 GPY, DOB+CSM, DG18,
 SCG 4
 (0.4% L-sorbose, 0.1% Triton-X) (100 mg/L
 Streptomycin, Chloramphenicol) 가 .

Sequence 분석 및 계통해석

(주) (Seoul, Korea)
 ITS sequencing
 (Kim and Kim, 2015). MEGA 5.2
 (Tamura *et al.*, 2011) neighbor-joining method (Saitou
 and Nei, 1987) UPGMA method
 DDBJ/EMBL/GenBank
 Accession numbers (LC272609~LC272922, 314
 entries)

결 과

해양수질의 분석 결과

2016 12

(pH)

8.01((I : 7.8-8.3)

13.67-15.39°C

15.4°C ,

(DO) 7.52-7.9 mg/L

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
(mg/L)												
1253	431	191	1.54	0.06	0.96	0.031	1.422	0.365	ND	1.124	0.234	47.248

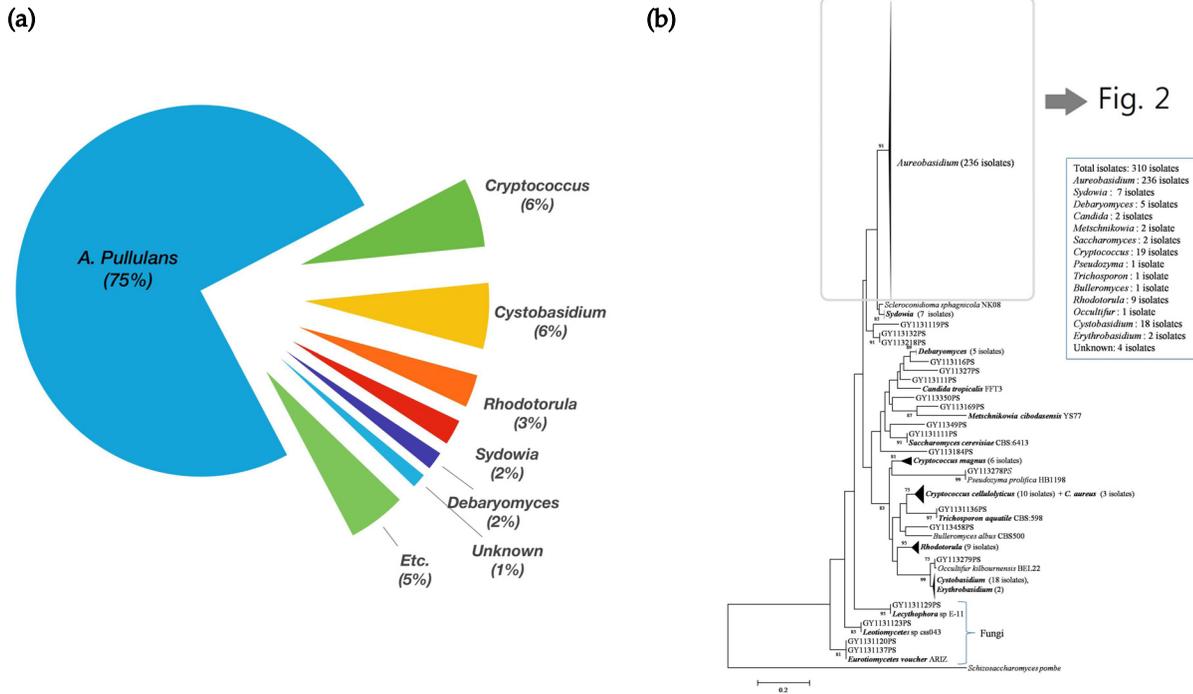


Fig. 1(a). A Pie chart showing the yeast component of East-sea. 1(b). Molecular phylogenetic tree constructed by neighbor-joining method using the sequences of representative yeast isolates from seawater and related yeasts. The numerals represent the confidence levels from 1000 replicate bootstrap samplings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

7.89 mg/L
 I (7.5 mg/L
 (COD) 1.02-
 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
), *Saccharomyces* (2), *Metschnikowia* (2) ,

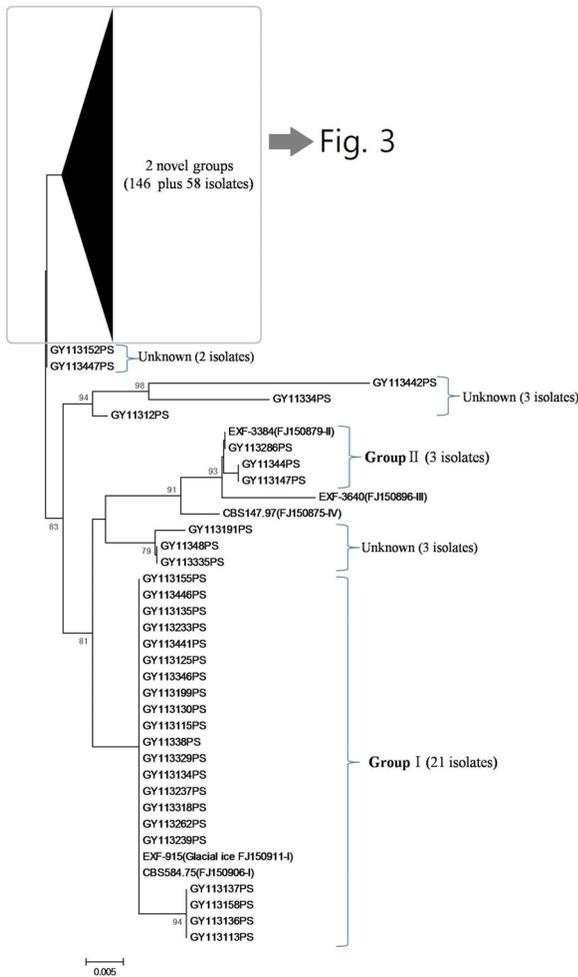


Fig. 2. Molecular phylogenetic tree constructed by neighbor-joining method using the sequences of representative yeast isolates from seawater and related yeasts.

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).

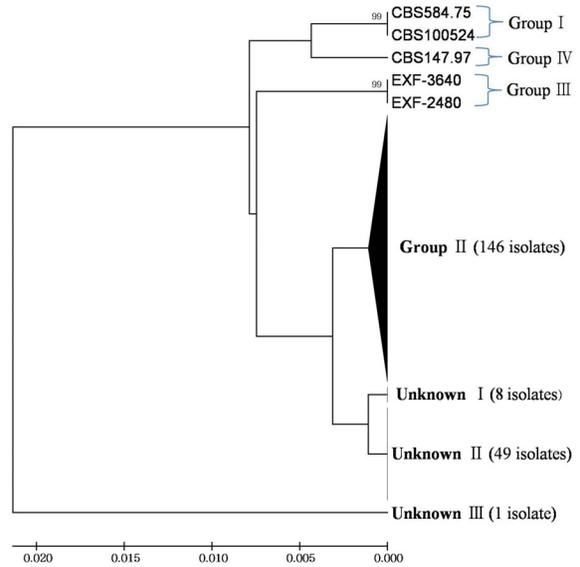


Fig. 3. Molecular phylogenetic tree constructed by UPGMA method using the sequences of representative yeast isolates from seawater and related yeasts.

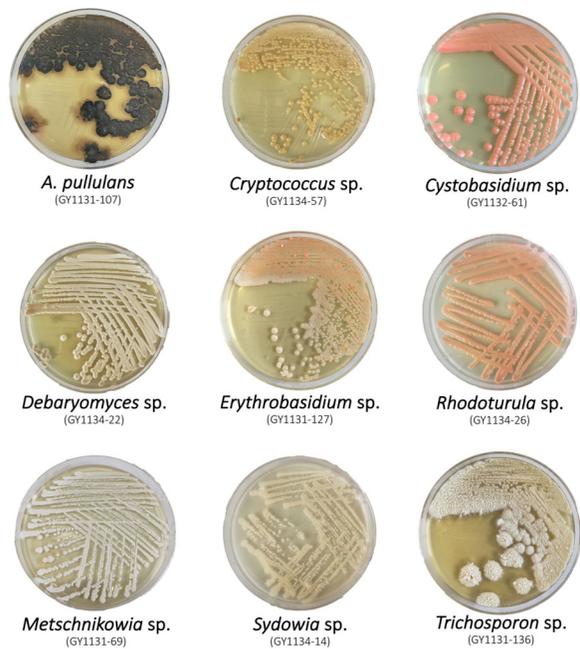


Fig. 4. Various yeasts isolates cultured on GPY growth medium from East-sea.

해수에서 분리한 효모의 형태학적 관찰

. 가
A. pullulans 가

. (球形) 가

, 가
(filamentous) . *Cryptococcus* sp., *Debaryomyces*

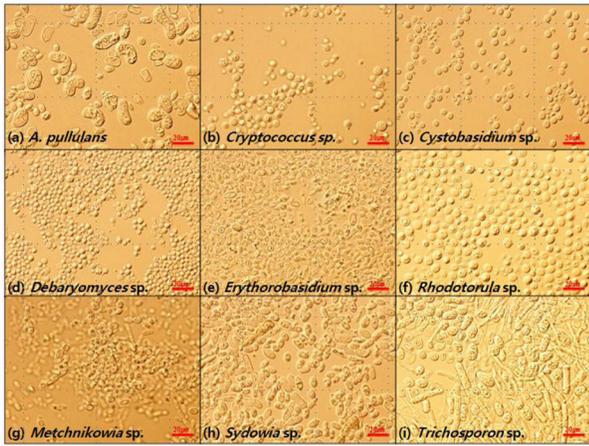


Fig. 5. Morphological observation with a microscope (Nikon Eclipse 80i, Tokyo, Japan).

sp., *Metschnikowia* sp. 가
 가 . *Cryptococcus* sp. , *Debaryomyces*
 sp. *Metschnikowia* sp. . *Erythrobasidium*
 sp., *Cystobasidium* sp., *Rhodotorula* sp.
 가
 , 가 . *Sydowia* sp. 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,
 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 A.
pullulans
 가 (Kim *et al.*, 2016).
*A. pullulans*가
 가
 가
 Table 2 30
 (Kutty and Philip, 2008)

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

가 , Table 1 가 가

Acknowledgement

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