Yeasts in the Flowers of Wild Fleabane [Erigeron annus (L.) Pers.]

Jong-Shik Kim¹*, Dae-Shin Kim²
¹Gyeongbuk Institute for Marine Bio-Industry, Uljin 36314, Korea
²World Heritage and Mt. Hallasan Research Institute, Jeju Special Self-Governing Provincial Government, Jeju 63122, Korea

Abstract

BACKGROUND: Yeasts associated with fleabane flowers were identified using isolation methods previously applied in yeast biotechnology. A culture-based approach was required for isolation of many yeast strains associated with fleabane.

METHODS AND RESULTS: We spread homogenized fleabane flowers onto GPY medium containing chloramphenicol, streptomycin, Triton X-100, and L-sorbose. We isolated 79 yeast strains from the flowers of wild fleabane, and identified the yeasts via phylogenetic analysis of isolates from agar plates. The yeast species included 39 isolates of Aureobasidium pullulans, 17 of the genus Candida, 14 of the genus Rhodosporidium, 6 of the genus Cryptococcus, and 3 of the genus Rhodotorula.

CONCLUSION: Yeast isolates associated with fleabane flowers included A. pullulans (39 isolates) and other yeast species (40 isolates). Such yeast isolates may have biotechnological potential.

Key words: Fleabane, Erigeron annus (L.) Pers., ITS gene, Wild yeast

Introduction

Erigeron annus (L.) Pers. is an annual or biennial herb of the Compositae, and is a North America native pioneer species that often colonizes disturbed areas such as pastures, vacant fields, roadides, railways, and waste areas; the herb was introduced to Korea in 1910 (Park, 2009). Flowering occurs in June and July, cephalization is white, the ligulate flower is pistillate, and the tubular flower is yellow with a long pappus (Korea National Arboretum, 2012). The leaves are edible and are commonly used in folk medicine to treat indigestion, stomach ache, diarrhea caused by enteric pathogens, and hypoglycemia (Lee, 1996; Lee, 2003; Yoo, et al., 2008). Little is known, however, about the ecological roles of plant yeasts.

Yeasts play roles in many complex processes of various ecosystems such as plant tissues (stems, flowers, and fruits); insects; soils; aquatic environments; and extreme environments (Fonseca and Inácio, 2006; Raspor and Zupan, 2006; Botha, 2011). Industrial attributes of yeasts include the primary roles they play in many food fermentations yielding beers, ciders, wines, sake, distilled spirits, bakery products, industrial enzymes, and agricultural products (Deak, 2009; Tamang and Fleet, 2009).

In this study, we characterized the yeast species associated with fleabane flowers. Previous studies have attempted to do the same, but their sampling strategies were casual or insufficient. Moreover, only minimal phylogenetic analyses of cultural yeast isolates have been performed in the past (Halloran et al., 2013); therefore, our results provide a basis for
Materials and Methods

Yeast isolation

Erigeron annus (L.) Pers. samples were aseptically collected from a residential area in Uljin with the aid of autoclaved scissors and forceps, and placed in clean plastic bags. Flower samples were stored in a cooler during transfer to the laboratory (in Uljin) and processed the same day. Each sample was washed three times with 10 mM potassium phosphate buffer and stored in an autoclaved container. Several washed flower samples were placed in tubes (Falcon Plastics, Los Angeles, CA, USA) filled to 10 mL with 10 mM potassium phosphate buffer and homogenized using an autoclavable hand homogenizer (T10 Basis; IKA, Staufen, Germany). Homogenized samples (1 mL) were placed on sterile solid media, plated using a glass spreader, and incubated at 25°C for 2-5 days. Yeast colonies that grew on large plates (Nunc Bio-assay dishes, 245 × 245 × 25 mm, Thermo Scientific, Roskilde, Denmark) were selected with autoclaved toothpicks and inoculated into 96-deep-well plates (Assay Block, 2 mL/well, 96 well square v-bottom; Costar, Cambridge, MA, USA) prior to liquid culture at 25°C for 48 hours (Choi et al., 2013). The media used for screening included (all % values are w/w): dichloran-glycerol 18% (DG18) agar (MB Cell, Seoul, Korea); dropout base (DOB) with complete amino acid supplement mixture (CSM) agar (MP Bio, Santa Ana, CA, USA); GPY agar (4% glucose, 0.5% peptone, 0.5% yeast extract, and 1.5% agar); and Sabouraud chloramphenicol gentamicin (SCG) agar (MB Cell). Antibiotics (100 mg/L of both chloramphenicol and streptomycin) were added to each medium to repress bacterial growth, and 0.1% (v/v) Triton X-100 and 0.4% (w/v) L-sorbose were added to repress fungal growth. Yeasts were cultured on DG18, DOB with CSM, GPY, and SCG agar media in square plates (245 × 245 × 25 mm). All colonies from plates yielding multiple colonies were picked and cultured separately. In total, 79 individual isolates were transferred to fresh plates three times and then processed for sequencing of the internal transcribed spacer (ITS) genes.

Sequencing and phylogenetic analysis

The detailed methodology of sequencing and phylogenetic analysis has been previously published (Choi et al., 2013). The primer set used to amplify ITS genes from the yeast strains were the previously described ITS1 (5′-TCCGTAGGTGAACCTGCG-3′) and ITS4 (5′-TCCTCCTCCGTTATATGCAG-3′) primers (White et al., 1990). For polymerase chain reaction (PCR), DNA was extracted from yeast colonies growing on agar plates using the InstaGene Matrix method, according to the manufacturer’s protocol (Bio-Rad Laboratories, Hercules, CA, USA). PCR was performed with 20 ng genomic DNA as template in a 30-μL reaction volume containing EF-Taq DNA polymerase (Solgent, Daejeon, Korea). The PCR program included the following steps: 95°C for 5 minutes; followed by 35 cycles of 95°C for 2 minutes, 55°C for 60 s, and 72°C for 60 seconds; and a final extension step for 10 min at 72°C. Amplification products were purified using a multiscreen filter plate (Millipore Corp., Bedford, MA, USA). Sequencing was performed using a PRISM BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). Hi-Di formamide (Applied Biosystems) was added to DNA samples containing the extension products. The mixtures were incubated at 95°C for 5 minutes followed by 5 minutes on ice, and analyzed on an ABI Prism 3730XL DNA Analyzer (Applied Biosystems). DNA sequencing was performed by Macrogen Inc. (Seoul, Korea). Nucleotide sequences for the 79 isolates reported in this paper were deposited in DDBJ(DNA Data Bank of Japan)/GenBank under the following accession numbers: LC018741-LC018819.

Phylogenetic analysis of Aureobasidium pullulans interspecies

The nucleotide sequences of the ITS genes were aligned using the ClustalW2 program of the EMBL-EBI website. A BLAST search was used to identify the GenBank sequences most closely related to those of the yeast isolates. Phylogenetic trees were constructed using the neighbor-joining method, using MEGA5 for Windows (Tamura et al., 2011), and featured bootstrap analyses of 1,000 samples. Evolutionary distances were calculated using the Kimura two-parameter method (Saitou and Nei, 1987).

Results and Discussion

Here, yeast isolates colonizing fleabane flowers were isolated and phylogenetically analyzed. Seventy-nine strains from whole yeast isolates were identified
Fig. 1. (A) Pi chart, and (B) a phylogenetic tree of yeast isolates from flowers of the wild fleabane *Erigeron annus* (L.) Pers., based on internal transcribed spacer sequences. Strains described in the present study are in bold font. The numerals are the confidence levels derived from 1,000 replicate bootstrap samplings.
Yeast in the Flowers of Fleabane

Fig. 2. Aureobasidium pullulans interspecies. A neighbor-joining tree of A. pullulans isolates from flowers of the wild fleabane Erigeron annus (L.) Pers., based on internal transcribed spacer sequences. Strains described in the present study are in bold font. The numerals are the confidence levels derived from 1,000 replicate bootstrap samplings.

The strains included, A. pullulans (39 isolates), Candida (17 isolates), Rhodosporidium (14 isolates), Cryptococcus (6 isolates), and Rhodotorula (3 isolates) (Fig. 1). Shown in Fig. 1 is a summary phylogram of the yeast isolates. A. pullulans, Candida spp., and Rhodosporidium spp. dominated the yeast composition of the plant flower, representing 49%, 21%, and 18% of the isolates, respectively. The least abundant yeasts were Cryptococcus spp. (8%) and Rhodotorula spp. (4%). Among the isolates were several species, including C. tropicalis (11 isolates), C. parapsilosis (6 isolates), Rhodosporidium azoricum (13 isolates), and R. fluvial (1 isolate) for which there was little prior knowledge regarding their habitat and function (Käppeli and Fiechter, 1977). Karatay and Dönmez (2010) showed that C. tropicalis and R. mucilaginosa produce biodiesel in media containing molasses, while Rhodosporidium isolates were also shown to produce biodiesel in a snow crab study (unpublished data). As shown in Fig. 1, this is the first yeast composition to be defined in fleabane flowers. Specially, five genera colonized the flowers, and future studies should explore community structure. In addition, A. pullulans, Candida, and Rhodosporidium were major colonizers, suggesting that yeast strains may be plant-specific.

Based on BLAST searches and phylogenetic analyses,
we found several interspecies of *A. pullulans* (Fig. 2). *A. pullulans* is known as black yeast because it produces melanin. Previous data showed that this yeast is the dominant species on the flower, leaf and stem of tiger lily, covering 97%, 35%, and 42% of each of these surfaces, respectively (Kim and Kim, 2015). To identify species and/or interspecies, deep-clade phylogenetic analyses of the *A. pullulans* isolates was performed, revealing that the yeast strains were most closely related to Group I (37 isolates) and Group II (2 isolates), while none belonged to Groups III or IV. In Group I, isolate F-97 was separated from the rest of the group. Isolates F-3 through F-99 and the remaining 25 isolates were not related to Group I. Generally, *A. pullulans* produces polysaccharides, including pullulan and β-glucan, which find industrial and medical applications (Yurlova and de Hoog, 1997; Cheng et al., 2011; Muramatsu et al., 2012). Recently, *A. pullulans* has been shown to produce (poly)malic acid (Nagata et al., 1993), lipase (Leathers et al., 2013), laccase (Rich et al., 2015), valuable lipids (Turk et al., 2004), and siderophores (Ma et al., 2012). *A. pullulans* from plant flowers have also been found to produce several biosurfactants, depending on their phylogenetic class (unpublished data).

The present study identified the main yeast isolates from fleabane, including a phylogenetic assessment of the *A. pullulans* isolates. However, the roles played by yeasts (including *A. pullulans*) in host plants still require further exploration. Future studies should address the distribution of yeast communities using an extensive sampling strategy for more robust group assessments.

**Acknowledgement**

This work was supported by the National Research Foundation of Korea (NRF), via a grant from the Korean government (MSIP) (no. NRF-2014R1A2A1A1052888).

**References**


siderophore extraction. Process Biochemistry, 47(12), 1807-1812.