*IOSR Journal Of Pharmacy And Biological Sciences (IOSR-JPBS)*
*e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 19, Issue 4 Ser. 1 (July. – August. 2024), PP 40-47*
*www.Iosrjournals.Org*

# Determination And Characterization Of Aloe-Yeast(Kjm-0002) Isolated From Volcanic Soils Of Aloe Farm In Mt. Agung And Mt. Halla

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***Abstract:***
*The purpose of this study is to isolate yeast from volcanic soil collected from Agung Mountain in Bali, Indonesia, and Hallasan Mountain in Jeju Island, Korea, for sequence analysis and phylogenetic analysis. In order to suppress the growth of bacteria and fungi, each volcanic soil sample was cultured in a medium containing chloramphenicol, streptomycin, 0.1% Triton X-100, and 0.4% L-sorbose. After isolating yeast, genomic DNA of the isolated strains was extracted for the sequencing, and a phylogenetic tree was builded.*
*Meyerozyma (56 strains), Rhodotorula (1 strain), Sporoblomyces (1 strain), Cryptococcus (9 strains) were isolated from AloveBali volcanic soil (AL-V), and Saccharomyces (41 strains) and Rhodosporidium (1 strain) were isolated from KJM Aloe volcanic soil (AL-S). In addition, the S. cerevisiae strain, which is genetically similar to the yeast strain native to oak, was isolated for the first time from volcanic soil in Hallasan Mountain, Jeju.*

***Key Word****: Yeast, Volcanic soil, Sequence analysis, Phylogenetic analysis.*

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Date of Submission: 16-07-2024 Date of Acceptance: 26-07-2024

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## I. Introduction

Yeast lives in various environments such as soil, aquatic environments, plants, insects, and extreme environments. They have an important influence on beverages, pharmaceuticals, enzymes, agriculture, and industries, which give great benefits to human diet[1,2,3,4]. In order to conduct yeast research, technology that suppresses the growth of bacteria and fungi and cultivates and isolates only yeast is required. There are various methods of inhibiting the growth of bacteria and fungi, and many antibiotics exist[5,6,7,8]. In this study, chloramphenicol and streptomycin were added to the medium to suppress the growth of bacteria, and 0.1% Triton X - 100 and 0.4% L - sorbose were added to suppress the growth of fungi[9,10,11,12,13,14].

Aloe vera is a perennial succulent plant that belongs to the lily family and has about 500 species. It is known to have antioxidant activity, antibacterial, anti-inflammatory, and tissue formation properties. Therefore it is used to treat and prevent diseases. In addition, aloe is used in various ways for cosmetic purposes. The species mainly grown in Korea are Aloe vera and Aloe avoresence[15,16,17,18,19,20,21].

Both Mount Agung in Bali, Indonesia, and Hallasan in Jeju Island, Korea were created by volcanic activity, and both are active volcanoes. Volcanic soil of Mount Agung is mainly composed of soil feels like volcanic ash, while that of Hallasan is composed of porous basalt. Therefore, compared to Mount Agung, the volcanic soil of Hallasan is drained better. This is the environmental condition necessary for the growth of succulents such as aloe, and there is actually an aloe farm on Jeju Island[22]. In addition, studies related to aloe and yeast have been conducted[23]. Therefore, in this study, it was thought that there would be a difference in the yeast present in the volcanic soil of Agung Mountain and Hallasan Mountain, so the yeast was separated from the two volcanic soil and identified. Since the identification, sequence analysis, and phylogenetic analysis of yeast from volcanic soil have been rarely studied worldwide, this study is expected to be used as important basic data in the industrial field using yeast.

## II. Material And Methods

**Material**

A volcanic soil collected from Mount Agung in Bali, Indonesia(AloveBali volcanic soil, AL-V) was provided from AloveBali Ltd., and a volcanic soil collected from Hallasan in Jeju Island, Korea(KJM Aloe volcanic soil, AL-S) was provided from KimJeongMoon Aloe Ltd.(Figure 1).


**Figure 1.** Volcanic soils.
**a)** A volcanic soil collected from Mount Agung in Bali, Indonesia(AloveBali volcanic soil, AL-V).
**b)** and a volcanic soil collected from Hallasan in Jeju Island, Korea(KJM Aloe volcanic soil, AL-S)

**Isolation of yeast from volcanic soil (AL-V, AL-S)**

Samples were collected from each volcanic soil using sterilized tweezers in a clean bench. Samples were washed three times using a 0.1 M potassium phosphate buffer and stored in a sterilized container until the experiment. The medium for yeast screening was DG18 agar (Dichloran - Glycerol 18%, MBcell, Seoul), DOB with CSM agar (MP bio, CA, USA), GPY agar (4% glucose, 0.5% peptone, 0.5% yeast extract, 1.5% agar), SCG agar (Sabouraud Glucose Agar, MBcell). 100mg/L of chloramphenicol and streptomycin were added to each medium to inhibit the growth of bacteria without affecting the growth of yeast, and Triton X -100 0.1% and L - sorbose 0.4% were added to suppress the growth of fungi. First, sterilize the DG18, DOB with CSM, GPY, and SCG agar medium and spray them onto a 245 × 245 × 25 mm square plate (Nunc Bio-Assay Dish, Thermo Scientific, Roskilde, Denmark). The collected samples were cut into small pieces suitable for crushing, and then placed in a sterilized container. 10 mM potassium phosphate buffer was added in container so that the total volume became 10 mL, and then crushed by using a sterilizable homogenizer (T10 basis, IKA, Germany). 1 mL of the well-crushed volcanic soil stock solution was spread using a sterilized glass spreader in the medium and then cultured in a 25°C incubator for 2 to 5 days.

**Sequence analysis and phylogenetic analysis (AL-V, AL-S)**

Only yeast was selected from each plate cultured as described above, and tertiary separation was performed to obtain a single strain. The entire yeast was separated from the plate that contains appropriate number of colony. Genomic DNA was extracted with InstaGene Matrix (Bio-Rad, Hercules, CA, USA) and PCR was performed with EFTaq DNA Polymerase (Solgent, Korea). Sequencing was performed with PRISM

BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA), and analysis was performed with ABI PRISM 3730XL DNA analyzer (Applied Biosystems, Foster City, CA) in Macrogen, Inc..

Sequence analysis results using ITS1 (5'- TCCGTAGGTGAACCTGCG -3') and ITS4 (5'- TCCTCCGCTTATTGATATGC -3') primers were aligned with sequence data of standard strains from NCBI using the Cluster W program[24].

The phylogenetic tree was completed using the neighbor - joining method of MEGA version 5[25,26]. And the base sequence information of the strain will be registered in GenBank in the future to be assigned a strain number.

**Sequencing of AL-S and genotype analysis**

Microsatellite genotypic data analysis of AL-S(yeast in KJM Aloe volcanic soil taken from Mt. Halla on Jeju Island) was used to collect approximately 250 genotypes of AL-S strains and to identify 75 AL-S strains for WGS. DNA was extracted using phenol/chloroform, and sequencing libraries were prepared using Genome Shotgun PCR Free 1.3 and Index TruSeq paired-end in Genome Sciences Center of Macrogen, Inc.. 150 base pairs, paired-end library was sequenced on HiSeqX-1.

**Genetic map of AL-S**

A genetic map was obtained from the sequencing result of AL-S by using R-pheat map package. The gene was blasted for readable global strains of interest in an assembly of a nucleotide sequence of 105 non-S288c clusters in 75 AL-S strains (blastn, bit score ratio of 0.4 and E-value <0.000001). MEGAHIT v1.2.9 with parameter "–no-mercy –prune-level 3 –min-count 5" was used to assemble it. After the contig was modified, scaffolding was conducted by using S288c reference genome R64-3-1 with ragtag v 2.01. Clusters not containing sequences were removed in 75 strains and the clusters that are inconsistent in sequencing quality, type, and depth are excepted. Non-S288c clusters were mapped in 75 AL-S strains, independent of 34 global strains.

## III. Result And Discussion

**Isolation of Meyerozyma strain from AloveBali volcanic soil (AL-V) and Saccharomyces strain from KJM Aloe volcanic soil (AL-S)**

In this study, yeast strains living in two types of volcanic soil were isolated and their taxonomic position was clarified by phylogenetic analysis through through sequencing of ITS genes.

Meyerozyma (56 strains), Rhodotorula (1 strain), Sporoblomyces (1 strain), and Cryptococcus (9 strains) were identified in AL-V (volcanic soil from AloveBali volcanic soil). In particular, in the case of Meyerozyma, it was found that two clades were largely formed (Figure 2 a).

Saccharomyces (41 strain) and Rhodosporidium (1 strain) were isolated from AL-S (volcanic soil of KJM Aloe). This study revealed that the types of yeast settled in AL-S are extremely limited to two types of yeast species, Saccharomyces and Rhodosporidium. And Saccharomyces was found to be Saccharomyces cerevisiae (Figure 2 b).

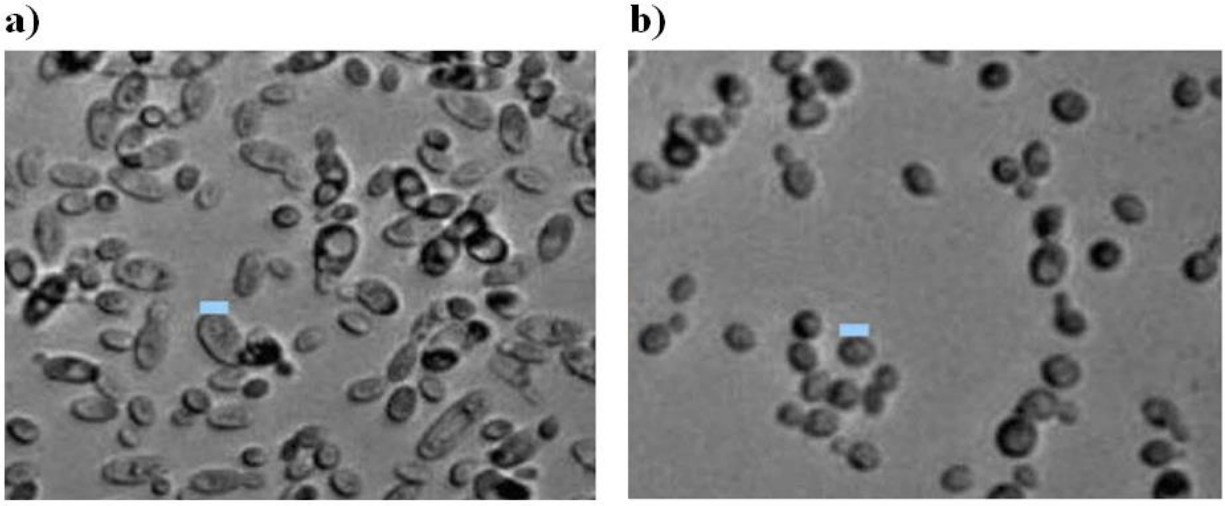



**Figure 2.** Optical microscopy result (magnification: 40×; H&E 200; bar=50 μm). **a)** A volcanic soil collected from Mount Agung in Bali, Indonesia(AloveBali volcanic soil, AL-V). **b)** and a volcanic soil collected from Hallasan in Jeju Island, Korea(KJM Aloe volcanic soil, AL-S)

**Sequencing of representative yeasts isolated from AL-V and AL-S**

As shown in Figure 3 and 4, the clusters of yeasts settled in AL-V and AL-S were revealed for the first time in this study. In particular, it is shown that four types of yeasts are settled in AL-V and two types are settled in AL-S, and further studies are required on the diversity of yeast communities. When more information is accumulated on these yeasts, the geological or geopolitical characteristic of AL-V and AL-S volcanic soil and the interrelationship of the yeast function settled there, or the application of its efficacy, will be possible.

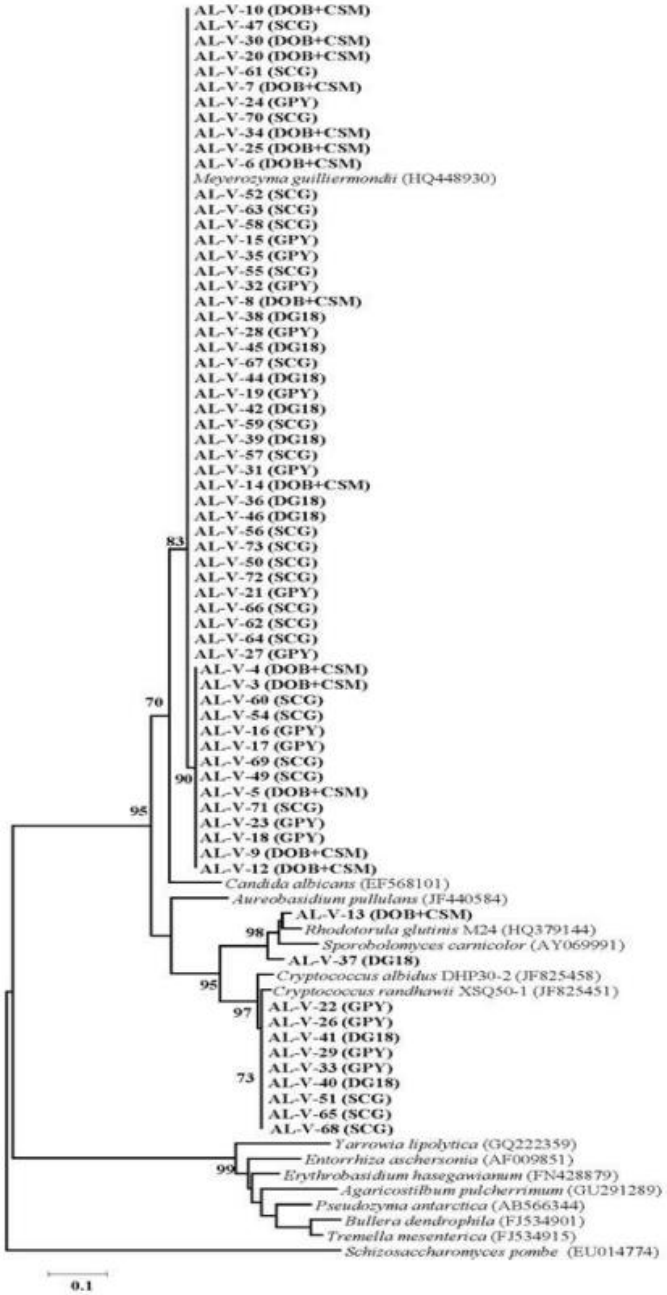



**Figure 3.** Molecular phylogenetic tree constructed by the Neighbor-Joining method using sequencing of yeast related to representative yeast isolated from AL-V. Isolated medium is shown in parentheses. The numbers shown indicate the confidence level of 1000 repetitive bootstrap sampling (frequency less than 75% is not shown)

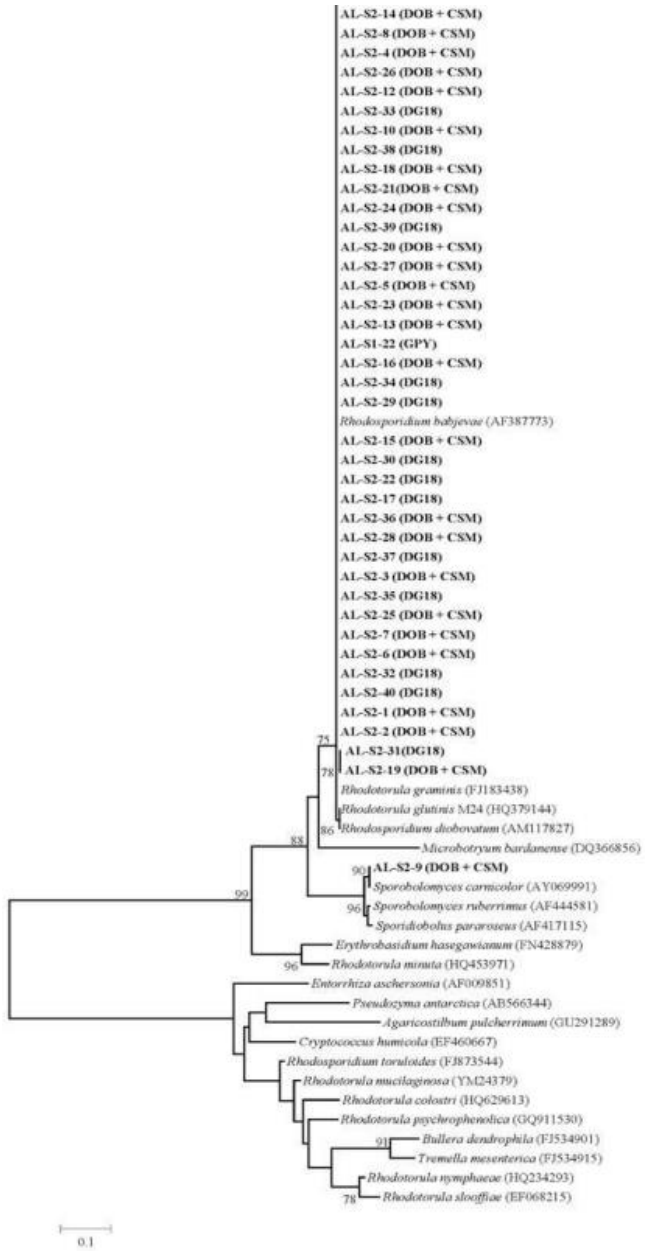



**Figure 4.** Molecular phylogenetic tree constructed by the Neighbor-Joining method using sequencing of yeast related to representative yeast isolated from AL-S. Isolated medium is shown in parentheses. The numbers shown indicate the confidence level of 1000 repetitive bootstrap sampling (frequency less than 75% is not shown)

**Analysis of the phylogenetic tree of AL-S**

A microsatellite assay based on 10 repressive societies was used to distinguish between commercial yeast strains and non-commercial strains. According to the results, phylogenetic trees for 75 AL-S strains were analyzed for WGS using the Illumina Hi-Seq platform. We obtained 150bp paired-end read with 205-fold genome coverage and an average of 15 million reads per genome. An average of 97% of sequencing reads were mapped to the reference genome of AL-S with an average density of 62,611 SNPs. As a result, it was identified as an Saccharomyces cerevisiae (S. cerevisiae) strain isolated from the wild oak native to Korea, and it was included in the phylogenetic tree of the oak strain and clustered near WE clade. The 34 strains of the AL-S strains belong to a new clade called PWCW clade. Moreover, it was confirmed that one of these strains clustered in the TPO clade, named S. cerevisiae KJM-0002 (tentative name) which is Isolated from AL-S (a volcanic soil of the aloe farm on Jeju Aloe Farm, provided by KimJeongMoon Aloe Ltd.). The S. cerevisiae KJM-0002 strain was isolated from Jeju aloe farm volcanic soil with more than 10 isolates, suggesting that it was identified as a novel yeast strain obtained by eco-friendly cultivation(Figure 5).

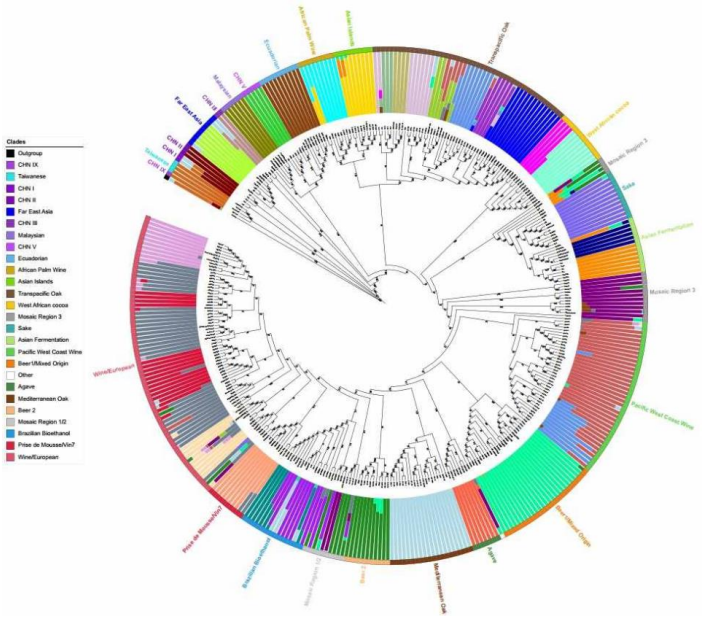
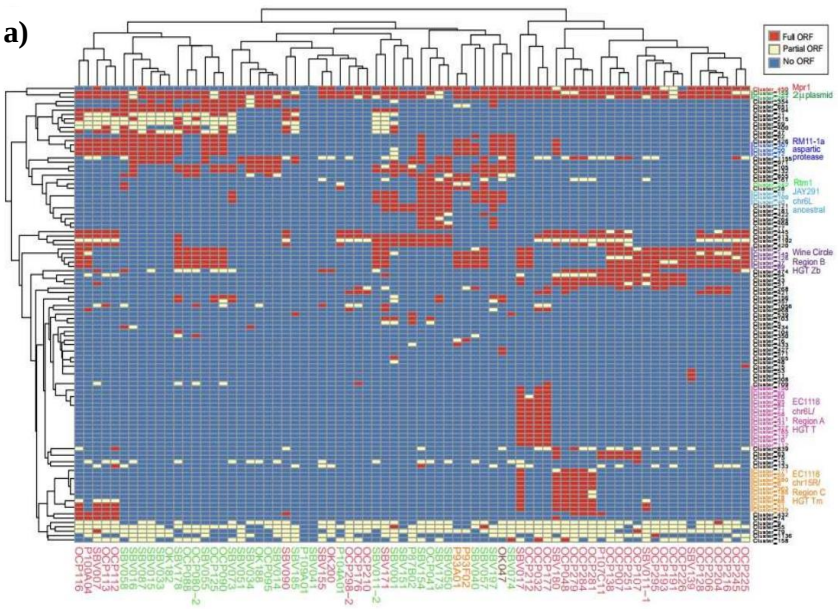



**Figure 5.** Genetic Map of S. cerevisiae KJM-0002 strain isolated from KJM Aloe volcanic soil (AL-S).

**Gene pangenome analysis from sequencing of S. cerevisiae KJM-0002 strains isolated from AL-S**

Analysis of the pangenome of the S. cerevisiae KJM-0002 strain isolated from AL-S revealed that 7,796 ORFs were contained and compared with 6,081 nonredundant ORFs of the S288c reference genome(Figure 6). The 1,715 ORFSs that were not expressed in the S288c genome were "non-S288c clusters genes" and these are predicted to be part of S. cerevisiae genome or acquired through HGT crossings from other yeast species. They were found in the specific natural environment of volcanic soil of Mt. Halla, Jeju. 5 gene clusters of the commercial strain, S. cerevisiae Z-bailii, were included and they are presumed to be originated from fermentation strains found in Korean traditional makgeolli and vinegar.

To identify the "non-S288c cluster gene" present in 75 BC strains of S. cerevisiae isolated from AL-S, it was mapped to the S288c genome. Subsequently, all remaining reads were newly assembled, ORFs were predicted, and ORFs with more than 97% nucleotide similarity to each other were then obtained by clustering, and the clusters were highly curated after comparison with two pangenomes (Figure 6). As a result, the gene pangenome obtained from sequencing of the S. cerevisiae KJM-0002 strain isolated from AL-S showed less HGT in the WE clade compared to PWCW (Figure 6 a), and the "non-S288c clusters gene" cluster was identified which gene clusters were present compared to the ORF of the "non-S288c clusters gene" predicted in 34 global strains (Figure 6 b).



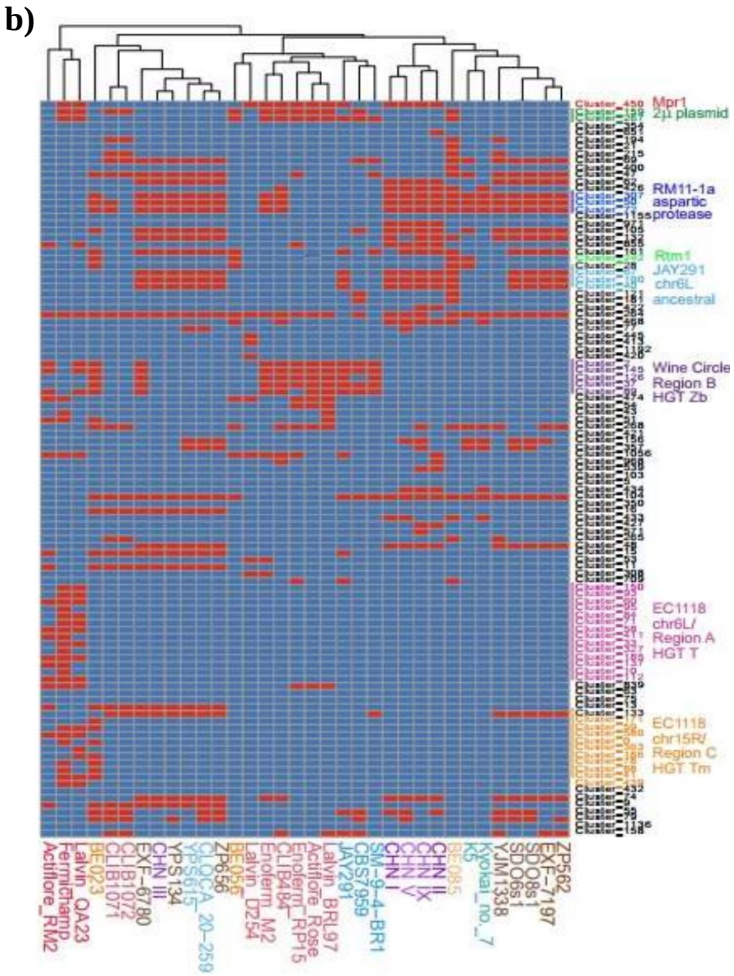



**Figure 6. a)** Gene pangenome. **b)** "non-S288c clusters gene" cluster. Both data were obtained from sequencing of S. cerevisiae KJM-0002 strains isolated from KJM Aloe volcanic soil (AL-S).

## IV. Conclusion

In this study, a screening method was used to separate only yeast while excluding bacteria and fungi. To test this, only yeast could be successfully separated volcanic soil of AloveBali(AL-V) and KJM Aloe(AL-S) by adding Triton X - 100 and L - sorbose to suppress fungi and antibiotics to suppress bacteria in a number of yeast selection media, DG18, DOB with CSM, GPY, and SCG. It is thought that we can quickly and easily explore the function of yeast and discover yeast by the large amount yeast screening method. And the study about the growth characteristics of volcanic soil cultivation crops (including Aloe) and fermentative activity using volcanic soil yeast is expected.

Saccharomyces (41 strains) and Rhodosporidium (1 strain) were isolated from KJM Aloe volcanic soil (AL-S) and Meyerozyma (56 strains), Rhodotorula (1 strain), Sporoblomyces (1 strain), Cryptococcus (9 strains) were isolated from AloveBali volcanic soil (AL-V). 41 S. cerevisiae strains were selected for whole genome sequencing using II llumina paired-end read based on microsatellite clustering data for the Saccharomyces strains isolated from KJM Aloe volcanic soil (AL-S), which are currently industrially available and recognized by regulatory agencies such as the Ministry of Food and Drug Safety among isolated yeast strains.

This study isolates an S. cerevisiae strain(KJM-0002) genetically similar to an oak native yeast strain from volcanic soil in Mt. Halla, Jeju for the first time.

## Acknowledgement

This work was supported by Hyeon-Ju Park, professor of Seoul National University.

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