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ISOLATION AND CHARACTERIZATION OF YEAST FROM FERMENTED SEMERU ARABICA WINE COFFEE CHERRIES

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ABSTRACT

Wine coffee is fermented coffee cherries with a wine-like flavor and aroma. The purpose of this study was to isolate, characterize ethanol-tolerant and thermotolerant yeast from Semeru Arabica wine coffee cherries and to determine the molecular identification of the selected yeast. In this study, the Semeru Arabica coffee cherries were fermented for 30 days.Yeast isolation was performed on samples collected each week during fermentation. The selected isolates were characterized for macroscopic and microscopic morphology, followed by ethanol and temperature tolerance analysis. The isolate with the most desirable characteristics, designated CW30, grew well at 30ºC but was sensitive to temperatures above 35ºC. The isolate grew well in media containing 5% ethanol but was sensitive to a 10% ethanol concentration. Based on nucleotide sequence analysis, CW30 exhibited 100% similarity with *Candida ethanolica* isolate 3-1-19.

Keywords: Fermented coffee, Semeru Arabica, Wine coffee, Yeast isolation

INTRODUCTION

Coffee is the most widely consumed non-alcoholic beverage in the world. It is made from the brewing of coffee beans that have been roasted and produced into powder. Coffee production in Indonesia is ranked 4th after Brazil, Vietnam, and Colombia, with a total of 624,000 tons (USDA Foreign Agricultural Service, 2021). The two most well-known coffee varieties are Arabica (*Coffea arabica*) and Robusta (*Coffea canephora*). Those varieties are categorized as the largest suppliers in the world coffee trade (Badmos *et al*., 2019). Arabica coffee has a strong taste and distinctive aroma and has a lower caffeine content compared to Robusta coffee, which is more bitter and has a higher caffeine content (Handayani, 2016). This gives Arabica coffee potential in processing the diversified results of wine coffee due to its milder bitterness and lower initial caffeine content compared to Robusta (Ismail Sulaiman *et al*., 2021). Therefore, Arabica wine coffee was used in this study.

According to Sulaiman and Hasni (2022), wine coffee is produced from the fermentation process of coffee cherries for 30–60 days and dried directly in sunlight for about 3–6 weeks. The fermentation process of wine coffee aims to eliminate bitterness so as to improve the quality of coffee products caused by compounds from the degradation of the mucous layer by microorganism activity and is often correlated with an increase in the selling price of products that have a taste, such as wine (Juanda *et al*., 2022). Microorganisms that have the potential in the fermentation process, such as yeasts, filamentous fungi, lactic acid bacteria (LAB), and acetic acid bacteria (AAB) (Huch and Franz, 2014), initiate the degradation process of the mucilage layer of coffee cherries composed of polysaccharides (pectin), which is fermented by LAB and yeast, using polysaccharides as substrates for their metabolism and produce simple saccharides. Those substances are ready to use substrate for yeast to perform alcoholic fermentation, which produces alcohol and CO_2. Alcohol and CO_2 will be converted by AAB into acetic acid (Haile and Kang, 2019).

Yeasts are microorganisms mostly isolated from fermented coffee cherries, but information on their effects on the development of coffee taste characteristics is limited (Pereira *et al*., 2014). The presence of yeast during the fermentation of coffee cherries depends on the coffee variety, processing method, moisture content, enzymatic activity of the colony species, competition from the substrate, environmental factors, and the antimicrobial activity of microorganisms (Desisa and Muleta, 2017). Exploration of yeast diversity during wine coffee processing is necessary to find out the potential yeast that exists in fermented Semeru Arabica wine coffee cherries. Previous research conducted by (Sulaiman and Hasni, 2022) indicated that yeast is more dominant at the beginning and after 20 days of fermentation. Yeast metabolism initiates the growth of other microorganisms (such as LAB and AAB) and inhibits the growth of mycotoxins in filamentous fungi. In this study, the conditions for yeast growth at the beginning of fermentation and after fermentation were followed by acetic acid bacteria with a fermentation period of 30 days due to fermentation to accommodate the succession of microorganisms. However, there is still a lack of information about yeast, which is limited to its influence on the development of taste characteristics in coffee. Therefore, the purpose of this study was to isolate, characterization shows that isolate has the potential to be ethanol-tolerant, thermotolerant yeast from Semeru Arabica wine coffee cherries and find out the molecular identification of selected yeast. The aim of get yeast isolates to high temperatures and ethanol for industrial needs. The yeast then can be applied for future applications as a starter in producing wine coffee and the use of high temperatures during the fermentation process and high ethanol levels during fermentation are the reasons why we are exploring high temperatures and ethanol-resistant yeast isolates.

MATERIALS AND METHODS

Materials

The material used for this study was Semeru Arabica coffee cherries obtained from Alir Coffee, Singosari, Malang. Primer sequencing materials NL-1 (5´-GCATATCAATAAGCGGAGGAAAAG-3´) and NL-4 (5´GGTCCGTTTTTCAAGACGG-3´), PCR Master Mix containing ddH2O, MyTaq Red Mix 2x (Bioline, BIO-25048), ± 10 ng DNA template, 0.4 µM of each primer, and 0.8% TBE agarose. Chemicals include YPGA (Yeast Extract Peptone Glucose Agar) (Merck) and YPGB (Yeast Extract Peptone Glucose Broth) (Merck), 0.05% chloramphenicol (Novachlor), and absolute ethanol (Merch). All materials were of analytical grade.

Equipments

The equipment used for this study includes 25 kg plastic bags, autoclaves, vortex (Thermo Scientific), laminar air flow, spectrophotometer (Thermo Scientific), nanodrop spectrophotometer, incubator (Binder), PCR (Bioline, BIO25048), microcentrifuge (Benchmark), cold centrifuge (Thermo Scientific), analytical balances (Scout Pro), electric stove (Maspion), microscope (Olympus), refrigerators, loop needles, bunsen, and glassware such as beaker glass, Erlenmeyer, spreader, stirring rod, measuring cup, test tube, and petri dish.

Research Design

This research employs an experimental approach by fermenting Semeru Arabica coffee cherries for 30 days. This extended fermentation period, compared to the 1-2 days used in regular coffee processing (Sulaiman and Hasni, 2022), aims to develop the characteristic taste and aroma associated with wine coffee. Isolation and screening were performed and followed by ethanol and temperature resistance analysis. The selected yeast was then identified by using molecular technique. Qualitative and quantitative descriptive methods were used in this study.

Research Stages

Semeru Arabica coffee cherries were sorted to separate high-quality from low-quality cherries. According to research by Aurelia *et al*., (2021), high quality cherries have thick, non-hollow flesh with a uniform red color. These high-quality cherries were then fermented in plastic bags. The fermentation process of Semeru Arabica coffee cherries was evaluated including monitoring the reduced sugar content. Yeast isolates were obtained through isolation and screening, followed by macroscopic and microscopic characterization. To identify ethanol- and temperature-tolerant yeasts, ethanol and temperature tolerance analyses were performed. Finally, the selected yeast was analyzed using a molecular technique.

Methods

Arabica coffee cherries for 30 days of fermentation; observations of reducing sugar before and after fermentation of wine coffee cherries (Dashtban *et al*., 2010); isolation of a single colony of yeast at room temperature using the streak plate method (Sari, 2020); macroscopic and microscopic observations of yeast isolates (Sari, 2020), ethanol tolerance and temperature tolerance tests were carried out (Kumari *et al*, 2019), and molecular identification of isolates conducted by PT. Genetics Science Indonesia (Delva *et al*., 2022).

The coffee wine fermentation process starts by soaking ripe Semeru Arabica coffee cherries. Fermentation then occurs in a sealed plastic bag with a small opening (approximately 10 cm) for controlled aeration. The bag is stirred periodically to ensure proper mixing. This controlled aeration creates a microaerobic environment suitable for the growth of certain microorganisms, particularly yeast, at their optimal temperature. Every 10 days during the 30-day fermentation period, the fermented coffee cherries are removed and dried in the morning sun.

The Nelson-Somogyi method uses arsenomolybdate and copper reagents. The principle of this method is an oxidation reaction between Nelson reagent and glucose that can form complex compounds after the addition of arsenomolybdate reagents reduced to blue molybdeen. The blue complex compounds formed were measured for absorbance using a UV-Vis spectrophotometer. A standard glucose curve was prepared by weighing 10 mg of anhydrous glucose plus 100 ml of aquades. Diluted to a concentration of 2, 4, 6, 8, 10 mg/ 10 mL. Five grams of samples were crushed and added to 45 ml aquades. Then followed by mixing and filtering with Whatman paper. Each concentration and filtrate was put into a test tube with 1 ml of standard solution and 1 ml of Nelson-Somogyi reagent in a ratio of 25 nelson A : 1 nelson B. Heated to boiling for about 20 minutes, then cooled to 25 ºC. Then, 1 ml of arsenomolybdate reagent and 7 ml of aquades were added. Cornering was carried out until the Cu_2O precipitate became soluble and homogeneous. The absorbance of each solution with a wavelength of 540 nm was measured. The absorbance results were recorded and plotted to obtain the standard concentration of glucose shown by the standard curve and equation (Dashtban *et al*., 2010)

The sample used in this study was the Arabica coffee commodity produced by Semeru. The samples were weighed at 5 g each, then dissolved into an Erlenmeyer containing 45 ml of sterile distilled water and homogenized with a stirring rod. Samples that have been homogeneous were taken as much as 1 ml of growing samples inoculated on YPG agar media (yeast extract 1%, peptone 2%, glucose 2%, agar 1.5%) + chloramphenicol 0.05% by the spread plate method and incubated at 27ºC for 2-4 days. The yeast isolate was taken in one loop and inoculated on 10 ml of YPG media containing 0.05% *chloramphenicol* and coated at 30ºC for 24 hours. The yeast isolates were grown on YPGA media, then 1 loop colony was taken, inoculated into YPGA media using the streak radian method, and incubated at a temperature of 27ºC for 24 hours. Colonies were selected on the basis of separate colonies, and identification was carried out Yeast selection was carried out based on the desired yeast character. The characteristics of yeast colonies are the colonies are not slimy, are white cream color, have a smooth texture, have a circular colony shape, have a convex surface, and have entire edges (Reis et al., 2013). To obtain thermotolerant yeast, the isolates were further incubated at 37 ºC, 40 ºC and 45 ºC. Incubation at different temperatures will affect the subsequent method since it is an initial parameter in the thermotolerant isolate (Sari, 2020).

Yeast isolates were then identified macroscopically and microscopically to provide a figure of the morphological characterization of yeast and can be used to determine yeast species that have been isolated. The yeast isolates were grown in the liquid media and on the agar plate. The samples were then incubated at a temperature of 27 ºC for 18–24 hours. Some macroscopic parameters were identified, among others: color, texture, surface appearance, colony shape, and colony edge (Sari, 2020).

After the isolate is identified, the isolate will be tested for the ethanol tolerance analysis of yeast isolates aims to determine the. ability of yeast to grow at different ethanol concentrations The isolates were grown on YPGA with different ethanol concentrations, namely 0%, 2.5%, 5%, 7.5%, and 10%. The plates were incubated at a temperature of 30ºC for 48 hours. The resulting growth was observed and spot test analyzed (Kumari *et al*., 2019). The temperature tolerance analysis of yeast isolates aims to determine the ability of yeasts to grow at different temperatures. The isolates were grown on YPGA by using the spotted test method. The plates were incubated at different temperatures of 30ºC, 37ºC, 40ºC and 45ºC for 72 hours. The resulting growth was observed and spot test analyzed (Kumari *et al*., 2019).

The isolate was grown on PGYA medium for 48 hours at a temperature of 30ºC. After the isolate grew well, the tube was sealed using parafilm, wrapped in aluminum foil to prevent contamination, and sent using fast delivery to PT. Genetics Science Indonesia for DNA sequencing, genomics, barcoding, and bioinformatics analysis. PT Genetics Science Indonesia used the Quick-DNA Fungal/Bacterial Miniprep Kit from Zymo D6005 research, designed to extract and isolate DNA quickly for 15 minutes. The determination of DNA concentration and purity was carried out using a nanodrop spectrophotometer. Continued DNA PCR amplification on ribosomal DNA regions D1/D2. Followed by electrophoresis analysis (0.8% TBE agarose) of PCR/amplicon products. Furthermore, DNA sequencing of isolates was carried out using the bi-directional sequencing method. DNA sequence assemblies in the D1/D2 region were then analyzed with nucleotide BLAST to predict the species alignment of CW30 isolates in the NCBI database. The BLAST results will be constructed into a phylogenetic tree by the neighbor-joining method of the NCBI BLAST Tree (Delva *et al.*, 2022).

RESULT AND DISCUSSION

1. Fermentation of Semeru Arabica *Wine Coffee Cherries*

Figure 1. Fermentation of Semeru Arabica Wine Coffee (a): Soaking of Coffee Cherries (A), Low Quality of Wine Coffee Cherries (B), Wine Coffee Cherries on 0 Day Fermentation (C), Wine Coffee Cherries After 10 Days (D), 20 Days (E), and 30 Days Fermentation (F)

Dominant yeasts in the fermentation process are facultative anaerobes, thriving in microaerobic conditions with an optimal temperature of 30°C. During the fermentation process Figure 1., the color of the coffee cherries will change darker in direct proportion to the fermentation time. This is because the fermentation process will produce heat (temperature

changes) resulting from the oxidation metabolism of sugar compounds in the pulp. According to Candrasari *et al*. (2019) the color change that occurs is the result of microorganism activity in degrading organic matter, which will produce alcohol and acids and release heat (exothermic reactions). This reaction will cause the diffusion of metabolites in seeds that cause seeds to die and continue with the occurrence of enzymatic reactions to the formation of aroma, taste, and color (Hui and Evranus, 2012).

Yeasts that commonly live in these facultative anaerobic conditions, for example, S. *cerevisiae*, convert sugars found in cherries into ethanol and carbon dioxide (Mariyam *et al*., 2022). At the time of the breakdown of sugars, sucrose is the main component, which will be broken down into glucose and fructose by yeast and LAB. After that, there was secondary fermentation in the form of lactic acid fermentation by LAB, which brings out the aroma of wine and complex taste. The fermentation process, followed by the drying or aerobic natural fermentation, would trigger the growth of AAB, which converts alcohol into acetic acid (Sulaiman and Hasni, 2022).

2. The effect of fermentation on reducing sugar content

The results of the sample sugar content can be seen in Table 1. An analysis of the sugar content of the sample was carried out to measure the concentration of reducing sugar in the Semeru Arabica wine coffee sample, which has a fairly high sugar content for yeast growth.

Table 1. Reducing the Sugar Content of Wine Coffee Cherries

Fermentation time (days)	**Reducing Sugar Content (%) Average ± Stdev**
0	0.61 ± 0.01
30	2.88 ± 0.28

Our results showed a surprisingly low initial reduction in sugar content (0.61%). This contradicts Melo *et al*. (2015) who reported that coffee cherry mucilage contains up to 30% pectic substances like glucose and fructose However, Pereira *et al* (2014) suggest this might be due to "T0 fermentation (fermented for 0 days)," where minimal simple sugars are present in the unfermented coffee filtrate because they haven't been fully broken down by pectinase enzymes. In contrast, T30 samples (fermented for 30 days) displayed significantly higher reducing sugar levels. This increase aligns with Pereira *et al*. (2014) who explain it as the combined effect of microbial pectinase activity: microorganisms producing pectinase enzymes hydrolyze pectin into glucose during fermentation. Moreover, fructose persistence: Oligofructose molecules, present in the mucilage, may not be completely broken down into their simpler form (fructose monomers). Supporting this concept, Ribeiro *et al* (2017) also reported increased reducing sugars at the end of fermentation. They attribute this to the activity of various enzymes (pectinolytic, cellulolytic, etc.) that hydrolyze polysaccharides in the coffee cherry. Additionally, Pereira *et al* (2022) highlight the "fructophilic" properties of specific yeasts (like *Candida* sp.) which can contribute to high residual sugar content in fermented products like coffee.

3. Yeast Isolate Obtained from Fermented *Semeru Arabica* Wine Coffee Cherries

The results showed that colonies only grew at temperatures between 27°C and 30°C, indicating sensitivity to high temperatures in these isolates. Colonies demonstrating successful growth were then selected using a streak-plating technique for 48 hours Figure 2. Based on the initial isolation and screening, a single isolate (CW30) was obtained from the fermented Semeru Arabica wine coffee sample and exhibited characteristics typical of yeast.

(a) **(b)**

Figure 2. Yeast isolate CW30 of Semeru Arabica Wine Coffee was Streaked on YPGA After Incubation at 30ºC for 48 h (a), CW30 was Observed Under a Microscope at 1000 Times Magnification (b)

4. Macroscopic and Microscopic Characterization of Yeast Isolates from Semeru Arabica Wine Coffee

Among several dozen isolated yeasts (data not shown), only one, designated CW30, was obtained from the fermented Semeru Arabica wine coffee sample. Based on the data in Tables 2a and 2b, CW30 isolates have potential similarity with the yeasts *Saccharomyces cerevisiae, Candida parapsilosis,* and *Torulaspora delbruecki* this is supported by the literature (Bressani *et al.*, 2020). Specifically, the isolate shares characteristics with *S. cerevisiae*. This yeast typically forms circular colonies with a convex surface, entire (smooth) edges, and a shiny, milky white appearance. Additionally, *Saccharomyces* reproduce asexually through multilateral budding and exhibit spherical to hemispherical cell morphology (Kurtzman, 2001; Knop, 2011).

Table 2a. Macroscopic Morphology of Yeast Isolate CW30 *Semeru Arabica* Wine Coffee

Morphology	Macroscopic
Color	White cream
Texture	*Smooth*
Surface	*Raised-convex*
Colony Form	*Circular*
Colony edge/elevation	*Entire*

Table 2b. Microscopic Morphology of Yeast Isolate CW30 *Semeru Arabica* Wine Coffee

Morphology Characteristics	Microscopic
Cell Shape	Round-semi round
Budding type	*Multilateral*
Hyphae formation	*Pseudohyphae*

This genus is usually grown from *wine production and* has the advantage of being able to live under anaerobic fermentation conditions as well as facultative anaerobes in some types of sugars and produce ethanol and CO_2 (Azizah *et al.*, 2012). In Robert's opinion (2021), the macroscopic morphology and microscopy of CW30 isolates have similarities with characters from the genus *Candida sp.* Because it has colonies that are white, cream, shiny, smooth, and do not form true hyphae (pseudohyphae). Types of species *Candida sp.,* namely *M. (Candida) parapsilosis,* usually grow on coffee fruits with high proteolytic activity during the *semi-dry* fermentation process, which can give caramel and fruity flavors and has high active compounds in coffee (Pereira *et al.*, 2022).

5. Temperature Tolerance of Yeast Isolate from Semeru Arabica Wine Coffee

Of the several tens of isolates (data not shown), there was only one yeast isolate isolated from the 30th fermented Semeru Arabica wine coffee sample.

Table 3. Temperature Tolerance of CW30 Isolate

Temperature (ºC)	Growth
27	+
30	+
35	-
40	-
45	-

Note: (+) grew well, (-) cannot grow

According to Table 3, the isolate CW30 was grown at 27°C as a control temperature since the optimal growth range for most yeasts is between 27°C and 30°C. As expected, good growth (+) was observed at both 27°C and 30°C. Temperatures of 35°C and 40°C were used to assess the isolate's initial tolerance to high temperatures. The 35°C treatment might have served as a pre-adaptation step for the 40°C challenge. However, no growth (-) was observed at either 35°C or 40°C, suggesting that CW30 may not be tolerant of high temperatures (Reis *et al*., 2013).

6. Ethanol Tolerance of Yeast Isolate from Semeru Arabica Wine Coffee

Based on the results obtained, there was still CW30 cell growth in 2.5%, 5% and 7.5% media, but no cell growth in media containing 10% ethanol Table 4. This result is because CW30 isolates are sensitive to media with 10% ethanol. This result is in accordance with Neto *et al.* (2013), who found that high ethanol tolerance depends on the strain with the maximum ethanol tolerance that allows for yeast growth of 10% because alcohol dehydrogenase and hexokinase enzymes are more sensitive to high ethanol, resulting in inhibition of phospholipid membranes where binding to membrane hydrophobics causes disruption of the transport system and causes membrane selectivity ability to decrease. The results of the ethanol tolerance test have not been clearly defined, although it is reported to be able to grow under specified conditions; this depends on the genetic complex CW30 isolates have.

Table 4. Ethanol t=Tolerance of CW30 Isolate

Ethanol concentation (%)	Growth
2.5	+
5	+
7.5	+
10	-

Note: (+) grew well, (-) cannot grow

These results were obtained according to Tikka *et al.*, (2013) the tolerance range of ethanol is 7-12%. The addition of ethanol will temporarily inhibit cell growth, causing delayed cell growth. Yeasts that are able to withstand high ethanol levels have membranes that are resistant to being responsive to ethanol. The ability to tolerate ethanol depends on the composition of the plasma membrane which plays a role in maintaining the membrane of the yeast cell.

7. Molecular Identification of CW30 Yeast Isolate

Results of nucleic acid extraction (genomic DNA) from molecular assay CW30 isolates were measured using a nanodrop spectrophotometer. The DNA purity value of the CW30 isolate was A_{260}/A_{280} 1.87. This was in accordance with the DNA purity value to proceed to the PCR with a range of A_{260}/A_{280} = 1.8–2.0 (Sambrook *et al.*, 1989).

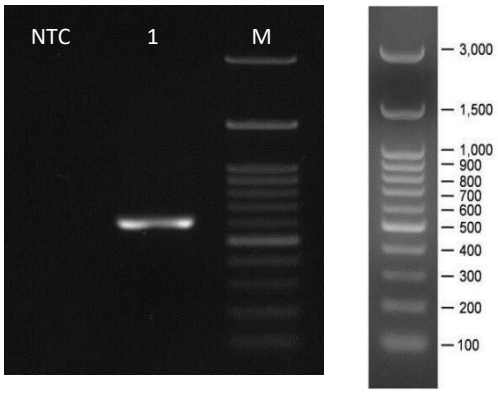

Figure 3. Electrophoresis Results from Amplification of CW30 Isolate with a Concentration of 0.8% Agarose Gel. Description: M (Marker 1Kb, Loaded 2.5 µl); 1 (CW30 Isolate)

The purified DNA was followed by DNA sequencing readings using the Bi-directional sequencing method. The results of CW30 isolate sequencing were shown in Figure 3**.** Results obtained from CW30 isolates with NL1/NL4 primers have a length query of 585 bp. The data was then analyzed using BLAST (Basic Local Alignment Search Tool) to determine the percentage of kinship, type, and strain of yeast isolate.

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
[Candida] ethanolica isolate 3-1-19 large subunit ribosomal RNA gene, partial sequence	1079	1079	99%	0.0	100.00%	MW969731.1
[Candida] ethanolica strain ZJ-21 26S ribosomal RNA gene, partial sequence	1072	1072	99%	0.0	99.83%	KY283163.1
Candida ethanolica partial 26S rRNA gene, isolate H5S11K28	1070	1070	99%	0.0	99.83%	FM180545.1
Pichia deserticola culture CBS:7122 large subunit ribosomal RNA gene, partial sequence	1064	1064	100%	0.0	99.49%	KY108790.1
Pichia deserticola culture CBS:7121 large subunit ribosomal RNA gene, partial sequence	1064	1064	100%	0.0	99.49%	KY108788.1
Pichia deserticola culture CBS:7119 large subunit ribosomal RNA gene, partial sequence	1050	1050	100%	0.0	98.98%	KY108789.1
Pichia deserticola NRRL Y-12918 28S rRNA, partial sequence; from TYPE material	1037	1037	97%	0.0	99.47%	NG_055106.1
[Candida] ethanolica NRRL Y-12615 28S rRNA, partial sequence; from TYPE material	1037	1037	97%	0.0	99.47%	NG_055105.1
Pichia deserticola isolate YP14 large subunit ribosomal RNA gene, partial sequence	1035	1035	97%	0.0	99.47%	OL636346.1
Candida ethanolica strain DM4106 26S ribosomal RNA gene, partial sequence	1033	1033	95%	0.0	100.00%	KM005170.1

Figure 4. Top 10 Sequence Homology Similarity Result (BLAST)

The results of sequence analysis using BLAST showed that CW30 has a base arrangement similar to *Candida ethanolica isolate 3-1-19* with an accession number of MW969731.1 and a 100% similarity Figure 4. The results of several previous studies say that *C. ethanolica* has a role in wine fermentation which can provide a high aroma concentration (Xu *et al*., 2022). Based on the kinships, a phylogenic tree was created using the Neighbor-joining tree CW30 method which is shown in Figure 5.

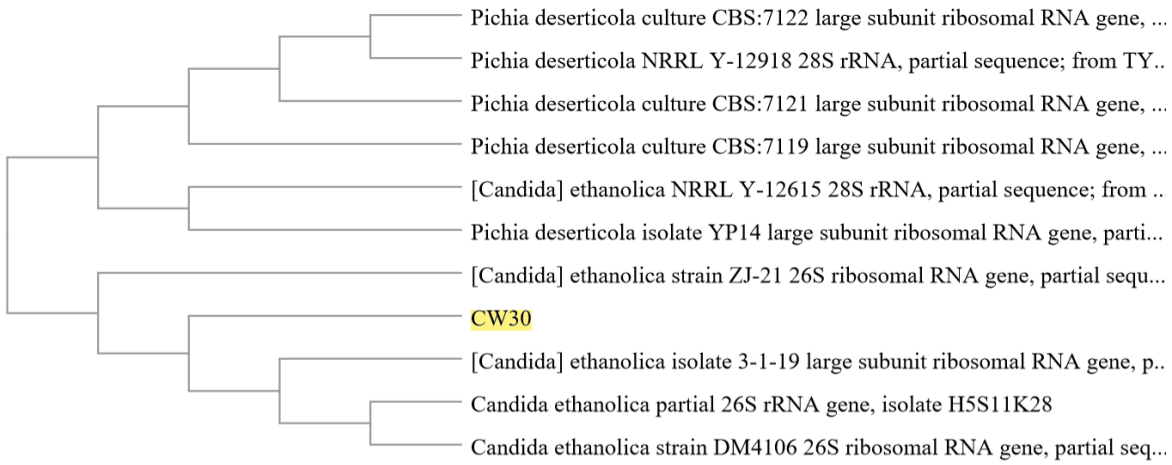

Figure 5. Phylogenic Tree of Isolate CW30

The results of the phylogenetic tree show two species that are synonymous based on the very similar D1/D2 sequence, *Pichia deserticola* and *C. ethanolica*. This is related to previous research by Daniel *et al*., (2009) on cocoa bean fermentation, it was found the *P. deserticola* and *C. ethanolica* species were almost the same but had differences in their nucleotide sequences.

CONCLUSION

The yeast isolates from fermented wine coffee cherries for 30 days namely CW30. Based on morphological characteristics, CW30 isolate is thought to have similarities between the yeasts *Saccharomyces cerevisiae, Candida parapsilosis*, and *Torulaspora delbruecki.* CW30 has a tolerance to the addition of up to 5% ethanol but is sensitive to 10% ethanol. The isolate was sensitive to temperatures of more than 35ºC indicated by a slowly growth. Based on molecular identification, CW30 has a 100% similarity with *Candida ethanolica* isolate 3-1-19 with accession number MW969731.1.

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