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OPTIMIZING AMOUNT AND IDENTIFICATION OF YEAST IN SALAK YEAST WATER (*Salacca edulis* Reinw cv Pondoh)

Optimasi Jumlah dan Identifikasi Yeast pada Salak Yeast Water (*Salacca edulis* Reinw cv Pondoh)

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ABSTRACT

Salak fruit is a tropical fruit spread across Southeast Asia and can potentially be a source of natural yeast by processing fruit yeast water. Salak fruit is known to be rich in carbohydrates, making it a potential natural habitat for microbial communities, especially yeast. This research aims to optimize the growth of yeast in the salak yeast water system by varying the sugar concentration treatment, the composition of the amount of fruit flesh, and the fermentation time, as well as identifying the yeast in the salak yeast water. The method used is to count the number of yeast colonies using the Total Plate Count method. The most optimal treatment results are then tested for pH, total sugar, alcohol, isolation and yeast identification. The research results showed that the composition of salak meat was 30%, adding 1% sugar, and fermentation for 6 days resulted in the most optimal growth of the microbial community with an amount of 3.1×10^6 cfu/ml. The pH test showed a result of 3.01, the alcohol content, namely ethanol, was 0.066855%, no methanol was detected, the total sugar test result in the yeast water was 2.08%, and it was identified in the yeast water that there were Hanseniaspora opuntiae and Candida sorboxylosa. The results show that salak yeast water (Salacca edulis Reinw cv Pondoh) can be used as a yeast water product to ferment food.

***Keywords**: fruit yeast water, identification, optimization, salak, yeast*

ABSTRAK

Buah salak merupakan buah tropis yang tersebar di AsiaTenggara dan memiliki potensi sebagai sumber ragi alami melalui pengolahan *fruit yeast water*. Buah salak ini dikenal kaya akan karbohidrat, menjadikannya habitat alami yang potensial bagi komunitas mikroba, terutama yeast. Penelitian ini bertujuan untuk mengoptimalkan pertumbuhan yeast pada sistem salak yeast water dengan memvariasikan perlakuan konsentrasi gula, komposisi jumlah daging buah, dan waktu fermentasi serta mengidentifikasi yeast yang ada dalam salak yeast water. Metode yang dilakukan yaitu meghitung jumlah koloni yeast dengan metode *Total Plate Count*, hasil perlakuan paling optimal selanjutnya diuji untuk pH, total gula, alkohol, isolasi, dan identifikasi yeast. Hasil penelitian menunjukkan bahwa komposisi daging salak sebanyak 30%, penambahan gula sebanyak 1%, dan fermentasi selama 6 hari menghasilkan pertumbuhan komunitas mikroba paling optimal dengan jumlah $3{,}1\times10^6$ cfu/ml. Uji pH menunjukkan hasil 3,01, kadar alkohol yaitu etanol sebesar 0,066855%, tidak terdeteksi adanya methanol, hasil uji total gula pada yeast water yaitu sebesar 2,08%, dan teridentifikasi dalam salak yeast water terdapat *Hanseniaspora opuntiae* serta *Candida sorboxylosa*. Dari hasil yang didapat menunjukkan *salak yeast water* (*Salacca edulis* Reinw cv Pondoh) memiliki potensi untuk digunakan sebagai produk *yeast water* yang dapat digunakan untuk memfermentasi bahan pangan.

Kata kunci: fruit yeast water, identifikasi, optimasi, salak, yeast

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INTRODUCTION

Salak is a native Indonesian product that can be planted in the lowlands (Harahap, Bayu, & Siiregar, 2013). Salak (Salacca edulis Reinw cv Pondoh) is a tropical fruit in Indonesia that belongs to the Arecaceae family. Salak fruit is popular because of its sweet and crunchy taste (Zuliatin and Mazidatul, 2021). Salak grows as a palm that can grow to a height of 6 m and is expected to be productive for an average of 50 years. Salak usually grows in the lowlands with high humidity (Saleh et al, 2018).

Salak fruit can be used as an alternative yeast water product, one of which is as a bread improver, namely through a fruit yeast water processing system to obtain natural yeast (wild yeast), which can be used for food processing. Yeast water is a mixture of water and fruit left to ferment to capture wild yeast in the environment (Jamie, 2021). Natural yeast (wild yeast) is found in all types of food, such as fruit, vegetables, cereals, herbs and flowers. These ingredients are added with water and then fermented for several days in a sterile glass at room temperature (Sanggramasari, 2018). Natural yeast has the potential to be a bread-raising agent. Bread that uses wild yeast is 100% healthy bread because it only uses beneficial microorganisms derived from natural ingredients and does not require additional or chemical ingredients such as bread improvers. Foods made using wild yeast are resistant to mold growth because they contain chemical compounds from natural fermentation (Ko, 2012).

Making Salak Yeast Water is quite simple, but it is necessary to optimize the fermentation process, namely the amount of fruit flesh, increasing the sugar concentration, and the length of fermentation time to get the maximum amount of yeast. Fruits are an abundant food source for yeast based on the availability of simple sugars. Yeast, especially Saccharomyces cerevisiae, is usually the dominant group (Dashko et al, 2014). The relatively abundant source of carbohydrates in Salak Pondoh is an indicator of the natural habitat of yeast (Saleh et al, 2018). The fermentation process of natural yeast uses fresh fruit, sugar, or honey (Ko, 2012). The addition of sugar can increase cell density and yeast growth.

Fruits have diverse yeast communities. In 2020, Sari succeeded in characterizing several yeasts from Pondoh salak fruit. Salak yeast water, which is fermented from salak pondoh fruit, will have a diverse yeast community like the fruit. Identification was carried out by looking at the morphology and molecules of yeast isolated from Salak Yeast Water (Prihartini and Ilmi, 2018).

Research (Kusuma, Ulfah, & Nur, 2022) shows that salak fruit has the potential wild yeast as a bread improver. Still, research has yet to reveal growth optimization and identification of the microbial community from salak fruit, especially yeast, through a fruit yeast water production system. This research aims to determine the optimal number of yeast community colonies through the fruit yeast water system by varying the sugar concentration treatment, the composition of the amount of fruit flesh, and fermentation time to reveal the potential use of snake fruit, especially organic salak pondoh fruit as a source of natural yeast through fruit yeast water processing. In this research, the most optimal characteristics of salak yeast water, including pH, total sugar, ethanol content, and methanol, were also tested to determine the potential application of the product. Yeast, which was successfully isolated from fruit yeast water, was also identified.

MATERIALS AND METHODS

Making Salak Yeast Water

Samples of salak fruit for making yeast water were taken from Ledoknongko Sleman at map coordinates 7°38'33.5"S110°21'36.0" E. The samples taken were ripe Pondoh salak fruit aged six months from one garden and obtained from several salak trees. Making snake fruit yeast water is done by washing and peeling the fruit. Next, the salak fruit was peeled, cut, and weighed according to the amount added to the distilled water and sugar solution, then closed tightly. The mixture of salak and sugar solution was then incubated for seven days at room temperature in the dark.

Optimization of Microbial Community from Salak Fruit

In this study, each treatment was made in three repetitions with treatments varying the composition of the amount of fruit pulp 20% 0.5% sugar, 20% fruit flesh 1% sugar, 20% fruit flesh 1.5% sugar, 25% fruit flesh 0 sugar, 5%, pulp 25% sugar 1%, pulp 25% sugar 1.5%, pulp 30% sugar 0.5%, pulp 30% sugar 1%, pulp 30% sugar 1.5%, flesh fruit 35% 0.5% sugar, fruit flesh 35% 1% sugar, fruit flesh 35% 1.5% sugar, fruit flesh 40% 0.5% sugar, fruit flesh 40% 1% sugar, and fruit flesh 40% sugar 1.5%. Each treatment variation was incubated for seven days, and sampling was carried out every day from days 3, 4, 5, 6, and 7 to determine the optimal growth time for the microbial community (Jamie 2021).

Calculation of the Number of Microbial Colonies

The number of microbial colonies was calculated using the Total Plate Count (TPC) technique using a pour plate on Bean Sprout Extract Agar (TEA) media. According to research, microbial colony counts were carried out by observation by taking samples every day from day 3 to day 7 of fermentation (Yumas and Rosniati, 2019). Sampling was conducted in salak yeast water in a dilution series of 10^{-3} and 10^{-4}, then continued with a pour plate and TPC. TPC testing was conducted by referring to research (Safrida, Raihanaton, and Ananda, 2019). The calculation of the number of cells refers to research (Pradikaningrum, 2015).

Data Analysis

Data analysis was collected using the IBM SPSS 25 program to analyze statistical data (Kurniyanti and Asri, 2022). Data analysis was carried out using the Kruskal-Wallis test referring to Quraisy et al, 2021.

Test pH

The pH test is carried out by shaking the yeast water sample and then reading the pH with a pH meter. Press the "Power" button to turn on the pH meter. Calibrate the pH meter using pH 4.01, pH 7.01, and pH 10.01 buffer solutions. Read the pH of the sample.

Total Sugar Test

LPPT (Lembaga Penelitian dan Pengujian Terpadu) Gadjah Mada University carried out the total sugar test using the Luff school method on salak yeast water in the treatment that showed the most optimal results to determine the total amount of sugar after fermentation. The total sugar test is carried out by calculating total sugar (%) using the formula for the difference in sugar content after and before inversion.

Alcohol Content Test (Ethanol and Methanol)

The alcohol content test, namely ethanol and methanol, was carried out by LPPT Gadjah Mada University using the gas chromatography method on salak yeast water in the treatment, which showed the most optimal results to determine the total amount of sugar after fermentation.

Yeast Isolation

The yeast was isolated using the pour plate method on BSEAC Media (Bean Sprouts Extract Chloramphenicol Agar) in duplo with a dilution of 10 0 to 105. To obtain a single colony with a four-quadrant streak, it was then transferred to an agar slant for storage.

Colony & Cell Morphology Observations

Colony morphology was observed based on texture, color, height and margins. Meanwhile, cell observations are carried out based on cell shape, reproductive type, and pseudohyphae or true hyphae.

DNA Isolation & Visualization of Isolation Results

Yeast DNA was isolated using the Wizard® Genomic DNA Purification Kit for yeast, and isolation results were visualized using gel electrophoresis.

PCR (Polymerase Chain Reaction) & Sequencing

The primers used were ITS1 (5'-TCCGTAGGTGAACCTGCGG-3'), ITS4 (5'-TCCTCCGCTTATTGATATGC-3), NL1 (5'-GCATATCAATAAGCGGAGGA AAAG-3'), and NL4 (5'- GGTCCGTGTTTCAAGACGG-3'). The PCR process uses the following

protocol: initial denaturation at 95°C for 2 minutes, denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, elongation at 72°C for 60 seconds and final elongation at 72°C for 5 minutes as many as 35 cycles (Sumerta and Kanti, 2017). The sequencing stages were carried out at the Genetics Laboratory Division, PT. Genetica Science Indonesia, Jakarta.

Phylogenetic Tree Construction

The raw data from the sequencing results were first analyzed using GeneStudio 2.2 to obtain consensus sequencing results using contig analysis (Febrianti et al., 2023). sequence as comparison data taken from NCBI https://www.ncbi.nlm.nih.gov/ in the BLAST. Phylogenetic tree construction was carried out using Mega XI with the "Neighbor Joining" option and NCBI with the "Distance tree of results" option.

RESULTS AND DISCUSSION

Salak Yeast Water

Salak yeast water can be made by adding sugar water to pieces of snake fruit flesh in a dark room at room temperature for 7 days. Manufacturing process Ko (2012) states that the way to make natural yeast is by adding water to pieces of fruit in a closed container and fermenting it at room temperature for several days (3-14 days). Salak yeast water is a liquid obtained from the fermentation of fruit extract, which is a liquid material containing yeast with the characteristics of the liquid having a tapai-like aroma, slightly sour taste, cloudy color and slightly thick (Riana, Cucu, and Ridawati, 2021).

Figure 1. Salak Yeast Water

In salak yeast water, microorganism activity is formed, characterized by the production of carbon dioxide gas, indicated by the presence of bubbles and sediment during the fermentation process from the remaining biomass of microorganisms (Figure 1). Changes in yeast water fermentation are caused by the activity of the microbial community, especially wild yeast. Wild yeast on the surface of the fruit will be captured into the water during the process of making yeast water (Jamie, 2021). Wild yeast uses sugar as a carbon source for its growth during fermentation and produces carbon full of active yeast. Yeast carries out fermentation, which will convert sugar into alcohol and carbon dioxide gas with the help of its enzymes. During fermentation, biochemical reactions occur involving various metabolic pathways in yeast cells, namely glycolysis and alcoholic fermentation. In glycolysis, sugar is broken down into pyruvate, producing small amounts of energy (ATP and NADH). Sugar is converted into energy and other compounds necessary for yeast cellular growth. The yeast will continue alcoholic fermentation, where the pyruvate is converted into ethanol and carbon dioxide. This process does not produce additional ATP but recycles the NAD+ consumed in glycolysis (Risky, Wijanarka, and Sri, 2019).

Optimization of Microbial Community from Salak Fruit

Optimization of the microbial community from salak fruit through the yeast water making system of salak was studied by observing the number of colonies in each treatment, variations in fruit flesh composition, sugar concentration, and fermentation time. Optimization results show that variations in fruit flesh composition, sugar concentration, and fermentation time influence the number of microbial colonies that grow.

Based on the research results, it was found that the most optimal number of microbial colonies was treated with variations in fruit flesh composition of 30%, sugar concentration of 1%, and fermentation time of 6 days, namely 3.1×10^{6} cfu/ml (Figure 2). The composition of fruit flesh can influence the number of microbial colonies obtained because the sugar content is a source of energy for microbes to grow. Salak fruit

contains 10 g/100 grams of sucrose, 2.4 g/100 grams of glucose, and 2.3 g/100 grams of fructose (Mazumdar, Pratama, Lau, Teo, & Harikrishna, 2019).

The composition of fruit flesh is an important factor in obtaining optimal microbial communities, especially wild yeast, through the fruit yeast water production system. Fruit flesh is a natural habitat for microbial communities, especially yeast. An optimal fruit pulp composition is needed to obtain wild yeast so that the microbial community, especially yeast from fermented fruit, can grow optimally (Saleh, et al., 2018). In addition, fruits contain sugar to support yeast growth. All fruit components, especially sugar, will be metabolized by yeast during its growth period (Budiarso and Amarantini, 2017).

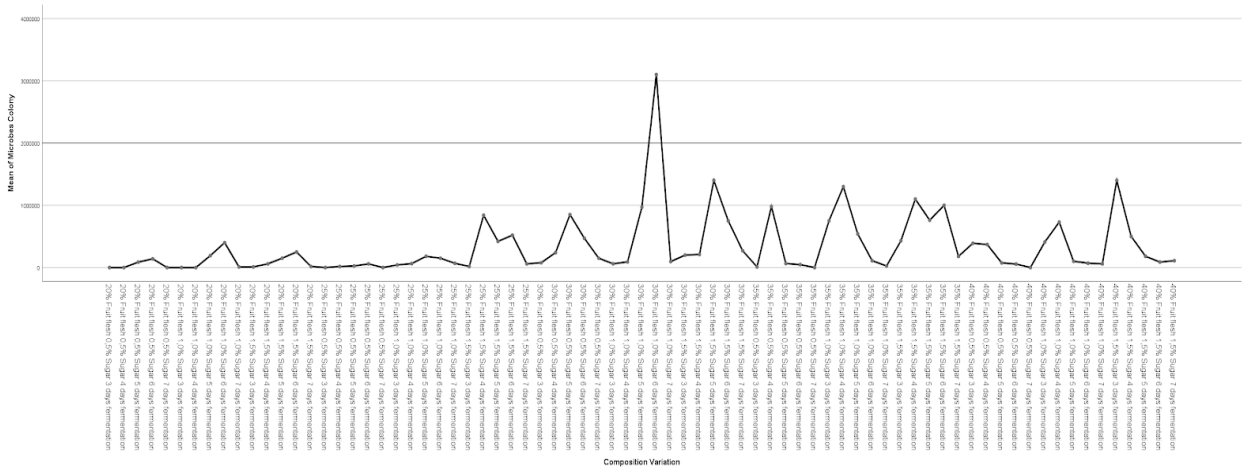


Figure 2. Mean of Yeast Colony Amount

Table 1. Kruskal-Wallis Test

Ranks			
Variations in Fruit Flesh Composition and Sugar Concentration Days 3-7		N	Mean Rank
Number of Colony	Fruit flesh 20% Sugar 0,5%	5	17.20
	Fruit flesh 20% Sugar 1,0%	5	24.30
	Fruit flesh 20% Sugar 1,5%	5	27.80
	Fruit flesh 25% Sugar 0,5%	5	12.60
	Fruit flesh 25% Sugar 1,0%	5	31.40
	Fruit flesh 25% Sugar 1,5%	5	43.70
	Fruit flesh 30% Sugar 0,5%	5	49.20
	Fruit flesh 30% Sugar 1,0%	5	47.40
	Fruit flesh 30% Sugar 1,5%	5	56.60
	Fruit flesh 35% Sugar 0,5%	5	26.00
	Fruit flesh 35% Sugar 1,0%	5	50.10
	Fruit flesh 35% Sugar 1,5%	5	61.40
	Fruit flesh 40% Sugar 0,5%	5	32.00
	Fruit flesh 40% Sugar 1,0%	5	40.90
	Fruit flesh 40% Sugar 1,5%	5	49.40
	Total	75	

Table 2. Statistic Test

Test Statistics	
	Number of Colony
Kruskal-Wallis H	31.832
df	14
Asymp. Sig.	0.004
a. Kruskal Wallis Test	
b. Grouping Variable: Variations in Fruit Flesh Composition and Sugar Concentration Days 3-7	

Based on the results of the statistic test above, the Asymp value is known. Sig is 0.004 < 0.05. Thus, it can be concluded that H0 is rejected and Ha is accepted, which means there is a significant difference between the treatment variations (Table 1&2).

Test the manufacture of salak yeast water on various composition variations, namely 20%, 30% and 40% fruit flesh; sugar of 0.5%, 1%, and 1.5% and a fermentation time of 7 days show that variations in fruit pulp composition of 30%, 1% sugar, and a fermentation time of 6 days produce the most optimal salak yeast water. The most optimal results were followed by product chemical characteristic tests, including pH tests, total sugar tests, and alcohol tests (ethanol and methanol) to learn more about the potential application of salak yeast water as a bread improver.

pH Test

Table 3. pH Test

Sample	pH			Average
	1	2	3	
Salak Yeast Water Fruit flesh 30%, Sugar 1%, 6 days fermentation	3.01	3.01	3.01	3.01

The acidity of yeast water in the fruit pulp treatment variation of 30%, sugar 1%, and fermentation time of 6 days obtained a pH of 3.01 (Table 3). This shows that yeast water has the potential to be a starter for bread improvement. pH testing on yeast water is carried out to ensure the product's safety for use as a bread improver. The low pH of natural yeast starter (<4) can inhibit the growth of pathogenic microorganisms, such as *Salmonella* and *Escherichia coli* bacteria. These pathogenic bacteria can cause diarrhoea, vomiting and food poisoning. Therefore, the natural yeast starter for making bread must have a low pH (Hannah, 2019). pH testing on yeast water can also be done to determine the maturity of the product. Yeast water with a low pH will indicate that the fermentation process has been completed. The fermentation process that has been completed is marked by a decrease in pH to acid (Hannah, 2019).

Total Sugar Test

Table 4. Total Sugar Test

Sample	Before Inversion (%)	After Inversion (%)	Total Sugar (%)
Salak Yeast Water Fruit flesh 30%, Sugar 1%, 6 days fermentation	0.57	2.65	2.08

The total sugar test in yeast water as a bread improver was carried out to determine the sugar content in yeast water. It is important to know the total sugar that needs to be added when making bread. The total sugar test results show that the total sugar is 2.08% (Table 4). This can be obtained from the 1% sugar added at the beginning of

making salak yeast water and the added salak fruit. Microorganisms will later utilize this sugar in fermentation to grow microorganisms and convert them into metabolite products such as alcohol and carbon dioxide. According to Mazumdar et al. (2019), snake fruit contains a total sugar of 11,850 – 17,220 mg/100 g of snake fruit or around 11.8 – 17.2%. Salak fruit contains 10,000 mg sucrose/100 mL fruit juice, 2,400 mg glucose/100 mL fruit juice, and 2,300 mg fructose/100 mL fruit juice.

Alcohol Level (Ethanol & Methanol)

Table 5. Methanol Test

Sample	Volume Sample (ml)	Final Volume (ml)	Concentration on reading the reading results of the tool (%)	Methanol Concentration Calculation Results (%)	Detection Limits (LoD) (mg/L)	Average Methanol Concentration (mg/L)
Salak Yeast Water Fruit flesh 30%, Sugar 1%, 6 days fermentation (a)	1	1	nd	nd	4.47	< 4.47
Salak Yeast Water Fruit flesh 30%, Sugar 1%, 6 days fermentation (b)	1	1	nd	nd	4.47	

The methanol test on yeast water is carried out to determine the methanol content in yeast water (Table 5). Methanol is a toxic substance to the body, so it is important to ensure that the yeast water used for food applications is safe for consumption. Based on the results of the methanol analysis in the salak yeast water sample, no methanol was found, indicating that yeast water can be used for food applications. This could be because the fermentation process in fruit will produce alcohol, especially ethanol, and it is hoped that there will be no methanol production. This is to research (Gunam, Ardani, & Antara, 2018) in the production of fermented alcoholic fruit drinks, namely, the methanol content in salak wine is very limited in quantity and is even expected to be non-existent because this substance is toxic to the body, especially to the nerves.

Table 6. Ethanol Test

Sample	Volume Sample (ml)	Final Volume (ml)	Concentration on reading the reading results of the tool (%)	Ethanol Concentration Calculation Results (%)	Average Ethanol Concentration (%)	Average Ethanol Concentration (mg/L)
Salak Yeast Water Fruit flesh 30%, Sugar 1%, 6 days fermentation (a)	1	1	0.06496	0.06496	0.066855	0.0668.55
Salak Yeast Water Fruit flesh 30%, Sugar 1%, 6 days fermentation (b)	1	1	0.06888	0.06888		
Salak Yeast Water Fruit flesh 30%, Sugar 1%, 6 days fermentation (c)	1	1	0.06672	0.06672		

The ethanol test is carried out to ensure the safety of using yeast water products. Based on chemical tests (Table 6), the ethyl alcohol content produced from salak yeast water shows that the ethanol content is 0.066855%, which is included in the quality standard for low alcohol wine by SNI (1999), namely for low alcohol wine, the maximum ethyl alcohol is 1.15%. The presence of alcohol is caused by the fermentation process, which breaks down sugar into alcohol and carbon dioxide, so it is one of the factors in the production of alcohol content (Breemer, Moniharapon, & Nimreskosu, 2016).

Yeast Isolation and Cell Morphology

Based on the research, 4 yeast isolates were obtained from snake yeast water from Salak pondoh (Salacca edulis Reinw). Select yeast isolates based on the most dominant morphological criteria among other colonies. Each isolate was coded Yis 1, Yis 2, Yis 3 and Yis 4. Characterization of yeast isolates refers to the book "The Yeast: A Taxonomy Study" based on colony and species morphology using macroscopic and microscopic observations. The results of macroscopic colony characterization can be seen in Table 7, and microscopic cell morphology can be seen in Table 8.

Table 7. Colony Morphology

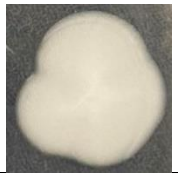
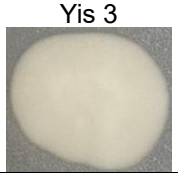
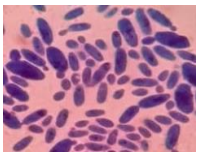
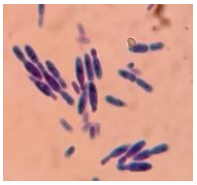
Isolate Code	Colony Morphology					
	Shape	**Color**	**Surface**	**Elevation**	**Margin**	**Texture**
Yis 1 	Circular	*white*	*Glistening*	*like dome*	*streak*	*Mucoid*
Yis 2 	Irreguler	*white*	*smooth*	*Flat*	*Undulating*	*viscous*
Yis 3 	Circular	*white*	*Glistening*	*like dome*	*streak*	*Mucoid*
Yis 4 	Circular	*white*	*Glistening*	*like dome*	*streak*	*Mucoid*

Table 8. Cell Morphology

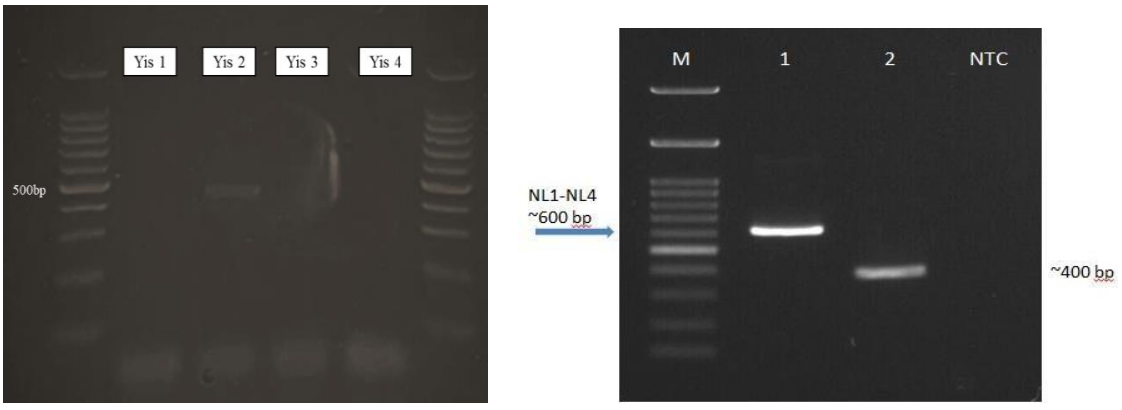
Isolate Code	Cell Morphology		
	Shape	**Reproduction Type**	**Psuodohyphae/True hyphae**
Yis 1 	*Ovoid, Apiculate, Elongate*	Monopolar & Bipolar	Psuodohyphae & True hyphae

Isolate Code	Cell Morphology		
	Shape	Reproduction Type	Psuodohyphae/True hyphae
Yis 2 	*Ovoid & Elongate*	Monopolar,Bipolar, Multipolar	Psuodohyphae
Yis 3 	*Ovoid, Apiculate, Elongate*	Monopolar & Bipolar	Psuodohyphae & True hyphae
Yis 4 	*Apiculate, Ovoid, Elongate*	Monopolar & Bipolar	Psuodohyphae & True hyphae

In general, Yis 1, 3 and 4, when viewed from the morphology of the colony and cells, are the same species, while Yis 2 is a different species. Isolates Yis 1, 3, and 4 are similar to the genus *Hanseniaspora*, while isolate Yis 2 has characteristics similar to the genus *Candida*. A suitable environment for yeast to grow is one of the main factors found in both species. Fruits are a suitable environment for yeast to live in because they have abundant carbon sources, so both yeast genera are suitable for growing in this environment. This aligns with Linawati's statement (2021) that the yeasts often found in fresh fruit and juice are *Candida, Dekkera, Hanseniaspora, Pichia, Saccharomyces*, and *Zygosaccharomyces* species.

PCR Visualization and Phylogenetic Trees

PCR was carried out using two pairs of primers. The first primer, primers ITS1 and ITS4, was used to see the relationship between isolates. The second primer, namely NL1 and NL4, was used to identify species by sequencing and then analyzed by building a phylogenetic tree.



(a) (b)

Figure 3. (a) Amplification result using primer ITS1 & ITS4 (b) Amplification result using primer NL1 & NL4

In Figure 3(a), the PCR results using primers ITS1 and ITS4 show that isolates Yis 1,3,4 were amplified in the same area, while Yis2 had different amplification results. This result is by the results of cell and colony morphology observations, which show that Yis 1,3,4 isolates have the same colony and cell morphology characteristics. Then, Figure 3(b) shows the amplification results using primers NL1 and NL4. Yis 1 and Yis 2 have different amplification results. Next, the sequencing results from PCR amplification using primers NL1 and NL4 were analyzed to build a phylogenetic tree.

Sequence Yis 1
1 AAGCGGAGGA AAAGAAACCA ACTGGGATTA CCTTAGTAAC GGCGAGTGAA GCGG-TAAAAG
61 CTCAAATTTG AAATCTGGTA CTTTCAGTGC CCGAGTTGTA ATTTGTAGAA TTT-GTCTTTG
121 ATTAGGTCCT TGTCTATGTT CCTTGGAACA GGACGTCATA GAGGGTGAGA ATCCCGTTTG
181 GCGAGGATAC CTTTTCTCTG TAAGACTTTT TCGAAGAGTC GAGTTGTTTG GGAATGCAGC
241 TCAAAGTGGG TGGTAAATTC CATCTAAAGC TAAATATTGG CGAGAGACCG ATAGCGAACA
301 AGTACAGTGA TGGAAAGATG AAAAGAACTT TGAAAAGAGA GTGAAAAAGT AC-GTGAAATT
361 GTTGAAAGGG AAGGGCATTT GATCAGACAT GGTGTTTTTT GCATGCACTC GCCTCTCGTG
421 GGCTTGGGCC TCTCAAAAAT TTCACTGGGC CAACATCAAT TCTGGCAGTA GGA-TAAATCA
481 TTAAGAATGT AGCTACCTCG GTAGTGTTAT AGCTTATTGG AATACTGCTA GCTGG-GATTG
541 AGGACTGCGC TTCGGCAAGG ATGTTGGCAT AATGGTTAAA TGCCGCCCGT CTT-GAACC

Sequence Yis 2
1 TGCATATCAA TAAGCGGAGG AAAAGAAACC AACCGGGATT GCCCCAGTAG CGGCGAGTGA
61 AGCGGCAGGA GCTCTAGTTT GAAATCTTCG GAGATGTAGA GACGGGGAGT GGG-CATTGGA
121 GTCCCCTGGA ACGGGGCGCG ACGCGAGGTG ACAGCCCTCG GAGACCAGCC CCCTGTCTGG
181 TGAGTCGAGT TGTTTGGGAA TGCAGCTCTA GTGGTGGTAT GCTCCATCTG CGGCTAAATA
241 TTGGCGAGAG ACCGATAGCA GACAAGTACT GTGAAGGAAA GATGAAAAGC ACTTTGAAAA
301 GAGAGTGAAA GAGGGCGTGA AATTGTTGAA AGGGAAGGGT GGGTTTCTGT GGA-GATCCAC
361 CGCCCGTCTT GAAACACGGA CCA

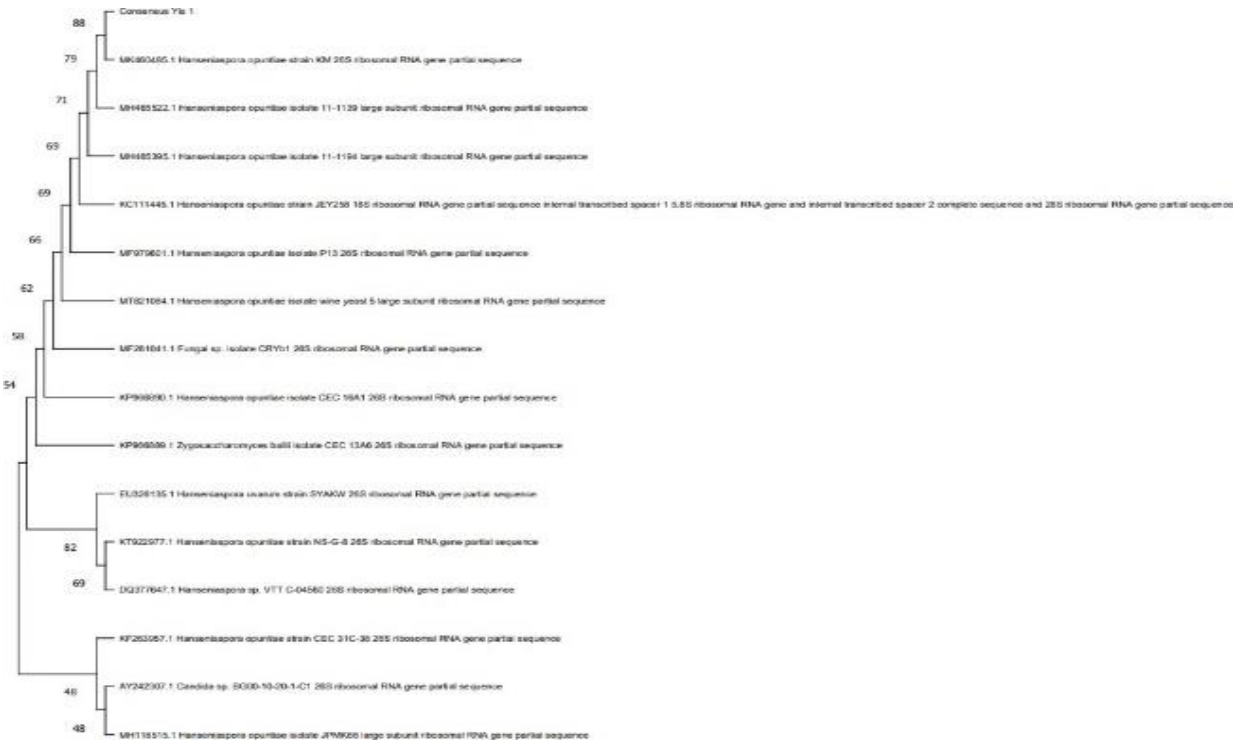


Figure 4. Phylogenetic Tree Construction Yis 1

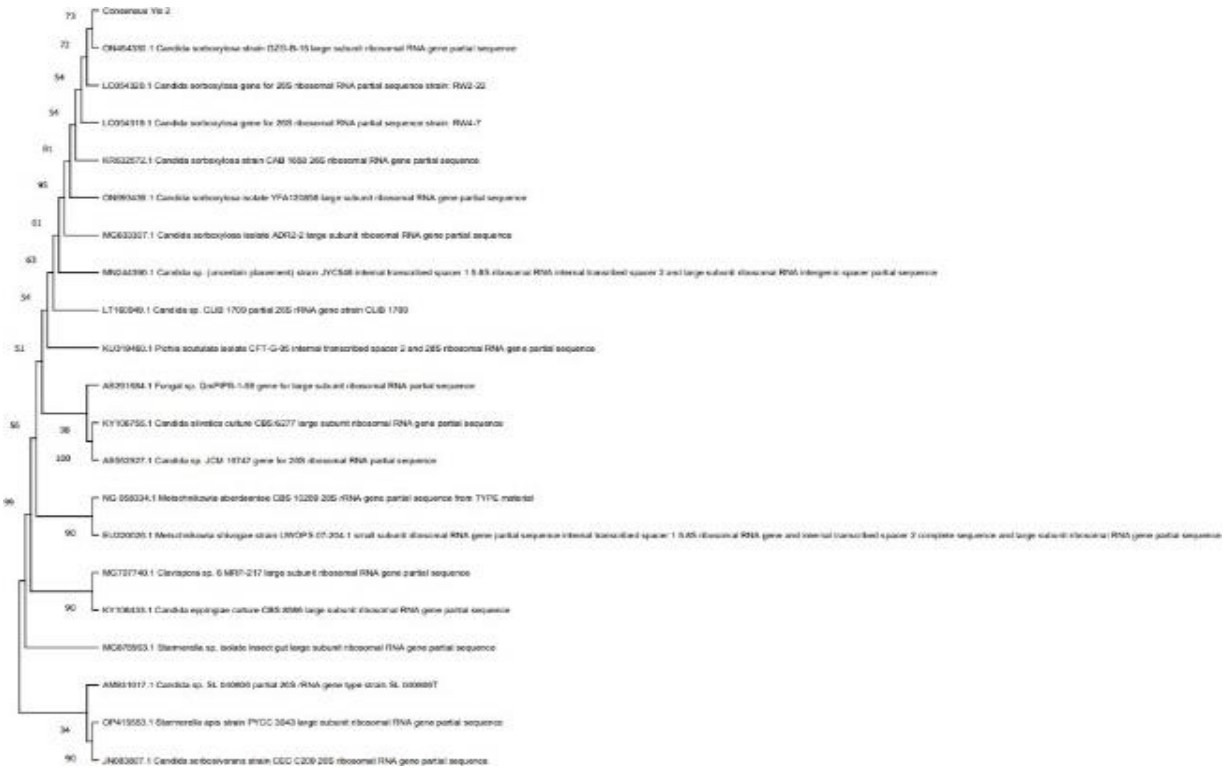


Figure 5. Phylogenetic Tree Construction Yis 2

Figure 4 and 5 shows that Yis 1 and Yis 2 are different species. The colony and cell morphology identification results, which continued to the molecular level, showed that the Yis 1 isolates as a representative of Yis 3 and 4 were a Hanseniaspora opuntia. Based on its morphological characteristics, Yis 1 is similar to Yis 3 and 4 then, if it is

drawn down to the molecular level, it also has the same base bitterness. When viewed based on its morphology, the Yis 2 isolate shows similarities to Candida species and the results of the phylogenetic tree construction show that Yis 2 is a Candida sorboxylosa.

CONCLUSION

The most optimal treatment for growing the number of microbial colonies from snake fruit is making fruit yeast water, varying the 30% fruit flesh, 1% sugar, and 6 days fermentation. Based on the chemical characteristics of the most optimal yeast water, it shows a pH of 3.01, the alcohol content, namely ethanol, is 0.067%, no methanol is detected, and the total sugar test in the yeast water is 2.08%. Yeast isolated from the salak yeast water processing system obtained 4 isolates with the codes Yis 1, Yis 2, Yis 3 and Yis 4. Isolates Yis 1, 3 and 4 were declared *Hanseniaspora opuntiae* species while Yis 2 was declared *Candida sorboxylosa* species.

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