



Isolation and Molecular Identification of Endophytic Mold *Schizophillum commune* in Red Dahlia (*Dahlia* sp. L) Tuber as Producing Inulinases

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Abstract

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BACKGROUND: Dahlia tuber as the source of isolates in endophytic mold produces Inulinases. Inulinase is used in industry to produce inulin to become fructose through an enzymatic reaction. Fructose from natural substances constitutes low calories used as diet in diabetes mellitus type 2 patients. An important phase is done obtain the inulinase enzyme through mold isolation.

AIM: The objective of this research was to isolate and identify the molecular of endophytic mold from red dahlia tuber which had the optimum inulinase activity.

METHODS: Mold isolation was done through the stage of surface sterilization and purification. Mold isolation which had the highest inulinase activity was identified in a molecular using the polymerase chain reaction technique with the Internal transcribed spacers ITS region. DNA amplification used primer ITS 1 and ITS 4.

RESULTS: The result of the study showed that there was five-mold isolation with the result of screening high inulinase activity found in UD3 (++) , UD4 (++) , and UD5 (+++), the value of inulinase enzyme activity of UD3 was 0.582716049, UD4 was 0.330864198, and UD5 was 0.685185185. The result of identifying UD5 molecular in eight DNA tapes was successfully amplified with 660 pb. The result of identifying molecular based on BLAST analysis found *Schizophillum commune* species

CONCLUSION: It was found that the highest inulinase activity was the species *S. commune*, which added new variants of inulinase enzyme from red dahlia tuber which could be used by industry to yield fructose through an enzymatic reaction.

Introduction

Dahlia is a flower that has bulbs from the Asteraceae family. Dahlia is a raw material that is high in inulin, which is about 15–20%. Inulin is a natural dietary fiber and low glycemic index that have a fructosyl compound at the terminal. Inulin is a substrate and inducer for the production of inulinase. From this enzymatic process, the biotechnological potential of high inulin feedstock is explored for the production of various important industrial products. Raw materials high in inulin can be used for the production of fructooligosaccharides (FOS) and fructose. Fructose is a low-calorie sugar that can be used by people with diabetes mellitus [1].

The development of modern biotechnology currently has the opportunity for the enzyme industry to treat all types of agricultural waste that will be used as high-value products, for example, the availability of cheap and abundant inulin from dahlia tubers as agricultural waste can be processed through hydrolysis of inulin by

targets on the β -2,1 linkage of inulin into fructose with D-fructose content is more than 95% [2]. Hydrolysis of inulin by one step using inulinase gives a yield of approximately 90–95% fructose better than conventional fructose production from starch requires at least three enzymatic steps involving amylase, glucoamylase, and glucose isomerase and yields only 45% fructose [3].

Inulin besides being able to be processed as an ingredient in high-fructose and FOS syrups; it can also be used for various products such as sorbitol, citric acid, single-cell oil, single cell protein, bioethanol, lactic acid, glutamic acid, and various other products [4].

Fructose and FOS are the main products of inulin. Inulin is a natural fiber that is low in calories, safe to use for diabetics, besides that it can improve the growth of intestinal flora, increase iron absorption in children, overcome digestive problems such as irritable bowel syndrome, obesity, lower cholesterol, and overcome other health problems [5].

Inulin content in addition to being found in dahlia tubers is also found in chicory, Jerusalem artichoke,

potato tuber Yaco'n, Asparagus, onion, garlic, wheat, and banana which can be processed pharmacologically as a prebiotic to improve the intestinal microbial environment, regulate blood sugar, regulate blood lipids, antioxidants, anticancer, immune regulation, and so on. Meanwhile, in the food industry, inulin can be used as thickeners, fat substitutes, sweeteners, and water-retaining agents [6].

The management of inulin can be done enzymatically by microbial inulinase. A number of microbial groups such as bacteria, fungi, and yeasts has been exploited for inulinase. It is of the several species that have the best growth against environmental and high osmotic conditions as producers of inulinase are fungi [7].

Inulinase is an enzyme produced by plants, animals, and microbial sources that are induced by inulin. Inulin is a substrate and an effective carbon source as a producer of inulinase. Potential sources of inulinase-producing microbes are bacteria, fungi, and yeasts. However, fungi can be the main choice because they can grow on better substrates and require lower costs. So that this enzymatic reaction is very beneficial because it produces various important industrial products from these inulinolytic enzymes such as fructose and FOS [8].

Inulinase was developed today because of its cheap and abundant supply. These inulinase-producing microorganisms can be cultured in an inulin-rich medium, wherein inulin from dahlia tubers can be used as an inducer and a carbon source for inulinase-producing. Fungal growth as an inulinase producer needs attention, especially in temperature and pH regulation. Temperature and pH play an important role in the best inulinase production, which is in the range of PH 4–6.5 [9].

The results of the previous studies found that plant materials containing inulin such as dahlia, garlic, and Jerusalem artichoke could be used as a carbon source to produce the best inulinase compared to the purified substrate. Inulinases produced from these plant materials have higher profits and lower production costs when compared to purified substrates [10].

Another study also found that chicory root as a substrate produced the highest inulinase in *Aspergillus niger* among of 12 the tested strains with a clear zone diameter on agar plates after hydrolysis of inulin used iodine solution was 40 mm and the value of inulinase activity was 102.3 U/mL [11].

An important phase should be done to obtain a big-scale inulinase enzyme through mold isolation. To isolate inulinase in a great amount from plants is somewhat difficult, so this research is important to do to find out the type of endophytic mold which had the highest inulinase activity through isolation and identification of endophytic mold molecular in red dahlia tuber. It was also aimed to get a new isolate of

endophytic mold which had the highest inulin activity so that it could get a new variant of the inulinase enzyme which could be used in industry to produce fructose enzymatically.

Materials and Methods

This type of study is a descriptive study. Identification was carried out by morphological, microscopic, and molecular observations to determine the type of inulinase-producing endophytic mold in red dahlia. This study was performed in Mei 2021 at the Biology Molecular Laboratory of the Faculty of Medicine, Prima Indonesia University. This study has been approved by Health Research Ethics Committee from Prima Indonesia University.

Isolation of inulinase producing endophytic mold

Endophytic mold isolation producing inulinase obtained from red dahlia tuber from Berastagi, North Sumatera in Indonesia. The initial step is to sterilize dahlia tubers in stages, which is done twice in a row using 96% ethanol for 30 s, 1% NaOCl for 1 min, and 70% ethanol for 30 s. Dahlia tubers were then cut into small pieces, placed on a petri dish containing potato dextrose agar (PDA), and incubated at room temperature. The mycelium that grew from the dahlia tubers was then transferred to agar in a new PDA dish with a mixture of 1% inulin and 250 g cloramphenicol as an anti-bacterial, where each isolates with codes UD1, UD2, UD3, UD4, and UD5, and they were incubated at the room temperature of 30°C in 72 h or until pure cultures are obtained. Each pure culture of the fungus was observed for color and colony morphology macroscopically and microscopically.

Inulinase activity screening

Screening inulinase activity in the isolates was screened using PDA medium and 1% of inulin powder, after isolates were incubated at 28–30°C for 5–7 days; a qualitative inulinase test was carried out by adding Lugol's solution to see the hydrolysis of inulinase, Lugol iodine was poured into the petri dish for 3–5 min and removed after that aquabidest was poured to remove the remaining Lugol's iodine. The clear zone indicated inulin hydrolysis by the mold. Positive results are indicated by the presence of a clear zone around the endophytic mold, while negative results are indicated by the absence of a clear zone [12].

Inulinase activity grouped into three categories: Low activity with the ratio of the area of clear zone toward the colony area of <1, was given a symbol (+),

moderate activity <1–2 (++)), high activity with the ratio >2 (+++). The isolates with the highest inulinase activity were further identified molecularly [13].

Isolation inulinase enzyme (Crude enzyme)

Fermentation culture was prepared by taking a number of mold isolates dissolved in 25 mL of potato dextrose broth (PDB) medium at pH 5.0. It was incubated in a shaking incubator at the velocity of 160 rpm for 72 h at the temperature of 30°C. To extract inulinase crude enzyme of 2.5 mL, fermentation culture was put into Erlenmeyer flask containing 25 mL, inulinase enzyme isolation medium consisted of 1% of inulin, $(\text{NH}_4)_2\text{PO}_4$ 0.5%, MgSO_4 , 7 H_2O 0.05%, and FeSO_4 0.015% pH 5.0. It was then incubated for 120 h in a shaking incubator at the velocity of 160 rpm at the temperature of 30°C. From this incubation, a suspension of inulinase enzyme will be produced which is then centrifuged at 3.500 rpm for 15 min. The filtrate was a *crude* inulinase enzyme [14].

Determination of the standard curve

The making of fructose calibration curve is a standard fructose concentration that was prepared in some reaction tubes with the graded concentration of 20–90 mL of the fructose main solution of 1.000 mg/L plus 1 mL of reagent Nelson. Reaction tubes were heated in the water heater for 30 min. Reaction tubes were cooled in running water until the temperature was 25°C, added with 1 mL of Arsenomolibdat solution, stirring until all the precipitate was dissolved, 7 mL of aquadest was added and mixed again until it was homogeneous. Absorbance was measured with an ultraviolet-visible (UV-Vis) spectrophotometer at a wavelength of 499 nm [15].

Inulinase enzyme activity test

Testing for inulinase activity was carried out by preparing a test tube containing 0.1% inulin solution, 0.5 mL of the *crude* enzyme, 1 mL of 0.1 M acetate buffer at pH 5.0, and then incubated at 35°C for 15 min. After that, 1 mL of Nelson's reagent was added and reheated for 30 min, after being cooled, 1 mL of Arsenomolybdate solution, and 7 mL of distilled water were added and stirred until homogeneous. The determination absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 499 nm [16].

DNA isolation

DNA isolation was done in each mold isolate obtained from the result of purification. DNA isolation, that is, mycelium was inoculated in 10 mL of PDB medium in the vial tube, and then grow it rotary shaker

in 72 h. Then, the harvesting of mycelia was done by centrifugation method with a velocity of 13.000 rpm in 15 min. The supernatant was removed, and only its pellets were taken out. The pellets were washed with 500 μL TE buffer (with pH 8) and 300 μL extracted buffer. 150 μL of sodium acetate pH 5.2 was added after it was incubated at the temperature of 20°C within 10 min. After that, it was centrifugated with a velocity of 13.000 rpm in 5 min, and its supernatant of 600 μL was taken out and moved into a new microtube. Isopropanol was added to the microtube containing supernatant with the same volume and centrifugated with a velocity of 13.000 rpm in 10 min. After that, DNA was washed with 500 μL of ETOH (70% ethanol) and dried up and dissolved with 20 μL of buffer TE, and kept in a freezer at the temperature of -20°C [17].

Polymerase chain reaction (PCR) amplification and elektroforesis

DNA as a result of extraction was amplified using Esco Swift MaxPro thermal cycler. Gen internal transcribed spacers (ITSs) were amplified using ITS 1 Primer and ITS 4. Total volume PCR was made 50 μL consisted of containing 2 μL of DNA isolate, 10 μL of each primer concentrating 2 μmol , 5 μL dNTP concentrating 2 mM, 10 μL buffer + 5 μL MgCl_2 , 7.75 μL H_2O , and 0.25 μL Go Taq DNA polymerase. The reaction begins with a hot star at 95°C for 5 min, denaturation at 94°C for 1.5 min, annealing process at a temperature of 53°C for 1 min, and an extension process at 72°C for 3 min. This PCR reaction process lasts for 35 cycles. The primer used was universal ITS 1/ITS 4 primer, ITS 1 as a reverse primer, and ITS 4 as forwarding primer. Primer ITS 1 is 5' (TCC GTA GGT GAA CCT GCG G) 3' and primer ITS 4 is 5' (TCC TCC GCT TAT TGA TAT GC) 3' [18].

Analysis of the amplified DNA was carried out by electrophoresis using 0.8% agarose gel for DNA extraction, while PCR amplification used 1.2% agarose gel. Electrophoresis was carried out by taking 3 μL of 100 bp marker and loading dye 1 μL then resuspended and put into wells in 1.2% agarose gel. The PCR product from the sample was then taken as much as 3 μL , and put into a well in 1.2% agarose gel, then electrophoresed for 30 min with a voltage of 110 volts. Then, the gel was soaked with ethidium bromide after the electrophoresis was completed and analyzed with a UV transilluminator [19].

DNA sequencing

The amplified DNA was used for DNA sequencing steps based on the dideoxy nucleotide chain termination method (Macrogen Inc., South Korea). The results of DNA sequencing were then compiled using the Bioedit program and analyzed using the BLAST program using information from Genbank (<http://www.ncbi.nlm.nih.gov>) [20].

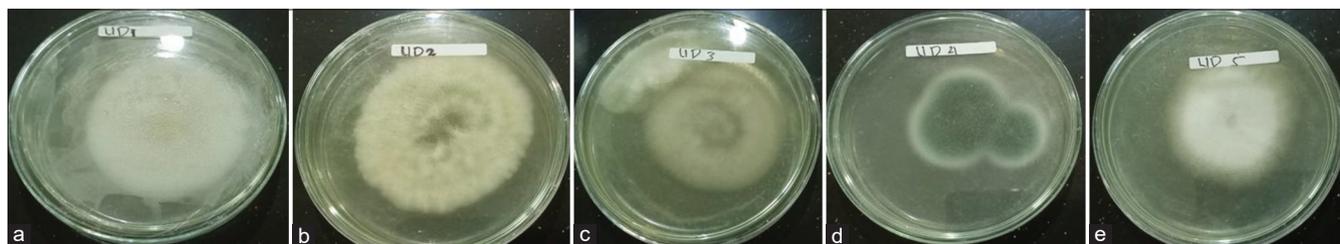


Figure 1: Macroscopic mold morphology from endophytic mold isolation of red dahlia root: (a) UD1, (b) UD 2, (c) UD 3, (d) UD 4, and (e) UD 5

Result

The result of mold isolation of red dahlia tuber showed that five types of mold which were given their labels as UD1, UD2, UD3, UD4, and UD 5 are shown in Figure 1. After the result had been obtained, each of the mold types was treated with pure breeding as stock culture. From the mold isolate, observation was done macroscopically and microscopically. Coloring with lactophenol cotton blue was done in microscopic observation Figure 2. The result of macroscopic and microscopic observation is shown in Table 1.

The result of screening of inulinase activity was done three repetitions with pure media dripped with Lugol iodine and cleaned up its remaining Lugol with aquadest so that inulinase activity measurement was obtained by measuring clear zone in the mold as it is shown in Table 2 below. It was followed by measuring inulinase activity in the mold isolate which had the highest inulinase: UD 3, UD 4, and UD 5 as they are shown in Table 3.

After the inulinase activity test had been done, it was followed by identifying the molecular with ITS primer. Primer used to amplify rDNA and the result of DNA sequencing with ITS 1 and ITS 4 is shown in Table 4 and Figure 3.

The result of identifying molecular is shown in Tables 5 and 6.

The result of measuring the highest inulinase in UD 5 was 0.685185185 and molecular identification was done with ITS 1 primer and ITS 4 primer which showed that UD 5 which had the highest inulinase activity was mold with species *S. commune* from the Schizophillaceae family.

Discussion

The endophytic mold isolated from red dahlia tubers had five isolates, each of which was labeled UD1, UD 2, UD3, UD4, and UD 5. Each isolate had almost the same color, namely, white, and among the isolates, the isolates were white, which has the highest activity is UD 5, namely: 0.685185185, and from the results of molecular identification with the ITS1 and ITS4 regions, it was successful to identify the endophytic mold species *S. commune* (Figure 5).

This study explores the types of inulinase-producing endophytic molds that will be used by the industrial world to produce fructose and FOS through an enzymatic process so that inulinase is an important isolate in industry and pharmaceuticals as a basic ingredient for various foods and medicines.

Inulinase is a very important hydrolytic enzyme whose contents are composed of fructose monomer units with β -D-2,1 glycosidic linkages and have glucose moiety at the reducing end joined by an α -D-1,2 glycosidic linkage. Inulinase can be categorized into two types, namely, endoinulinase and exoinulinase. Exoinulinase cleaves β -2,1 bonds sequentially to produce fructose, while endoinulinase cleaves randomly and hydrolyzes internal bonds in inulin to produce FOS [21].

Fructose can be classified as a natural sweetener with low calories; it has a level of sweetness of 70% which is higher than sucrose so that it is safe to be consumed by diabetes patients. Fructose can also be used as an alternative sweetener as the substitute for artificial sweetener. Another important application of the inulinase enzyme, among others, is for prebiotic production such as inulooligosaccharides and FOS. It

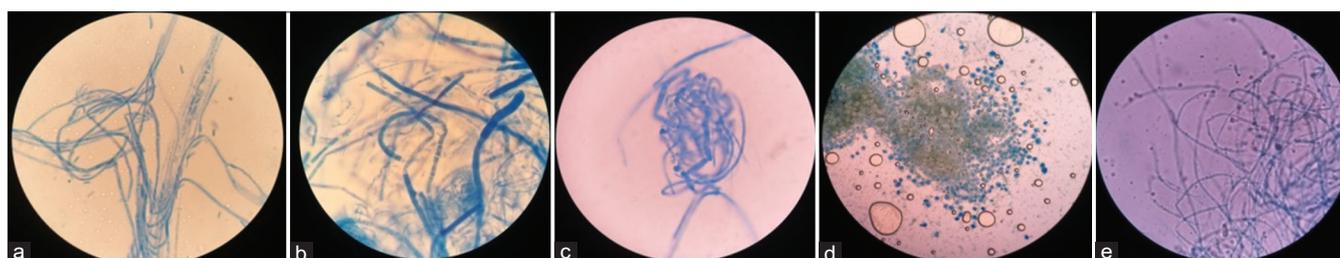


Figure 2: Microscopic mold morphology from endophytic mold isolation of red dahlia root: (a) UD1, (b) UD 2, (c) UD 3, (d) UD 4, and (e) UD

Table 1: Result of observation of macroscopic and microscopic endophytic mold morphology

No	Types of Mold	Macroscopic Observation	Microscopic Observation
1.	UD1	Upper part: Milky white hyphae, white meselenium, yellowish spore, and thick colony Reverse: Yellowish hyphae, brownish spore, thick colony, and in order	Septate hyphae, non-branchy miselenium, transparent hyphae stem, and spore (+)
2.	UD2	Upper part: White hyphae, greenish spore, dense colony, and in order Reverse: Yellowish hyphae, brownish spore, thick colony, and in order	Eptate hyphae, branchy miselenium, transparent stem, and conidium globusa
3.	UD3	Upper part: White hyphae, black spore, dense colony, fibrous, and in order Reverse: Yellowish hyphae, black spore, dense colony, and in order	Septate hyphae, branchy miselinium, transparent stem, batang, and spore (-)
4.	UD4	Upper part: White hyphae, green spore, thick colony, and in order Reverse: White hyphae, yellowish spore, thick colony, and in order	Non-setate hyphae, branchy miselenium, conidium globusa, and spore (+)
5.	UD5	Upper part: Cottonish white hyphae, yellowish spore, thick colony, and in order Reverse: Yellowish hyphae, yellowish spore, thick colony, and in order	Non-setate hyphae, non-branchy miselenium, transparent stem, and spore (+)

can also be used to produce ethanol, pollutant, and sorbitol [22].

Table 2: Inulinase activity screening

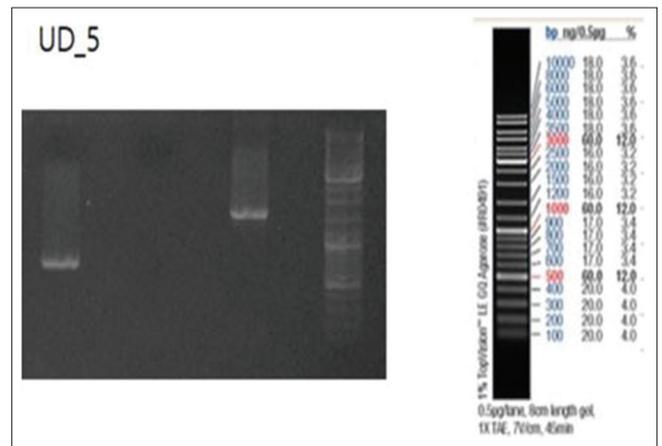
No	Types of mold	Area of clear zone	Explanation
1.	UD 1	<1-1	(+): Low activity
2.	UD 2	>1-2	(+): Low activity
3.	UD 3	>2-3	(++): Moderate activity
4.	UD 4	>3-4	(+++): Moderate activity
5.	UD 5	> 4	(+++): High activity

The results of this study produced the highest inulinase enzyme at UD 5, from five isolates produced through isolation of red dahlia tuber molds labeled UD1, UD2, UD3, UD4, and UD5. The result of identifying molecular showed that the type of *S. commune* was found which indicated that it was a new isolate that would add a new variant of inulinase enzyme that could be used by industry to produce fructose enzymatically from the inulin powder of red dahlia tuber due to the importance of increasing beneficial food production for health by exploring the available local natural resources.

Table 3: Result of inulinase activity measurement

Isolat Code	Absorbance Sample	Fructose Concentration	Inulinase Enzyme Activity
UD3	1736	157.3333333	0.582716049
UD4	1532	89.33333333	0.330864198
UD5	1819	185	0.685185185

DNA amplification product fragments are sequenced in the ITS1 and ITS 4 regions. The nucleotide sequence in fungi can change because the ITS region has a high evolutionary rate. This situation can be used to determine the different species. Fungi with the same sequence of nucleotide bases can be concluded as the same species. Furthermore, the DNA sequencing Bioedit program will provide complete answers to these types of species by producing complete DNA sequences from the endophytic fungi. It is shown in Figure 4. So that the results of the study found *S. commune* which has a 99% similarity based on Genbank molecular information.

**Figure 3: Results of DNA amplification of red dahlia tuber endophytic molds using ITS1/ITS 4 primer pair. M, 100 bp DNA ladder****Table 4: Primer used to amplify rDNA in endophytic mold**

Sequencing Primer Name	Primer Sequences	PCR Primer Name	Primer Sequences
ITS1 5'	(TCC GTA GGT GAA CCT GCG G) 3'	ITS1 5'	(TCC GTA GGT GAA CCT GCG G) 3'
GAA CCT GCG G) 3'		ITS4 5'	(TCC TCC GCT TAT TGA TAT GC) 3'
ITS4 5'	(TCC TCC GCT TAT TGA TAT GC) 3'		
TAT TGA TAT GC) 3'			

A previous study found that endophytic mold from red dahlia tuber which had the highest activity was *Aspergillus clavatus*, the mold which had superiority in yielding the biggest inulinase enzyme and had better inulinase activity in inulin hydrolysis in the 3rd day within 30 h of 66.96% [23]. However, in this research, it was found that there was a new variant, *S. commune*, which had the highest inulinase activity of 0.685185185. Besides that, the previous research also found that *S. commune* had the potency of good antioxidant which could be the source of good nutraceuticals even though it contained 2.0 mg/g phenol [24].

Table 5: The identities of endophytic mold

Subject	Score	Identities
Accession	Description	Length Start End Coverage Bit E-Value Math/Total Pct. (%)
AB369910.1	Schizophyllum commune	660 1 652 98 1194 0.0 651/653 99

In general, the produced inulinase activity was correlated with the amount of reducing sugar produced. The more amount of reducing sugar produced is the higher the inulinase activity and vice versa [25].

Table 6: The strain of endophytic mold

Kingdom	Family	Genus	Species
Eukaryota	Schizophyllaceae	Schizophyllum	Schizophyllum commune

The results of this study are different from the previous studies which found sources of inulinase from fungi such as the discovery of *Aspergillus fumigatus* [26], and *Penicillium* sp., as well as other sources of inulinase from bacteria such as *Pseudomonas* sp., *Bacillus* sp., and *Arthrobacter* sp, and source inulinase from yeasts such as *Saccharomyces* sp., *Kluyveromyces* sp., and *Candida* sp. [27].

Whereas from the result of this research, it was found that new isolate which produced the

DNA Squence of UD 5 ITS 1

CTACAGGGACTGCGGAGATCATTAACGAATCAAC AAGTTCATCTTGTCTGATCCTGTGCACCTTATGTAGTC
 CCAAAGCCTTCACGGGCGGGCGGTTGACTACGTCTACCTCACACCTTAAAGTATGTTAACGAATGTAATCAT
 GGTCTTGACAGACCCTAAAAGTTAATAACAACCTTCGACAACGGATCTCTTGGCTCTCGCATCGATGAAGAACG
 CAGCGAAATGCGATAAGTAATGTAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTTCGCGCCTTTGGTATT
 CCGAGGGGCATGCCTGTTTTGAGTGTCTATAAATACCATAACCTCTTTTACTTCCGTCGAGAGTGGCTTGAAGTG
 GAGGTCTGCTGGAGCCTAACGGAGCCAGCTCCTCTTAAATGTATTAGCGGATTTCCCTTCCGGGATCGCGTCT
 CCGATGTGATAATTTCTACGTCGTTGACCATCTCGGGGCTGACCTAGTCAGTTTCAATAGGAGTGCCTTC
 CAACCGTCTCTTGACCGAGACTAGCGACTTGTGCGCTAACTTTTACTTGCCTCAAATCAGGTAGGACTACCCGCTGAA
 CTTAAGCATATCAATAAGGCGGATCGCAAGGAAACGTAAGGGGGAGGGGGCGGGGGGGGGGGGGAGGGGGGGGGCGG
 GGGACGCCCGCCGCGGCCACCCAACGAACCCAAGAAGTGAAGCGAAGCAGAGGGCCACCCGCGGAGAAACAACC
 CACACGCCGCGACAAAAGAAACCCAGCCCCAAGCTATTGATCAATATCACGGTGGGGGACGACTTACTCTT
 CACTAATCCATCTTTTTCGGAGAAGTGTGAAGGATGCAATATCTTATCCACTGGATGCGACAGTTAACCTTTAAAATACGT
 CCTAGAATAGGCAATAGGGA TATCCCGCCGTCTAAATCTTTTGTGCTTCGCCGCGGACGGCATCGTAATAAGTTATTATT
 CCCGTAATCAGATTAGAAATCTGTGCTACATTA TATGCTATCACGGGATAGGTACACAGCCTACAC
 CGAAGATATTTAATCCGAGTTTGATATATACGTACCGGTAAGTGAATGCATA

DNA Squence of UD 5 ITS 4

GGACCGGGATGCTACCTGATTTGAGGTGAGTCAAAAGTTAGCGCACAAAGTCGCTAGTCTCGGTC
 AAGAGACGTTTGAAGCAGACTCCTATTGAAACTGACTAGGTGAGCCCGAGATGGTCAACGACGTA
 GAAATTATCACATCGGAGACGCGATCCCGCAAGGGAAATCCGCTAATACATTTAAGAGGAGCTGGCTCCG
 TTAGGCTCCAGCAGACCTCCACTTCCAAGCCACTCTCGAGACCGAAGTCAAAGAGGGTTGATGGTATTTAAT
 GACTCAAACAGGCATGCCCTCGGAATACCAAGGGCGCAAGGTGCGTTCAAAGATTCGATGATCACTGAAT
 TCTGCAATTCACATTACTTATCGATTTTTCGCTGCGTTCTTCATCGATGCGAGAGCCAAGAGATCCGTTGTCGAAAGTTG
 TATTAATTTTTAGGGTCTGTCAAGACCATGATTACATTCGTTAACATACTTTAAGGT
 GTGAGGTAGACGTAGTCAACCGCCGCCGTGAAGGCTTTGGGACTACATAAGGTGCACAG
 GATCAGAACAAGATGAAGTTGTTGATTGTTAATGATCCTTCCGCAGGTTACCTACGGAAAC
 CTTGTTACGATTTTAACTTCCCAC

Figure 4: Alignments of DNA sequence of UD 5 verified with pairwise alignment using Bioedit program

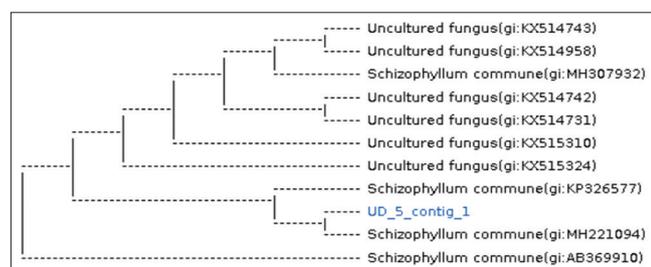


Figure 5: Neighbor-join tree (NJ) from the ITS sequence

highest inulinase, *S. commune*, so that in the future, it is expected the industrial business can use the inulinase enzyme from mold in producing fructose and FOS from the inulin of red dahlia tuber in big scale.

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