

IDENTIFICATION OF CANDIDA SPECIES

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**IDENTIFICATION OF CANDIDA SPECIES
BY ASSIMILATION AND MULTIPLEX-PCR METHODS**

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ABSTRACT

The species of *Candida*, responsible for causing candidiasis infection, require fast and accurate identification, so that drugs can be delivered right. A total of 16 glycerol stock samples, obtained from the woman's prison at Palembang, were cultivated and rejuvenated on a Sabouraud Dextrose Agar (SDA) medium and identified by using the API 20C AUX kit. The results have identified *C. glabrata*, *C. crusei*, *C. parapsilosis*, *C. albicans*, *C. Tropicalis*; two samples have not been clearly identified. The result from the API 20C AUX kit method were then compared to the results from the fermentation method in a previous study. Samples which were not fit or not identified were reanalyzed by using Multiplex-PCR. Prior to performing Multiplex PCR, DNA of *Candida* samples were isolated by using the heating method and the presence of DNA was confirmed by using a spectrophotometer. Primer pairs used in the Multiplex-PCR method were the universal primer ITS1-ITS2, and the specific primers CA3 and CA4. The results identified *C. tropicalis*, *C. glabrata*, and *C. parapsilosis* about 218, 483, and 229 bp, respectively. The match of both methods (the assimilation method, using the API 20C AUX Kit and the Multiplex-PCR method) was 70 %, which can support the use of assimilation methods for the identification of *Candida* species. The assimilation method using API 20C AUX Kit needs to spend about 2 days for identification, while the conventional method or a fermentation method needs 14 days.

Keywords: *Candida* species, assimilation, API 20C AUX, Multiplex PCR, fermentation.

INTRODUCTION

Candida is a yeast group, which consists of hundred of species. It is the one of pathogenic microorganisms which requires fast identification. The infection caused by *Candida* is called candidiasis. It represents many diseases caused by fungi and can be found in a range of ages or gender. More than 150 species of *Candida* were identified, at least 70 % of the infections were caused by *Candida albicans* [1]. As many as 17 species

that can live in the human body have been found, for example, *Candida albicans*, *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis*, *Candida krusei*, *Candida kefyr*, *Candida guilliermondii*, *Candida lusitanae* and *Candida dubliniensis*. *Candida* species live in the human body in the gastrointestinal tract, mucous membranes, respiratory tract, vagina, urethra, skin, and under the nails of the fingers and toes [1].

There are several methods that can be used for identification of *Candida* species, one of them is the

assimilation method. The principle of identification with the assimilation method is identifying *Candida* species by observing its growth on carbohydrates such as glucose, galactose, sucrose, maltose, lactose, and raffinosa [2].

There are two ways to apply the assimilation method - the conventional identification method and the automated manner (Kit). The assimilation conventional method was demonstrated by Wicherham and Burton in 1975. It needs a lot of time, about 1 - 14 days. The development of the method aimed to get results fast and in a precise manner. Thus, it was discovered how to use kits for the identification in 1978 [3]. The kit is known as API (Analysis Profile Index) 20C AUX (bioMerieux Vitex, Inc.), which has the same principle as the conventional assimilation method. The Kit API 20C AUX involves 19 assimilation reactions for identifying yeasts, and provides accurate results after incubation for 72 hours [4]. Yeast will only grow if it matches with the substrate that has the appropriate carbon source. The principle of this tool is realized by comparing the reaction of the sample with the control.

The sample of *Candida* which used in this study was isolates in a glycerol stock form, obtained from vaginal swab of patients at the Women's Prison Palembang. The sample of *Candida* were isolated and identified by using the fermentation method by Susilawati [5]. Identification by fermentation took a long time - about 21 days, so then [8] make the time shorter, the identification was done by using the API 20C AUX kit. The identification of *Candida* species with assimilation method, using the API 20C AUX kit took only 48 hours. The reasons why the assimilation was chosen, was that the assimilation method is a gold standard that used as a guide for many other methods for *Candida* identification [2].

The results of this study are expected to verify the results that have been obtained previously by the fermentation method. If there are samples that have different identification results by fermentation method and the assimilation method using Kit API 20C AUX, they will be reanalyzed with the Multiplex-PCR. Multiplex-PCR has been chosen because it can amplified more than one DNA fragment in one run for a single PCR mixture [6].

EXPERIMENTAL

Materials and Methods

A set of glass equipment, an analytical balance, laminar air flow, hotplate, a set of micro pipettes, an autoclave, bunsen burner, API 20C AUX Kit yeast identification system (BioMerieux), spectrophotometer Uvmini-1240, a freezer, needle ose, centrifuge Universal 320R, vortex Thermolyne, PCR Bio-Rad, a set of electrophoresis Bio-Rad, the LIAS Slite-140 Gel Documentation, incubators and shakers incubator N-Biotek.

Candida isolates

Candida isolates in a stock glycerol form, obtained from women with clinical candidiasis vaginalis at Women's Prison Palembang), peptone, dextrose, bacto agar, sterile distilled water (water without chemical additives that can liberate gases such as Cl₂, CO₂, etc.), bromthymol blue, agarose, substance intercalator ethidiumbromide 0.1%, TAE buffer 10X Promega, Bench Top marker 1Kb DNA Ladder Promega, GoTaq Green Master Mix 2X, Universal primer pair ITS1 (TCCGTAGGTGAACCTGCGG) and ITS2 (GCTGCGTTCTTCATCGATGC); a specific primer pair CA3 (GGTTTGCTTGAAAGACGGTAG) and CA4 (AGTTTGAAGATATACGTGGTAG).

Sterilization of Equipments

All equipments were washed thoroughly before usage and then dried in an oven at temperature about 37°C until they were completely dry. A petri dish, a tip, a needle ose, erlenmeyer flasks and all the tools that had been wrapped, were sterilized in the autoclave at temperature of 121°C and pressure of 1 atm, for 15 minutes.

Preparation of Sabouraud Dekstrosa Agar Media (SDA)

A total of 65 g of a mixture (10 g peptone, 40 g dextrose, and 15g agar-bacto) was dissolved into 1 L of distilled water by heating on a hotplate. The solution was then sterilized in the autoclave at temperature 121°C and pressure 1 atm, for 15 minutes. The media, with the same volume was poured into a sterilized petri dish. The process must proceed in sterile conditions in a laminar

air flow. The media was ready to use. In a solid form, the media can be stored at 4°C.

Preparation of Sabouraud Dekstrosa Liquid Media

One gram of peptone and 4 g glucose, were mixed in an erlenmeyer flask. 120 mL of sterile water were added and the mixture was heated, until dissolved. Media was cooled for a few moments then transferred to 10 test tubes with the same composition. Media was sterilized by put it into autoclave at temperature 121°C, the pressure is 1 atm for 15 minutes. Media was ready to use, media can be stored at 4°C.

Cultivation of *Candida* on SDA

The sample of *Candida* was grown aseptically in the laminar air flow. Yeast *Candida* was scratched on the surface of SDA media with an aseptic needle, then incubated at temperature 30°C for approximately 24 hours.

Rejuvenation of *Candida* on SDA

Candida colonies were took by using an aseptic needle, then moved slowly onto the same media (SDA), then incubated at 30 °C for 20 hours.

Identification of spesies *Candida* using the API 20C AUX Kit (BioMerieux)

Preparation of the Strip

An incubation box was prepared. About 5 mL of distilled or demineralized water were distributed onto the honey-combed wells of the tray.

Preparation of the Inoculum

Colonies of *Candida* were taken using a sterilized pipette in the API 20C AUX Kit suspension medium, then stirred with a vortex. The turbidity of the Kit API 20C AUX suspension medium was compared to a 2McFarland standard (cultivated turbidity should approach the standard). If it has approached the standard, the suspension medium is ready for use, but it should be used immediately after preparation. An ampoule media kit API 20C AUX was opened slowly and then mixed with 100µL API 20C AUX Kit suspension medium which

was mixed with *Candida*, homogenized with a pipette and taking care to avoid the formation of air bubbles.

Inoculation of the Strip

The wells of the strip were filled with the Kit API 20C AUX solution medium that had been homogenized with the kit API 20C AUX suspension media. The wells were filled from one side to avoid the formation of air bubbles, not stuffed too full or too empty (a flat surface). Tray was sealed and then incubated for 48 hours at temperature $29 \pm 2^\circ\text{C}$.

Reading the Strip

After 48 hours of incubation, the growth in each well was compared to the 0 cupule, which was used as a negative control. A well, more turbid than the control indicates a positive reaction to be recorded on the results sheet. In order to minimize the risks of contamination when reincubation was necessary, the lid was removed only when reading the strip and replaced immediately.

Result Interpretation: The result of API 20C AUX Kit was compared with the Numeric Profile Table, supplied from the company.

Identification with Multiplex-PCR

DNA Extraction [7]

A total of 1.5 mL *Candida* sample was put into an eppendorf tube, centrifuged for 15 minutes at 5000 rpm and temperature of 4°C. The pellets were taken, 500 µL distilled water was added and centrifuged at 5000 rpm, 4°C for 5 minutes. The pellets were taken again, and this step was repeated twice. Next, the pellets were added to 200 µL of sterile distilled water, mixed with a vortek for 1 minute and heated into boiling water for 5 minutes. This step was repeated three times. In the final step, the tubes were centrifuged for 10 min, 5000 rpm and 4°C, the supernatants were collected and thus DNA was isolated.

The Measurement of the DNA concentration

Two cuvettes were prepared. The first cuvette was filled with 5 mL of sterile water as a blank and the second cuvette was filled with the sample, containing

500 µL *Candida*, which had been isolated, and 4.5 mL sterile water. A quartz cuvette was filled with the blank then UV-Vis spectrophotometer was tared. The sample was measured by using a UV-Vis spectrometer at wavelength about 260 nm. The data obtained is used to find the concentration of *Candida* in the samples by using the formula [8]:

$$\text{Concentration (C)} = [\text{Absorbance } \lambda A_{260} \times \text{C factor} \times \text{dilution factor}]$$

Note: C Factor = 50 µg/mL (DNA double helix unit conversion).

Multiplex-PCR and Electrophoresis [9]

Primer sequences (ITS1, ITS2, CA3 and CA4) were amplified using following conditions: 95°C for 60 seconds, then 35 cycles at 94°C for 30 seconds, 60°C for 60 seconds, 72°C for 45 seconds followed by one cycle at 72°C for 5 minutes. The DNA product of Multiplex-PCR was separated by electrophoresis on agarose gel (1 % concentration). Electrophoresis was performed in an electrophoresis apparatus, containing 1x TAE (Tris-Acetic acid-EDTA). A total of 5 µL DNA were put in wells on an agarose gel, 1kb DNA ladder marker was used as a marker size of DNA bands in the gel electrophoresis. The electrophoresis was performed at voltage of 100 volts and run for 45 minutes. Furthermore, the DNA bands were detected by using the Gel Doc.

RESULTS AND DISCUSSION

Candida Growth on Sabouraud Dektrosa Agar (SDA)

The *Candida* samples, obtained from glycerol stocks at temperature about -45°C in the freezer were refreshed on a SDA agar plate. SDA is used for fungi cultivation. It provides nutrients needed for growth of *Candida*, such as nitrogen, vitamins and carbon. The growth of *Candida* can be visible after incubation for 24 hours with the temperature maintained at 30°C.

The morphology of the *Candida* colonies can be directly seen. The colony has a rounded shape, a bit protruding, smooth and slippery. It would be wrinkled when the colony was old, yellowish white in color, and the smell was like *tape*. If the *Candida* growth looked



Fig. 1. Colonies of *Candida* sp that were cultivated on a SDA agar plate, and incubated for 24 hours at temperature 30°C.

like in the figure, the cultivation of *Candida* could go to further treatment, so they were rejuvenated and species identified.

Identification of *Candida* Species by the Assimilation Method, Using the API 20C AUX Kit

The API 20C AUX Kit is an identification system for *Candida* species that does not require a long time and a lot of mixtures of chemicals. It provides 19 different sugar assimilation reactions (D-glucose, glycerol, calcium 2-keto-gluconate, L-arabinose, D-xylose, adonitol, xylitol, D-galactose, inositol, D-sorbitol, methyl-α-D-glucopyranoside, N-acetyl-glucosamine, D-cellobiose, D-lactose, D-maltose, D-sucrose, D-trehalose, D-melezitose, D-raffinose) and a 0 well is a negative control for the comparison.

The identification, using an API 20C AUX Kit, is based on the principle of conventional assimilation, which has become the standard in any *Candida* species identification method. Each species of *Candida* requires carbon sources from different carbohydrates as a source of energy for cell growth. The need of different carbohydrates source becomes the basic identification for the assimilation method by every *Candida* species. Fig. 2 shows the positive identification for *Candida albicans*. *C. albicans* can grow on the substrate with successive sugars, such as D-glucose, calcium 2-keto-

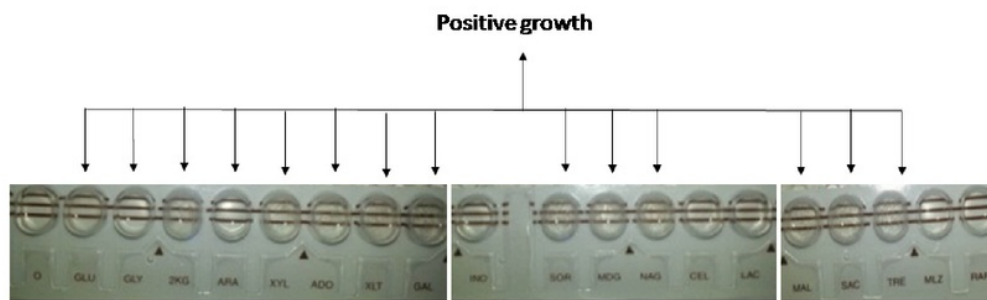


Fig. 2. Identification result of isolate code number 44, using the API 20C AUX Kit and identified as *Candida albicans*.

gluconate, D-xylose, adonitol, xylitol, D-galactose, D-sorbitol, methyl- α D glukopiranosida, N-acetyl-glukosamin, D-maltose, D-sucrose, and D-trehalose. The turbidity was between 100 and 76 %. Growth also occurs in substrates, such as glycogen and L-arabinose, where turbidity is between 25 and 1 %.

Based on the sugars that can be utilized by *C. albicans* as an energy source, it has been observed that *C. albicans* can use several types of sugars from the group of the carbohydrates such as monosaccharides, disaccharides, and polysaccharides. Glucose can be directly used by *Candida albicans* to perform metabolism in the cells. The first pathway of glucose metabolism in yeast is glycolysis. Glucose through enzymatic reactions breaks down glucose (6 carbon atoms), which becomes pyruvic acid (3 atom C), these reactions produce energy. Pyruvic acid furthermore enters the Krebs's mitochondria cycle and the electron transport.

On Fig. 2 *Candida albicans* can also take advantage from the disaccharide sugars and polysaccharides as a source of energy, since *C. albicans* have the enzymes which can hydrolyze sugars into its monomers and then follow the same pathway as with glucose. *Candida albicans* showed a positive reaction to disaccharides such as maltose and sucrose, and did not show growth on the disaccharide lactose. Similar results were obtained by other research [10]. The negative reaction indicated that *C. Albicans* did not have a specific enzyme to process carbohydrates as substrates. Similar observations were also made for other *Candida* samples. The results of the identification of *Candida* samples are shown in Table 1.

It has been reported that the identification with the conventional method, and using API 20C AUX Kit have in common as much as 97 %. From identification point of view, the Kit API 20C AUX requires less time and does not require addition of any chemicals, so it is more practical to use for rapid identification. In this study, the similarities obtained between the conventional fermentation method and the assimilation method, using Kit API 20C AUX, are shown in Table 1 [11].

In previous research, identification using the fermentation method has been done. Its results are shown in Table 1. They are used for comparison with

Table 1. Identification results of spesies *Candida* by the asimilation method, using the API 20C AUX Kit.

IsolateCode	Organism	
	Asimilation (API 20C)	Fermentation*
2	Not Identified	<i>C. tropicalis</i>
4	<i>C. parapsilosis</i>	<i>C. tropicalis</i>
5	<i>C. albican</i>	<i>C. albican</i>
6	<i>C. crusei</i>	<i>C. crusei</i>
18	Not Identified	<i>C. glabrata</i>
19	<i>C. glabrata</i>	<i>C. crusei</i>
20	<i>C. glabrata</i>	<i>C. crusei</i>
39	<i>C. crusei</i>	<i>C. crusei</i>
44	<i>C. albican</i>	<i>C. albican</i>
53	<i>C. glabrata</i>	<i>C. crusei</i>
61	<i>C. albican</i>	<i>C. albican</i>
63	<i>C. glabrata</i>	<i>C. crusei</i>
65	<i>C. parapsilosis</i>	<i>C. crusei</i>
77	<i>C. tropicalis</i>	<i>C. tropicalis</i>
79	<i>C. parapsilosis</i>	<i>C. crusei</i>
80	<i>C. glabrata</i>	<i>C. albican</i>

*Reference: Susilawati, 2014

the results obtained from the assimilation method, using this kit. Table 1 also shows that the species of *Candida* that can be identified by using the assimilation method are species of *Candida glabrata*, *Candida crusei* and *Candida albicans*, while the other *Candida* species can not be identified as a whole by this Kit.

Some factors that caused that could not be clearly identified, because the former observations were not using the API database software, but were analyzed manually. The API database Software is used to ease the observation of the results for the identification with the API 20C AUX Kit, based on the figures in the numeric profile table. The level of turbidity in the API 20C AUX Kit can be distinguished by the number that appears on the API database software, then the number can be adapted to the numerical table to determine the type of *Candida* species.

In this research, the identification by the assimilation method with the API 20C AUX Kit has a difference from the results by the fermentation method obtained in the previous research [5], especially for the species *C. glabrata* and *C. crusei*. A total of 16 samples glycerol stock of *Candida* were grown on media SDA, 6 samples had the same identification results by the fermentation method, while 10 other samples showed difference between the two methods. These differences between the two methods were caused by the different treatment of the *Candida* samples. The sample for the fermentation method had been obtained from vaginal swab and then directly used for identification. The vaginal swab can consist of several species of *Candida* and the *Candida* species that have identified by this method were dominant or the majority in the swab sample.

For the assimilation method, using the API 20C AUX Kit, samples were cultivated and rejuvenated stock glycerol, thus allowing *Candida* species, that were not dominant in the stock, to be able to grow into dominant *Candida* species on SDA media. So the results between the fermentation method and assimilation method using the API 20C AUX kit would be different.

Most *Candida* species grow on glucose, but have the distinction of growing also on other sugar. These differences are because each *Candida* species have different genes, so the ability to process carbon as an

energy source for its growth is also different. It is a basic feature of the assimilation method to differentiate between any species of *Candida*. Observations could be clearly conducted in this study. For example, *Candida glabrata* is growing on glucose, glycerol, also trehalose, while *Candida crusei* grows on glucose, glycerol, and N-acetyl glucosamine.

The results of the sample identification with fermentation method was not suitable or could not be identified, using the API 20C AUX Kit. So, we did a re-analysis using multiplex-PCR to reassure the identification results. A comparison of the results between identification of *Candida* species with the API 20C AUX kit and the Multiplex-PCR is shown in Table 2.

Yeast identification using Multiplex-PCR

Prior to the identification with multiplex-PCR, the DNA sample was isolated, using the heating method. The existence of DNA, which had been isolated, was confirmed by measuring the concentration with the UV-spectrophotometer at wavelength 260 nm. Pure DNA can absorb ultraviolet light due to the existence of purine bases and pyrimidine. DNA double helix absorbs

Table 2. The reanalyzed result of the identification of *Candida* species by using Multiplex-PCR.

Isolate Code	Identification	
	Assimilation (API 20C Kit)	Multiplex-PCR
02	Not Identified	<i>C. tropicalis</i>
04	<i>C. parapsilosis</i>	<i>C. parapsilosis</i>
18	Not Identified	<i>C. tropicalis</i>
19	<i>C. glabrata</i>	<i>C. parapsilosis</i>
20	<i>C. glabrata</i>	<i>C. glabrata</i>
53	<i>C. glabrata</i>	<i>C. glabrata</i>
63	<i>C. glabrata</i>	<i>C. glabrata</i>
65	<i>C. parapsilosis</i>	<i>C. parapsilosis</i>
79	<i>C. parapsilosis</i>	<i>C. parapsilosis</i>
80	<i>C. glabrata</i>	<i>C. glabrata</i>

the UV light at 260 nm, while the protein contaminants and phenol absorb at wavelength of about 280 nm. The working principle of a spectrophotometer is irradiation with UV light which is absorbed by the nucleotide in the solution.

Multiplex-PCR was used for amplification of the DNA of *Candida*. It was used to amplify more than one sample in a single reaction. The primer pairs, used in the study, were fungi-primary universal (ITS1 and ITS2) and specific primaries for *Candida* and *Candida albicans* (CA3 and CA4). It is stated that the ITS primer is located between the 18S gene and 28S rRNA genes. The ITS primer used is ITS1, which is targeted between 18S and 5.8S gene, and ITS2 which is targeted between 5.8S gene and the 28S gene of the rRNA [12]. The universal primary produces DNA fragments from various species of *Candida*, while the primer pairs CA3 and CA4 produce specific DNA fragments for each species of *Candida*, especially *Candida albicans*.

The DNA that has been amplified, was then confirmed with electrophoresis. Electrophoresis is a method for separation of charged molecules by using an electric field (electro) and porous metrics (foresis). Molecular separation is based on the size of the base pairs of DNA. Electrophoresis is performed with 1 % agarose gel (TAE 1x and agarose) as a pore to separate DNA, once is being electrified, then the gel is stained with an Ethidium Bromide (EtBr) dye. EtBr will be bound between two double-stranded DNA, so the band of DNA in agarose will cause fluorescence, because the EtBr dye contains fluorescence substances.

Fig. 3 shows the results of the amplification with multiplex-PCR. The sample was run by using 1% agarose gel. The results, obtained by the *C. tropicalis* is about 218 bp for both samples, isolate 4 with codes 2 and 18 (Table 1), These samples were not identified with the API 20C AUX kit. Other samples, isolate with codes 4, 19, 65 and 79 were identified as *C. parapsilosis*, which has a DNA fragment about 229 bp. Isolates with codes 20, 53, 63 and 80 were identified as *C. glabrata*, having fragment size of 483 bp. The results, obtained in the reanalysis with multiplex-PCR were as described hereunder (Table 2).

Based on our data, the identification by Multiplex-

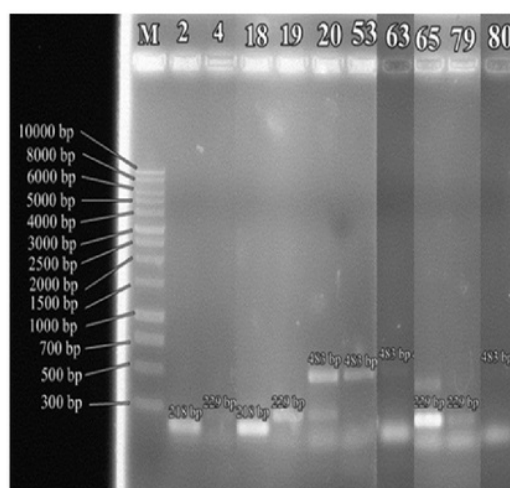


Fig. 3. The result of Multiplex-PCR for the *Candida* sample.

PCR has differences with the conventional fermentation, but it was almost similar to the assimilation method, applied with the API 20C AUX Kit. From the reanalysis results with Multiplex-PCR, the isolate 4 has different identifications by the Multiplex-PCR and the assimilation method. The assimilation method identified it as *C. glabrata* and the Multiplex-PCR identified it as *C. parapsilosis*. Isolates 4 was identified as *C. Glabrata*, considering that identification generated by assimilation methods are very clearly visible and clearly distinguishable from the other species of *Candida*.

The final three results (Table 3) showed that the similarity of the assimilation method using API 20C AUX Kit and Multiplex-PCR was about 70%, and the Multiplex-PCR confirmed the samples of *Candida* that were not identified with the API 20C AUX Kit.

From the three methods of identification of *Candida* species, the assimilation method is more preferable because it has been the gold standard for identification of *Candida* species. In addition, this method has a good accuracy and a short identification time. When compared with the conventional fermentation method, the assimilation method with API 20C AUX Kit has practical carbohydrate sources. When compared with the Multiplex-PCR method, the assimilation method using Kit API 20C AUX is cheaper.

Table 3. Results from the identification by fermentation and by assimilation using the API 20C AUX Kit, and the Multiplex-PCR methods.

Isolate Code	Result		
	Fermentation	Assimilation (API 20C Kit)	Multiplex-PCR
02	<i>C. tropicalis</i>	Not Identified	<i>C. tropicalis</i>
04	<i>C. tropicalis</i>	<i>C. parapsilosis</i>	<i>C. parapsilosis</i>
18	<i>C. glabrata</i>	Not Identified	<i>C. tropicalis</i>
19	<i>C. crusei</i>	<i>C. glabrata</i>	<i>C. parapsilosis</i>
20	<i>C. crusei</i>	<i>C. glabrata</i>	<i>C. glabrata</i>
53	<i>C. crusei</i>	<i>C. glabrata</i>	<i>C. glabrata</i>
63	<i>C. crusei</i>	<i>C. glabrata</i>	<i>C. glabrata</i>
65	<i>C. crusei</i>	<i>C. parapsilosis</i>	<i>C. parapsilosis</i>
79	<i>C. crusei</i>	<i>C. parapsilosis</i>	<i>C. parapsilosis</i>
80	<i>C. albican</i>	<i>C. glabrata</i>	<i>C. glabrata</i>

The growth of *Candida* in each of the dehydrated sugar substrates can show how it is metabolized. *Candida* can gain the energy from the different carbon sources and conduct the chemical reactions in the cells of an organism. *Candida* has a heterotrophic yeast metabolism that can assimilate the organic carbon into organic carbon else. A heterotrophic metabolism cannot assimilate inorganic carbon or carbon compounds which have one carbon (eg, CO₂). Organic carbon compounds can create new cell materials such as simple sugars, organic acids, sugars bound alcohols, short-chain polymers and long chains containing carbon, up to complex compounds such as carbohydrates, proteins, lipids and nucleic acids

The primary substrate in yeast metabolism is carbohydrate which can be oxidized to chemical energy, and is available in the cell as ATP. Carbohydrates provide almost all the carbon source for the assimilation. The

initial stage of the metabolism of carbohydrates is with mono- saccharides, while disaccharides or the trisakarida group must be hydrolyzed before hand, outside the cell. Transport of monosaccharides through the membrane is made by permease, a specific transport protein.

Candida species other than fermentation (gas formation and acid) can also make the process of assimilation. Assimilation is done by utilizing carbohydrate sources in the environment to be transformed into a source of energy (ATP), which is then used for the process of cell growth.

CONCLUSIONS

- The assimilation method, using API 20C AUX Kit can identify clearly *Candida* species such as *C. glabrata*, *C. crusei*, *C. parapsilosis*, *C. albican*, *C. tropicalis*.
- The reanalyzing of samples that do not match to the fermentation method or are not identified by the API 20C AUX Kit resulted in identification of *C. Tropicalis* 218 bp, *C. Glabrata* about 483 bp, and *C. parapsilosis* 229 bp.
- The results of the assimilation method, using the API 20C AUX Kit and the Multiplex-PCR had 70% match, indicating that use of assimilation method for the identification of *Candida* species can be done quickly and accurately.

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