EnvironmentAsia
The international journal by the Thai Society of Higher Education Institutes on Environment

EnvironmentAsia 13(3) 2020 70-79
DOI 10.14456/ea.2020.43
ISSN 1906-1714; ONLINE ISSN: 2586-8861

The Natural Lipolytic Yeast *Candida* sp. RMUTSB-27 Isolated from Pineapple for Treatment of Cooking Oil Contaminated Wastewater

Pitchya Tangsombatvichit[1*], Kanjana Pisapak[2], and Paweena Suksaard[1]

[1]*Department of Science (Microbiology), Faculty of Science and Technology Rajamangala University of Technology Suvarnabhumi, Pranakhon Si Ayutthaya, Thailand*
[2]*Department of Science (Chemistry), Faculty of Science and Technology, Rajamangala University of Technology Suvarnabhumi, Pranakhon Si Ayutthaya, Thailand*

*Corresponding Author, E-mail: pitchya.t@rmutsb.ac.th
Received: February 6, 2020; Revised: May 28, 2020; Accepted: June 24, 2020

Abstract

Some yeast species can be applied for treatment of oil-contaminated wastewater. Oil contamination is one of the most serious problems in agro-industrial countries. Fat, oil and grease (FOG) are waste products from household food preparation and industrial processing. The aims of this study are to isolate natural lipolytic yeasts from ripe pineapple and select for high lipase activity on Tween 80 agar. The result showed that yeast isolate No.27 had high lipase activity. It had oval-shaped cells with circular form, and the entire margin of the creamy colored colony had raised elevation and a rough surface. The isolate was identified as *Candida* sp. using the ITS specific sequencing region and named *Candida* sp. strain RMUTSB-27. This isolation grew well on YP medium containing 2% cooking oil waste as well as 2% glucose at 32°C. For oil-contaminated treatment, 2% palm oil waste mixed with wastewater was added *Candida* sp. RMUTSB-27 showed significantly reduction in pH, fat, oil and grease, chemical oxygen demand (COD), and total Kjeldahl nitrogen (TKN) for 4 days. Our results indicated that the yeast *Candida* sp. RMUTSB-27 is beneficial for lipid uptake and degradation of oil-contaminated wastewater and could be utilized for future green environmental treatment.

Keywords: Ripe pineapple; Natural lipolytic yeast; Oil-contaminated wastewater treatment; Yeast *Candida* sp.

1. Introduction

Oil-contaminated wastewater is now considered as the most serious problem in agro-industrial countries. Fat, oil and grease (FOG) are waste products from household preparation and industrial processing activities that impact the environment. Oils and fats are lipids and composed of fatty acids, triacylglycerols, and lipid-soluble hydrocarbons as one of the most important components of FOG (Husain *et al.,* 2014). Oils and fats cause environmental damage to water and aquatic organisms. These substances form an oil layer on the water surface that reduces biological activity and leads to decreased dissolved oxygen levels (Abd El-Gawad, 2014).

For oil-contaminated wastewater treatment, one of the conventional techniques to remove oil and grease is by using oil and grease traps and skimming tanks; however, the disadvantage of this method is the low efficiency of removal. The remaining oil causes clogging of the pipes in treatment units and these require periodical cleaning and replacement, leading to increased maintenance (Husain *et al.,* 2014). An alternative method of oil-contaminated waste management involves using microorganisms that can produce lipase

enzymes and accumulate oils and fats in their cells. Advantages of this method include environmental safety and low-cost in the production stage. Microbial lipase activity can eliminate the wastewater pretreatment process through the production of enzymes from microorganisms. Ibegbulam-Njoku *et al.,* (2014) proposed a new approach to degrade organic matter using a commercial mixture of lipase enzymes to cleanse holding tanks, grease traps, and other systems. *Pseudomonas aeruginosa* T1 isolated from a hot spring in Hokkaido, Japan efficiently degraded different types of fats and oils, including edible oil wastes, with high capacity to utilize free fatty acids. When induced by salad oil and oleic acid, this Gram-negative, rod-shaped bacterium secretes extracellular lipase (Hasanuzzaman *et al.,* 2004).

Yeasts are one of the alternative microorganisms for oil-contaminated wastewater treatment. Different yeast communities have diverse habitats and populations, and changes in their component species should reflect conditions within habitats. *Trichosporon* sp., *Rhodotorula* sp., *Candida* sp. and *Cryptococcus* sp. can be used as indicator fungi to measure water pollution. Yeasts also have effective degradability of some refractory substances and organic poisons (Qadir, 2019). Some yeast strains are used in wastewater treatment because they can uptake lipid, fat, and oil into cells and produce lipase enzymes. The acetyl-CoA metabolism in yeast is highly compartmentalized and occurs in the cytosol, mitochondrion, peroxisome and nucleus. In the peroxisome, acetyl-CoA can then be generated from β-oxidation of fatty acids. Peroxisomal acetyl-CoA can then be used as a substrate in the glyoxylate cycle (Krivoruchko *et al.,* 2015). Some yeasts can convert organic matter into non-toxic and single cell protein, which has high efficiency for wastewater treatment (Yang and Zheng, 2014). Wastewater that has high concentrations of chemical oxygen demand (COD) and biochemical oxygen demand (BOD) causes serious environmental pollution. To solve this problem, yeast treatment technology can be used to quickly reduce the oil content in wastewater from 10,000 mg/L to 100 mg/L as a green alternative to the pretreatment process (Hu *et al.,* 2003). Mafakher *et al.,* (2010) investigated the ability of yeasts *Yarrowia lipolytica* (M1 and M2) produce valuable products such as lipase and citric acid. These yeast strains produced maximum lipase levels of 11 U/mL (*Y. lipolytica* M1) and 8.3 U/mL (*Y. lipolytica* M2), on olive oil fermentation medium, and high levels of citric acid at 27 g/L (*Y. lipolytica* M1) and 8 g/L (*Y. lipolytica* M2), on citric acid fermentation medium. These abilities of yeasts can be used in oil waste treatments in many agro-industries. Moreover, the yeast *Y. lipolytica* is an oleaginous yeast that uses synthetic and food waste-derived volatile fatty acids as substrates (lipid degradation) and can adapt to survive under the high temperature and acidity involved in the biodiesel production process (Gao *et al.,* 2017). *Y. lipolytica* is frequently isolated from different fermented food, soils, sewage, and natural environments. It also reduces organic acid products in olive mills, palm oil mills, and wastewater treatments. These applications involve yeast lipase activity. Production of lipase in *Y. lipolytica* is limited and its genetic modification might not be accepted; however, it can still benefit the environment and waste treatment through many processes and applications (Aloulou *et al.,* 2007).

Nowadays, biological wastewater treatment methods are important for environmental safety. They are better than chemical treatment methods currently used in households and industries. Hence, the objectives of this experiment were to isolate and characterize lipolytic yeasts from ripe pineapple *(Ananas comosus)* fruit that is popular and cheap in Thailand. Yeasts were found from ripe pineapple in the course of natural fermentation. Next, one isolated yeast was selected that exhibited a high level of lipase activity. This isolated yeast was investigated for its cooking oil waste utilization efficiency. Finally, the potent isolated yeast was examined for its capability in oil-contaminated wastewater treatment compared to the well-known oleaginous yeast *Yarrowia lipolytica* TISTR5212.

2. Materials and Methods

2.1 Yeast isolation and lipase activity test

Ripe 'Phulae' pineapple from Ayutthaya Market, Thailand was peeled, cleaned with distilled water and fermented overnight for analysis. 10 g samples were cultured on a liquid medium containing 1% yeast 2% peptone (YP medium) and 2% oleic acid. The medium was shaken at 150 rpm at ambient temperature (32-33°C) for 24 hours. Next, the samples were serially diluted and spread on YP medium plates with 2% oleic acid containing 0.05 mg/mL of chloramphenicol to inhibit bacterial growth (30-300 colonies). The colonies were selected and streaked on YP medium plates with 2% oleic acid for five generations. The method was modified from Tan Gana *et al.,* (2014) by selecting observed colonies on Tween 80 agar containing calcium chloride. Lipase activity in the colonies was compared to that in *Saccharomyces cerevisiae* (non-lipase producing strain). The plates were then incubated at temperature of 32°C for 3 days in triplicate. Lipase activity was indicated by the appearance of a visible precipitate resulting from the deposition of calcium crystal salts formed by fatty acid liberated by extracellular lipase.

2.2 Isolated yeast strain morphology, physiology and sequencing

The yeast strain was selected based on high lipase activity and determined by standard morphological and physiological methods. The strain was analyzed under an optical microscopy (Olympus) to identify the yeast strain colony as described in Tan Gana *et al.* (2014). Then, DNA in the selected yeast strain was extracted using a DNA extraction method modified from Tangsombatvichit *et al.* (2015) and purified. Finally, the internal transcribed spacer (ITS) region was identified using ITS1 5' TCCGTAGGTGAACCTGCGG 3' and ITS4 5' TCCTCCGCTTATTGA TATGC 3' (Macrogen sequencing).

2.3 Spot test of used household oil wastes

S. cerevisiae, isolated *Candida* sp. RMUTSB-27 and *Y. lipolytica* were grown in liquid yeast peptone dextrose (YPD medium) overnight. Cells were spun, suspended in distilled water and diluted to an optical density (OD_{600}) of 0.1. Next, they were serially diluted at 10^4, 10^3, 10^2 and 10^1 cells and spotted on appropriated plates. Finally, growth assays were monitored on oil wastes such as palm oil, lard oil, soybean oil, sunflower seed oil, and rice bran oil compared to the growth on glucose at temperature of 32°C for 4 days. Growth assays were conducted in triplicate (modified from Tangsombatvichit *et al.,* 2015).

2.4 The growth of selected yeast on used palm oil waste

Isolated *Candida* sp. RMUTSB-27 and *Y. lipolytica* were grown in liquid yeast peptone dextrose (YPD medium) overnight. Cells were spun, suspended in distilled water and diluted to an optical density (OD_{600}) of 0.1. Next, the yeast cells were inoculated in YP medium, adding 2% glucose or 2% palm oil waste with shaking at 150 rpm at ambient temperature (32-33°C) for 96 hours. One milliliter of each sample was taken and optical density was measured every 12 hours. All samples were performed in triplicate (Jarboui *et al.,* 2012).

2.5 Utilization of selected yeast for oil contaminated wastewater treatment

Wastewater from the canteen at Rajamangala University of Technology Suvarnabhumi (aerobic wastewater) kept in a refrigerator at 4°C was prepared for the oil wastewater treatment test. Two percent of palm oil waste from household cooking was added and homogenized with the wastewater in 3 L Erlenmeyer flask. Next, the isolated *Candida* sp. RMUTSB-27 and *Y. lipolytica* (lipase producing strain) were grown in liquid yeast peptone dextrose (YPD medium) overnight. Cells were spun, suspended in distilled water and diluted to an optical density (OD_{600}), starting with 0.1. Each yeast

was inoculated in 1.5L of oil wastewater containing 2% palm oil cooking waste, with shaking at 150 rpm at ambient temperature (32-33°C) for 96 hours. All samples were performed in duplicate. Sample parameters as temperature, pH, fat, oil, and grease (mg/L), COD (mg/L) and total Kjeldahl nitrogen (TKN) (mg/L) were investigated following the modified methods from Mueller *et al.* (2003) and Ling *et al.,* (2014), and compared to the sample with no yeast added.

The data were analyzed by one-way ANOVA. Statistical analyses were performed using SPSS version 23.0. The level of significance was set at $p < 0.05$.

3. Results and Discussion

3.1 Yeast isolation and lipase activity test

Yeast strains were isolated from fresh ripe pineapple. A total of 36 yeast isolates were grown on selective YP medium plates with 2% oleic acid. These isolated yeasts were purified and their lipase activities were observed. The isolated yeast No.27 grew on plates containing oleic acid oil at temperature of 32°C as shown in Figure 1 as well as other isolated yeasts (data not shown), while non-lipase producing yeast *S. cerevisiae* grew less on YP medium with 2% oleic acid plates (Figure 1). The isolated yeast No.27 had higher lipase activity than the other isolated yeasts (data not shown) indicated by the appearance of a visible precipitate, while no precipitate appeared in *S. cerevisiae* (Figure 2). Alami *et al.* (2017) reported that when screening lipolytic yeast for lipase production, Candida W3.8 expressed the highest lipolytic potential. Lipase activity was observed by precipitate formation in Tween 80 agar medium. Lipolytic activity is determined based on the precipitation zone formed around colonies. Buzzini and Martini (2002) reported that yeasts isolated from tropical environments showed 61% of positive strains for enzymatic lipase activity such as *Pichia anomala* and *Candida maltosa*. Lipase production and lipid utilization occurred in some yeasts such as *Candida* sp., *Y. lipolytica, Trichosporon,* and *Kluyveromyces lactis*. Purified lipase requires oleic acid for hydrolysis of triglycerides (Vakhlu and Kour, 2006). Eighteen different isolates were found in spoiled milk sweet samples. Lipase producing yeasts and clear zones were observed around two isolates indicating a positive result for screening lipase activity on Tween 80 agar medium (Raj *et al.,* 2016).

3.2 Isolated yeast strain morphology, physiology and sequencing

At this step, the isolated yeast No. 27 strain was focused. Colony morphology of the isolated yeast No.27 had a creamy color with circular form and entire margin. The surface of the colony was rough in appearance, with raised elevation on the medium. Furthermore, isolated yeast No.27 cells were oval shaped under the microscope (400x magnification)

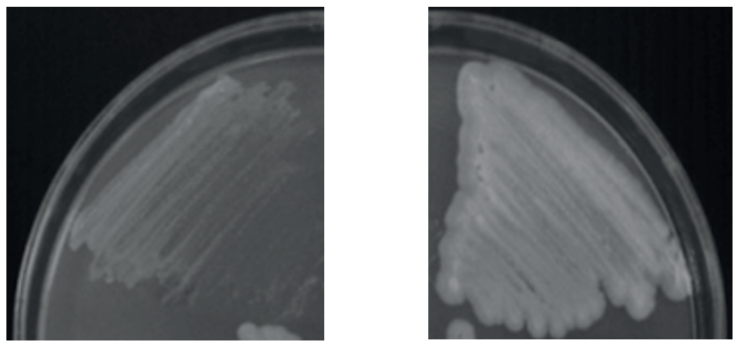

Figure 1. (A) yeast *S. cerevisiae* (non-lipase producing strain) grown on YP medium with 2% oleic acid compared to (B) isolated yeast No.27, incubated at a temperature of 32°C for 3 days, a representative in triplicate.

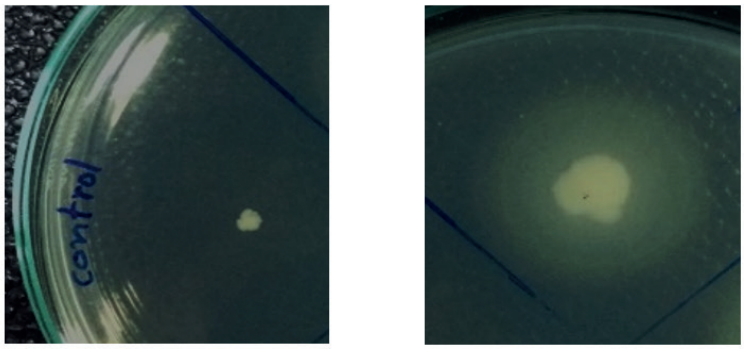

Figure 2. Lipase activity of (A) yeast S. *cerevisiae* (non-lipase producing strain) on tween80 agar test compared to (B) isolated yeast No.27, incubated at a temperature of 32°C for 3 days, a representative in triplicate.

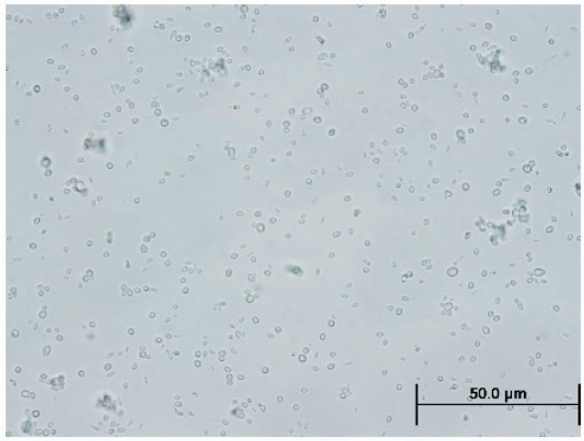

Figure 3. The isolated yeast No.27 morphology under compound light microscopy at 400x magnification.

(Figure 3). Morphology of the two yeast isolates presented in Raj *et al.* (2016) had a smooth surface, while still indicating lipase activity on the selective plate. *Y. lipolytica,* formerly referred to as *Candida lipolytica,* is an aerobic yeast that is found in oil-polluted environments. This yeast can utilize a wide range of carbon sources such as glucose, fatty acids, oils, olive mill wastewater, and organic acids (Gao *et al.,* 2017). The isolated yeast No.27 was molecularly identified using ITS specific sequencing. This indicated that it was the same genus as *Candida* sp. We named it *Candida* sp. RMUTSB-27 (Figure 4).

The phylogenetic tree showed that the yeast *Candida* sp. RMUTSB-27 had a close relationship with *Candida tropicalis* (Figure 4). Jarboui *et al.* (2012) reported that the yeast *Rhodotorula mucilaginosa* isolated from olive mill wastewater was molecularly identified using 18S RNA sequencing. Several yeasts have been proven to be producers of lipase enzymes. *Candida* sp., *Y. lipolytica* and *Pichia* sp. are most commonly used commercially to produce lipase enzymes (Larios, *et al.,* 2004).

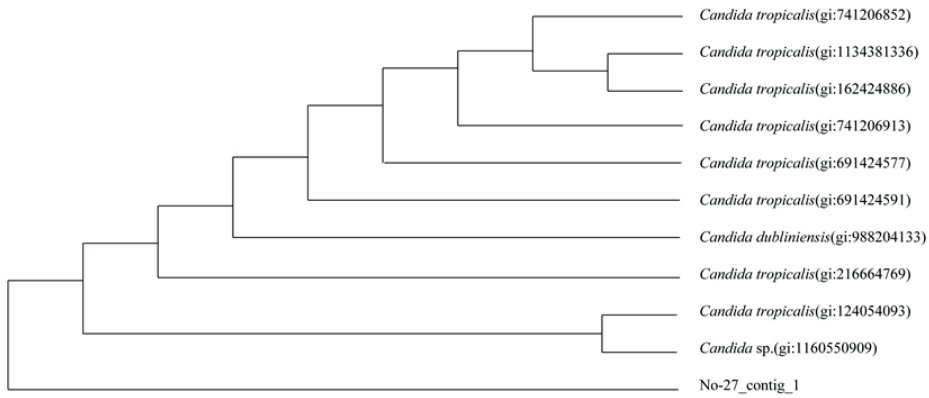

Figure 4. The isolated yeast No.27 identified the phylogenetic tree using ITS specific sequencing as *Candida* sp.

3.3 Spot test of used household oil wastes

The isolated yeast No.27 was observed that it could utilize oil wastes from households. Lipid degradation was observed using the spot test method. The results indicated that the isolated yeast *Candida* sp. RMUTSB-27 could grow on a medium containing 2% of palm oil waste, lard oil waste, soybean oil waste, sunflower seed oil waste, and rice bran oil waste as well as 2% glucose (normal carbon source). Moreover, the isolated yeast Candida sp. RMUTSB-27 grew as well as *Y. lipolytica* (Table 1). In contrast, *S. cerevisiae* grew slightly or no grew on the cooking oil wastes, while it could grow on 2% glucose (Table 1). The oleaginous yeast *Candida parapsilosis* could uptake or synthesize fatty acid involved in *OLE1* that leads to rapid accumulation of lipid droplets in the peroxisome. Deletion of *Candida OLE1 (ole1*Δ/Δ) results in impaired growth on oleic acid in yeast cells compared to that in wild-type cells since the *OLE1* gene is important in the β-oxidation of fatty acid utilization (Nguyen and Nosanchuk, 2011).

3.4 The growth of selected yeast on used oil waste

The isolated yeast *Candida* sp. RMUTSB-27 could grow on 3% waste cooking palm oil at an optical density equal to 2.0 in 36 hours as well as the oleaginous yeast *Y. lipolytica* (Figure 5). However, both yeast strains grew better on 3% waste cooking palm oil than 2% glucose, as shown in Figure 5. Progressive cell growth of yeast *R. mucilaginosa* increased the ability of the yeast to grow on olive mill wastewater as the sole carbon and energy source (Jarboui *et al.,* 2012). Oleaginous yeasts can use lipids as substrates and accumulate lipid droplets in their biomass. These yeasts can also utilize a wide range of fatty acids for growth via a β-oxidation process in the peroxisome (Krivoruchko *et al.,* 2015). Therefore, our result indicated that the natural isolated yeast *Candida* sp. RMUTSB-27 was more beneficial for lipid uptake and synthesis in cells for growth as well as *Y. lipolytica* and other oleaginous yeasts.

3.5 Utilization of selected yeast for oil contaminated wastewater treatment

All used oil-contaminated wastewater treatments indicated similar results at ambient temperature of 33°C (Table 2). The pH of oil wastewater treatment adding *Candida* sp. RMUTSB-27 isolate decreased from 8.6 to 6.9 as well as for *Y. lipolytica* (Table 2), while treatment of only oil-contaminated wastewater showed pH 8.6. Interestingly, our results presented that the isolated yeast *Candida* sp. RMUTSB-27 significantly reduced fat, oil and grease from 9,521.5 ± 1.5 mg/L to 350.0 ± 1.0 mg/L (96.3% reduction), COD from 7,684.0 ± 4.0 mg/L to 3,841.5 ± 1.5 (50.0% reduction),

Table 1. Spot test of isolated yeast *Candida* sp. RMUTSB-27 on the growth of YP medium added 2% glucose (GLU) or YP medium added 2% used oil wastes from household as palm oil (PO), lard oil (LO), soybean oil (SO), sunflower seed oil (SSO), and rice bran oil (RBO) compared with yeast *Saccharomyces cerevisiae* (negative control) or *Yarrowia lipolytica* (positive control), incubated for 4 days, in triplicate.

YEAST STRAINS	2% OF SOURCES TYPES					
	GLU	PO	LO	SO	SSO	RBO
S. cerevisiae	++++	+	-	+	+	+
Candida sp. RMUTSB-27	++++	++++	+++	++++	+++	++++
Y. lipolytica	++++	++++	+++	++++	+++	++++

Remark: – no growth, + slightly growth, +++ moderated growth, ++++ good growth

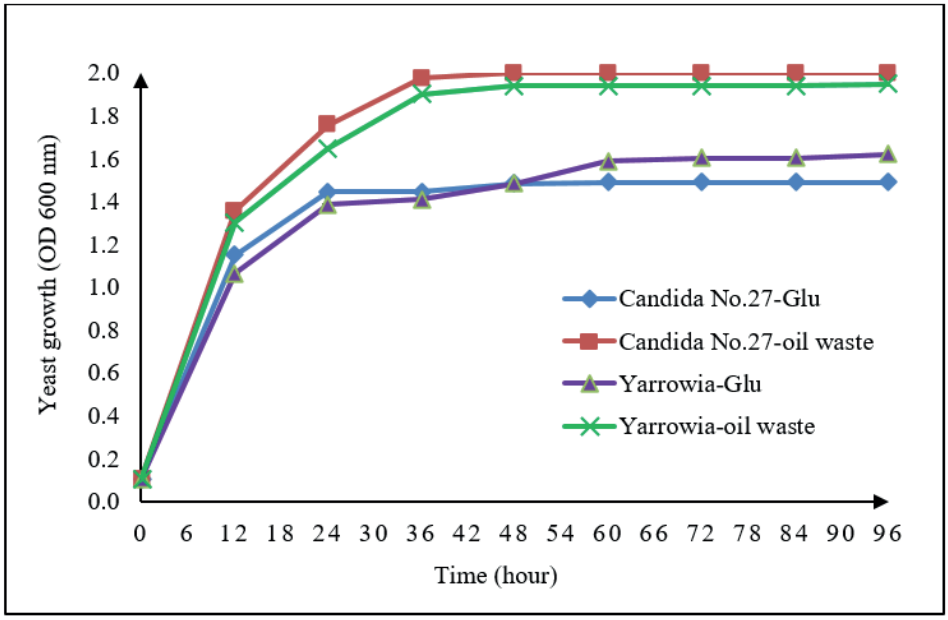

Figure 5. The isolated yeast *Candida* sp. RMUTSB-27 on growth of YP medium added 2% glucose (Glu) or YP medium added 3% waste cooking palm oil compared with yeast *Yarrowia lipolytica* (positive control), in triplicate.

and TKN from 458.5 ± 1.5 mg/L to 134.5 ± 2.5 mg/L (70.7% reduction) compared to treatment of only oil-contaminated wastewater (Table 2). As well *Y. lipolytica* reduced fat, oil and grease from 9,521.5 ± 1.5 mg/L to 450.5 ± 1.5 mg/L (95.3% reduction), COD from 7,684.0 ± 4.0 mg/L to 3,685.0 + 3.0 mg/L (52.0% reduction), and TKN from 458.5 ± 1.5 mg/L to 152.5 ± 1.5 mg/L (66.7% reduction) as shown in Table 2.

The trend for application of yeast in wastewater treatment is widely adapted because the yeast grows fast, has high metabolic efficiency, uses various carbon sources and shows a broad range of potential prospects. Oleaginous yeasts (*Candida, Rhodotorula, Trichosporon, Lipomyces*, and *Yarrowia)* have often been applied in industrial wastewater treatments, domestic sewage and other fields (biodiesel) (Qadir, 2019).

Table 2. Results of oil wastewater treatment using isolated yeast *Candida* sp. RMUTSB-27 compared with *Yarrowia lipolytica.* The wastewater was added 2% palm oil cooking waste from the household. Each parameter was analyzed in duplicate, for 4 days.

CONDITION	CHARACTERISTICS				
	TEMP. (°C)	pH	FAT, OIL AND GREASE (mg/L)	COD (mg/L)	TKN (mg/L)
No yeast	33.0 ± 0.0	8.6^{a} ± 0.0	$9,521.5^{a}$ ± 1.5	$7,684.0^{a}$ ± 4.0	458.5^{a} ± 1.5
Candida sp. RMUTSB-27	33.0 ± 0.0	6.9^{b} ± 0.0	350.0^{c} ± 1.0	$3,841.5^{b}$ ± 1.5	134.5^{b} ± 2.5
Y. lipolytica	33.0 ± 0.0	6.9^{b} ± 0.1	450.5^{b} ± 1.5	$3,685.0^{b}$ ± 3.0	152.5^{b} ± 1.5

^{a,b,c} significant differences 95% Duncan's multiple range test (p<0.05)

Nine mixed yeast strains isolated from industrial wastewater have been used for biological processing treatment of soybean oil wastewater. From an influent concentration of COD, BOD and oil at 39,300 mg/L, 18,200 mg/L and 11,900 mg/L, respectively, the removal efficiencies of COD, BOD and oil-contaminated using the wastewater treatment process of yeasts were all more than 93% (Chigusa *et al.,* 1996). The yeast *R. mucilaginosa* was studied for its ability to degrade phenolic compounds (highly toxic in the environment) and growth on olive mill wastewater. This isolated yeast reduced COD and phenols. At an initial COD concentration of 6,500 mg/L, the yeast strain decreased COD to 4,520 mg/L in 3 days of treatment (Jarboui *et al.,* 2012). Nowadays, technological combinations between microalgae and yeasts are used for mixed wastewater treatment. Microalgae produce oxygen for the yeasts, while yeasts provide carbon dioxide for the microalgae. The yeasts uptake organic matter and the microalgae require nitrogen and phosphorus from the wastewater. These processes greatly improve both lipid production and removal of organic matter and nutrients from the wastewater. The lipid content and yield achieved were 63.45% and 4.60 g/L, while removal rates of COD, total nitrogen (TN), and total phosphorus (TP) were 95.34%, 51.18% and 89.29%, respectively (Ling *et al.,* 2014). Thus, the yeast *Candida* sp. RMUTSB-27 can be combined with microalgae for more efficient oil wastewater treatment in the future.

4. Conclusion

The results from this study showed that the yeast strain isolated from ripe pineapple has the ability to degrade various cooking oil wastes. This yeast was identified as a Candida sp., using ITS specific sequencing. Cell growth was observed on used cooking palm oil, lard oil, soybean oil, sunflower seed oil and rice bran oil from households as the sole carbon source. The isolated yeast strain could be applied in used oil-contaminated wastewater treatment to reduce water pollution for domestic and industrial purposes. Furthermore, this strain could also be applied in lipid production for biodiesel to replace crude oil supplies. Yeast oils have advantages of being non-competitive with human and animal food. They have a short process cycle, and are independent of climatic factors compared to plant and animal oils.

References

Abd El-Gawad HS. Oil and grease removal from industrial wastewater using new utility approach. Advances in Environmental Chemistry 2014: 1-6.

Alami NH, Nasihah L, Umar RLA, Kuswytasari D, Zulaika E and Shovitri M. Lipase production in lipolytic yeast from Wonorejo mangrove area. Proceeding of international biology conference 2016: Biodiversity and Biotechnology for human welfare, AIP Conference Proceedings 1854, 020001 2017; https://doi.org/10.1063/ 1.4985392.

Aloulou A, Puccinelli D, De Caro A, Leblond Y, Carriere F. A comparative study on two fungal lipases from *Thermomyces lanuginosus* and *Yarrowia lipolytica* shows the combined effects of detergents and pH on lipase adsorption and activity. Biochimica et Biophysica Acta 2007; 1771 (12): 1446–1456.

Buzzini P, Martini A. Extracellular enzymatic activity profiles in yeast and yeast-like strains isolated from tropical environments. Journal of Applied Microbiology 2002; 93 (6): 1020-1025.

Chigusa K, Hasegawa T, Yamamoto N, Watanabe Y. Treatment of wastewater from oil manufacturing plant by yeasts. Water Science and Technology 1996; 34 (11): 51-58.

Gao R, Li Z, Zhou X, Cheng S, Zheng L. Oleaginous yeast *Yarrowia lipolytica* culture with synthetic and food waste derived volatile fatty acids for lipid production. Biotechnology for Biofuels 2017; 10: 247-261.

Hasanuzzaman M, Umadhay-Briones KM, Zsiros SM, Morita N, Nodasaka Y, Yumoto I, Okuyama H. Isolation, identification, and characterization of a novel, oil-degrading bacterium, *Pseudomonas aeruginosa* T1. Current microbiology 2004; 49 (2): 108-114.

Hu JY, Ong SL, Ng WJ, Lu F, Fan XJ. A new method for characterizing denitrifying phosphorus removal bacteria by using three different types of electron acceptors. Water Research 2003; 37 (14): 3463-3471.

Husain I, Alkhatib M, Jami MS, Mirghani ME, Bin Zainudin Z, Hoda A. Problems, control, and treatment of fat, oil, and grease (FOG): A review. Journal of Oleo Science 2014; 63 (8): 747-752.

Ibegbulam-Njoku PN, Achi OK, Chijioke-Osuji CC. Use of palm oil mill effluent as fermentative medium by lipase producing. International Journal of Scientific and Engineering Research 2014; 5 (2): 1631-1640.

Jarboui R, Baati H, Fetoui F, Gargouri A, Gharsallah N, Ammar E. Yeast performance in wastewater treatment: case study of *Rhodotorula mucilaginosa.* Environmental Technology 2012; 33 (7-9): 951-960.

Krivoruchko A, Zhang Y, Siewers V, Chen Y, Nielsen J. Microbial acetyl-CoA metabolism and metabolic engineering. Metabolic Engineering 2015; 25: 28-42.

Larios A, Garcia HS, Oliart RM, Valerio-Alfaro G. Synthesis of flavor and fragrance esters using *Candida Antarctica* lipase. Applied Microbiology and Biotechnology 2004; 65 (4): 373-376.

Ling JY, Nip S, Cheok WL, de Toledo RA, Shim H. Lipid production by a mixed culture of oleaginous yeast and microalga from distillery and domestic mixed wastewater. Bioresource Technology 2014; 173: 132-139.

Mafakher L, Mirbafheri M, Darvishi F, Nahvi I, Zarkesh-Esfahani H, Emtiazi G. Isolation of lipase and citric acid producing yeasts from agro-industrial wastewater. New Biotechnology 2010; 27 (4): 337-340.

Mueller SA, Kim BR, Anderson JE, Gaslightwala A, Szafranski MJ, Gaines WA. Removal of oil and grease and chemical oxygen demand from oily automotive wastewater by adsorption after chemical de-emulsification. Practice Periodical of Hazardous Toxic and Radioactive Waste Management 2003; 7 (3): 156-162.

Nguyen LN, Nosanchuk JD. Lipid droplet formation protects against gluco/lipotoxicity in *Candida parapsilosis*: An essential role of fatty acid desaturase Ole1. Cell cycle 2011; 10 (18): 3159-3167.

Qadir G. Yeast a magical microorganism in the wastewater treatment. Journal of Pharmacognosy and Phytochemistry 2019; 8 (4): 1498-1500.

Raj A, Baby G, Dutta S, Sarkar A, Rao B. Isolation, characterization and antioxidant activity of lipase enzyme producing yeasts isolated from spoiled sweet sample. Scholars Research Library Der Pharmacia Lettre 2016; 8 (10): 129-135.

Tan Gana NH, Mendoza BC, Monsalud RG. Isolation, screening and characterization of yeasts with amylolytic, lipolytic, and proteolytic activities from the surface of philippine bananas (*Musa* spp.). Philippine Journal of Science 2014; 143: 81-87.

Tangsombatvichit P, Semkiv MV, Sibirny AA, Jensen LT, Ratanakhanokchai K, Soontorngun N. Zinc cluster protein Znf1, a novel transcription factor of non-fermentative metabolism in *Saccharomyces cerevisiae*. FEMS Yeast Research 2015; 15 (2): 1-16.

Vakhlu J, Kour A. Yeast lipases: enzyme purification, biochemical properties and gene cloning. Electronic Journal of Biotechnology 2006; 9 (1): 69-85.

Yang M, Zheng SK. Pollutant removal-oriented yeast biomass production from high-organic-strength industrial wastewater: A review. Biomass and Bioenergy 2014; 64: 356-362.