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Powerful Lipolytic Activity of Fungi Isolated from Coconut and Avocado Flesh on Different pH and Temperature

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Abstract: Lipase is widely applied in various fields of industries elevating its economic value. The demand for lipase keeps increasing open up working opportunities. Isolates showing lipolytic activity obtained previously are tested for their activity on different pH mediums: 7, 8, 9, and 10; and at different temperatures. The fungi are grown on SDA medium supplemented with Olive Oil, emulsifier, Tween 80, and Rhodamine B. The experiment was made in 3 replicates and is incubated at room temperature of 30°C. Lipase activity was calculated based on the clear zone around colonies observed on day 2 and day 3 after inoculation. The result shows that those isolates are highly active on various pH at RT, and the activity slightly reduces at 30°C. This result suggests that a wide range of their applications are at room temperature when pH is a limiting factor for the applications.

Keywords: Lipase Activity; Coconut; Avocado; pH; Temperature

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Introduction

Biocatalysts (enzymes) are replacing chemical catalysts in various commercial industries as increase human awareness of the environment (Kumari et al, 2019). Enzymatic reactions have several benefits in comparison to a chemical reactions. This reaction does not need high temperature, high pressure, or high energy. The reaction can be very specific, unnecessary products can be avoided, therefore no further processes are needed for the product. The enzyme can be immobilized spanning the use several times. The enzyme also can be used to remove waste to save the environment while itself is degradable (Pratush, et. al., 2013) as well as no harmful residue is produced (Hasan et al, 2010).

Lipases belong to the hydrolase group EC 3.1.1.3. plays a role in breaking down triglyceride fats into glycerol and fatty acids. Various bioconversion capabilities at extreme conditions such as high temperatures and pH, in limited water and in non-polar solvents (Patel, et al, 1996) lipase is a very applicable enzyme in various commercials industrial fields, including food, drink, medicine, health, petroleum, agriculture and many others (Andualema and Gessesse, 2012).

Lipase can also be used for environmental bioremediation in oil spill areas and household, hotel, restaurant waste, to remediate used cooking oil (Okino-Delgado, et al., 2017). The latter is certainly very beneficial for traders who use cooking oil a lot, besides being cost-effective, it is also healthy for their customers. Moreover, lipase is used as a biosurfactant that can be added to various household cleaning gents (Hisham, et al., 2019). Enzyme-based detergent has become a positive trend that is environmentally friendly (Olsen and Fahlot, 1988).

For industrial purposes, lipases are generally produced from microbes for several reasons: the cheap and abundance of a substrate, the stability, the easiness to scale up production, shortness of time production, and the independence of season; they all make lipase

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production costs low (Guerrand, 2017; Pratush, et al., 2013; Wiseman, 1995).

Enzyme demand in Indonesia is enormous, especially for food processing, beverages, leather tanning, paper factories, and other industries. So far, 99% of demand is fulfilled by imported enzymes. In the year 2015 only imported enzyme was about 2.5 tons and expensed more than Rp 187 M (BPPT, 2015), disregarding the presence of the production within. Even though the imported enzyme is very expensive, however, it is still a better choice as it has higher activity than the domestic one. The expense for the enzyme to produce Bio-ethanol, for example, it reaches 30% of the total production cost (Bušić, et al., 2018).

Lipase production can be increased in several ways, including the selection of the right substrate, suitable environmental conditions (pH, temperature), the type and concentration of nitrogen, and the addition of sugar with a certain concentration. The addition of Fe2+, Mg2+, Triton X100, Tween esters increases lipase production (Kumar, et al 2012). Each microbe, however, has its own needs and characteristics that should be understood to predict how/what situations enzymes can be produced using certain microbe and may describe the flexibility of its application fields. Temperature and pH are very important factors for growth, enzyme production, and enzyme activity. Enzymes purified from microbes often lose activity under alkaline conditions, the presence of surfactants, and high temperatures (Kumari, et al., 2019).

The increasing of lipase application, the characteristic of lipase that meets the need for the satisfactory operating system is a limiting factor, therefore the interest in finding new lipases that suit the application is still increasing (Gupta, et al., 2004).

Two pure fungal isolates recently isolated from coconut and avocado flesh show the lipolytic activity on screening medium. This report discusses their activity in a range of pH and temperature of the fungi.

Method

Isolates used were fungi recently isolated from coconut flesh and avocado flesh which showed lipolytic activity. The isolates were rejuvenated on a PDA medium before use.

The medium used for the experiment was SDA medium with the following addition: 1% olive oil as a carbon source and stimulant, 1% emulsifier and Tween 80 0.05% so that the oil is evenly mixed and Rhodamine B as an indicator, 0.2% agar as solidifier and pH adjusted to 7, 8, 9 and 10. The medium was autoclaved for 15 minutes at 121^{o}C and 1 ATM pressure. After the medium reached about 60^{o}C, the medium was poured into sterile petri dishes, about 25 mL medium per petri dish.

Fungal inoculation was performed using a sterile toothpick to transfer as little mycelium as possible and inoculated right in the middle of the medium. Experiments were made in triplicates and incubated at room temperature. The same experimental set was incubated at 30^{o}C. Observations were made on the 2nd and 3rd days after inoculation. The clear zone formed around the colony indicated the presence of lipolytic activity.

Calculation of activity in a petri dish was carried out by measuring the diameter of the clear zone minus the colony diameter then divided by the colony diameter (Kouker and Jeager, 1997). Data obtained was analyzed using Two Way ANOVA Test (SPSS).

Result and Discussion

The appearance of the clear zone on a petri dish is presented in Figure 1 and the amount of activity is presented in Figure 2. Figure 1 shows the clear zone of the isolate at different pH and different temperatures. The control medium shows a darker color (opaque) which occurs due to a mixture of oil and the components of the medium. The inoculated medium showed a bright zone as a result of which the oil had been degraded by the isolate, which also meant that the isolate was active in producing lipase.

The observation picture on day 2 is brighter than day 3 and confirmed by numerical data (not shown) and the activity shown in Figure. This observation seems to be false data, that can be explained as follows: the colony diameter increased and covered part of the clear zone where the oil had been previously degraded, while the medium area on petri dish is fixed, so there is no more room to show further activity resulting false observation. The observations on day 3, however, provide information that for further research, data recording should be carried out on day 2. Observations on the first day of data are not recorded because the growth was still very small and the clear zone has not been seen.

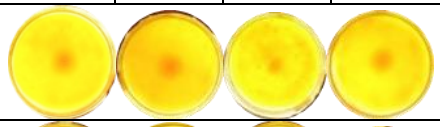
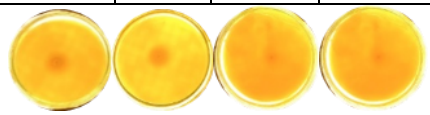
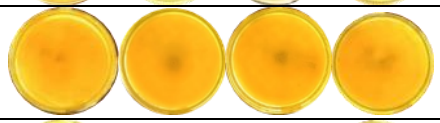
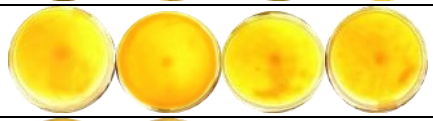
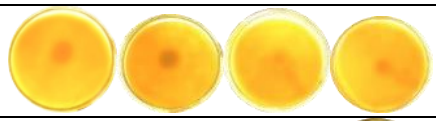
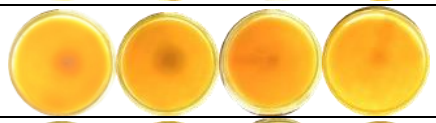
Isolate	Culture age	pH/RT				pH/30°C			
		7	8	9	10	7	8	9	10
CI	2								
	3								
AI	2								
	3								
CM									

Figure 1. The clear zone formed due to lipolytic activity by CI and AI isolates (shown 1 of three replicates).
Note: CI = Coconut Isolate, AI = Avocado Isolate, CM = Control Medium

The lipolytic activity of the isolates is presented in Figure 2. The lipolytic activity at room temperature ranged from 284 to 400%, while at 30°C it ranged from 151 to 245% multiples of the colony diameter. This difference is statistically significant p<0.00. Meanwhile, the differences in the lipolytic activity of both isolates in the medium with different pH were also significantly different statistically, p<0.009. However, further tests using LSD show that not all treatments are different one from another activities in the medium with a pH range of 7-10, this indicates that isolates are more flexible to be applied in the normal to high pH range. It can be seen on the petri dish that on day 2, the entire diameter of the petri has become a clear zone.

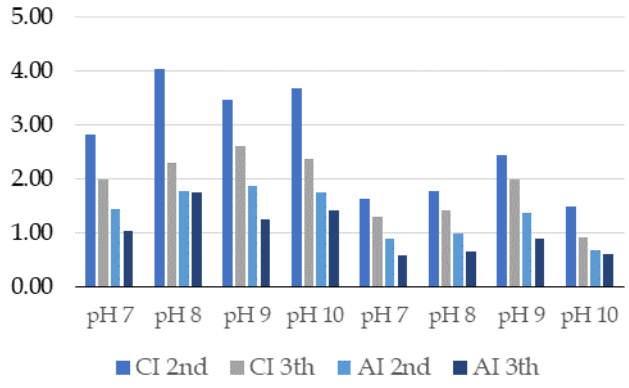


Figure 2. Lipolytic activity of CI and AI at room temperature (left) and 30°C (right) observed on the 2nd and 3rd days. CI = isolate from Coconut; AI = Isolate from Avocado. The quantities indicated are multiples of 100% of the colony diameter.

If it is assumed that the olive oil has run out within 2 days, it can be roughly estimated that for 1% olive oil in the medium, the content of the petri is about 25 ml, then the available oil is 250 micro liters/petri, which after calculating the fungal activity of 1 colony within each petri is 334 nM/min. Again this is a rough calculation adopted by the calculation method by (Kouker and Jeger, 1987). The right observation time to see the right time to get a complete clear zone in petri dishes needs to be done to get more accurate data, as well as the amount of medium that is poured into each petri dish. This simple method can be used for isolation and at the same time observing its activity, for data verification, it is necessary to carry out further testing by titration or spectrophotometry or more advanced methods such as GCMS. This data, however, show the very active isolat on such a high pH.

Statistically, there was nearly no significant difference between CI and AI isolates, p=0.0484. This indicates that both isolates have the same potential to be used as source organisms to produce lipase which can be carried out at normal to high pH conditions and incubated at room temperature for the best, but at 30°C the isolate still shows any activity. Further characterization of the lipases of the two isolates needs to be done such as stability and other characteristics.

For industrial applications (Essamri, et al., 1998), lipase from Candida and Aspergillus sp (Savitha and Ratledge, 1991) and Geotrichum sp. (Ginalska, et al., 2004) are particularly important. The isolate has not been identified yet, however, the activity may have possible applications for alkaline conditions for

example on detergent and its derivatives. It may be used such as soaking before washing or adding to powdered soap (Treves, et al., 1984), and in automatic dishwasher machines, lipase removes fatty residues and cleans clogged drains (Vulfson, 1994). Enzyme-based detergents are becoming a trend lately that deserves to be followed. In Europe, for an instant, detergent industries are the largest consumer of enzymes production (Hasan, et al, 2010) along with the increasing concern of the society toward the green environment. Some of the advantages of using an enzyme-based detergent include increasing the level of cleanliness of laundry, shortening washing time, lowering the washing temperature, reducing the use of chemicals, reducing the use of water all of which save energy, and is environmentally friendly (Olsen and Falhot, 1998). Enzymes purified from microbes often lose activity under alkaline conditions, the presence of surfactants, and high temperatures (Kumari, et al., 2019). Lipase isolates from coconut flesh and from avocados still have high activity at pH 10, maybe even more than 10 (needs to be roved), nevertheless detergent working at low temperature is beneficial for energy and environment and being sought of. Therefore, both isolates are potentially a good source for lipase production. Other applications are of course adapted to the needs, seeing that the lipases of the two isolates are quite flexible with the physical environmental conditions. Lipase flexibility to the chemical environment needs to be investigate.

Conclusion

Lipase of fungi isolated from Coconut and Avokado Flesh are highly active on pH normal to high alkaline (pH 10), best on room temperature, slightly decline on 30^{o}C.

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