POTENTIAL STRAINS OF THERMOPHILIC AND ORGANIC SOLVENT TOLERANT BACTERIA FOR LIPASE PRODUCTION USING BASAL MEDIUM OF PALM KERNEL CAKE

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ABSTRACT

Palm Kernel Cake (PKC) consists many nutrients that are useful to be applied in the industry and directed studies towards utilization of this substrate in order to produce enzymes like lipases. This enzyme can be applied in various type of industry including pharmaceutical industries, food industries, cosmetics industries and others. Using palm kernel cake (PKC) as source of thermophilic bacteria, 53 bacterial strains were found survived at temperature 65^0C. However, after restreak several times, only 17 strains were found as pure thermophilic strains. Preliminary screening both qualitative and quantitative was performed to all 17 potential thermophilic bacterial strains and showed that only 11 purified thermophilic strains are lipase producer. Strain PKC-P1 produced highest enzyme activity (11.13 U/g). Thermostable lipase purified from thermophilic bacteria strain was stable at temperature 65^0C and pH 8.

Keywords: Lipase, organic solvent tolerant bacteria, palm kernel cake (PKC), thermophilic bacteria, thermostable lipase.

INTRODUCTION

Enzymes extracted from different sources; microbes, vegetables or animal organ have been used for ages in variety forms. A large number of enzymes are being produced and sold for various purposes and their production are getting more attention in life science industry sector. Nowadays, enzymes being used in multiple areas like food, feed, detergent, tanning, textiles, laundry, fine-chemicals, cosmetics and pharmaceuticals industries and classified based on specific applications. These industrial applications view as over 80% of the global market of enzymes [1]. More than 500 industrial products are produced by using enzymes [2, 3]. The interests for this industrial enzyme has increased constantly driven by a developing requirement for sustainable solutions. Microbes have served and continue to serve as one of the largest and beneficial sources of many enzymes[4].

Lipase can be defined as carboxylesterases that catalyze hydrolysis and synthesis of long chain acylglycerols each. They are able to be produced by various microorganisms include fungi, bacteria, archea, eucarya, plants and animals [5] and can be applied in various type of industry including pharmaceutical industries, food industries, cosmetics industries and others. Industrial lipases mainly used as additives to washing detergents as well as in the food industry and also for transesterification reaction of triglycerides [6]. Besides that, lipases also have been used in biodegradation of plastics like polyhydroxyalkanoates (PHA) and polycaprolactone (PCL) [7].

Organic solvent tolerant bacteria are type of microorganisms that can survive in the existence of very high concentrations of organic solvents [8]. Their potential in industrial and environmental biotechnology have been studied as their enzymes retain activity in the presence of very high concentrations of organic solvents [9]. PKC is a solid wastes obtained from palm kernel seeds after process of oil extraction [10]. PKC consists many nutrients that make it able to be applied in the industry and directed studies towards utilization of this substrate in order to produce enzymes (for this research,lipase). PKC have been selected for microbial fermentation as it contains rich source of proteins as well as minerals [7]. There are many reports on the production, purification and characterization of microbial lipases with single property of thermostable or organic solvent tolerant which limit the complete reactions in synthesis of biodiesel or biochemicals. However, due to high cost of production of this enzyme, it becomes major limitation to be applied in large-scale industrial. In order to overcome this problem, the use of different microorganisms, supplements and substrates can contribute in getting the best combination to produce high value of lipases, using substrates and conditions that reduce the costs in industrial scale. The use of cheap raw materials would diminish the operating costs of the process [11].

This research involved the exploration of renewable PKC as potential fermentation medium and establishing the necessary process conditions in a solid state fermentation in the lab scale for discovery to novel TS-OST lipase that would have excellent tolerance and activity in presence of organic solvents with high temperatures for industrial applications. The work will be conducted to evaluate the potential bacterial strains from palm kernel cake (PKC) with the properties of thermostable and organic solvent (TS-OST) lipase in PKC based medium.

METHODOLOGY

Sample collection

Palm kernel cake (PKC) sample was collected from West Mill Sdn Bhd, Sime Darby Research Centre and compost sample was collected from laboratory of International Islamic University Malaysia (IIUM), Gombak, Malaysia.

Selection and purification of thermophilic strains

Approximately 0.1 g of the sample, PKC and compost were cultivated in 50 ml Luria-Bertani (LB) broth each, pH 7.0. Cultures were incubated at 65^{o}C, 150 rpm for 48 hours. In order to isolate bacteria with organic solvent properties, about 10% ethanol was added in the liquid media during cultivation. Sample of cultures were spread on the LB agar plate and incubated at 65^{o}C for 48 hours to identify thermophilic strains.

Screening of lipase producing bacteria

The screening process was completed by using two types of test: phenol red agar plate and tween 80 agar plate method.

Phenol red agar plate

Phenol red (0.01% w/v) along with lipidic substrate (olive oil) 1% (v/v), $CaCl_2$ 0.1% (w/v) and agar 2% (w/v) were used in order to prepare phenol red agar plate. 0.1 M NaOH was used to adjust pH to pH 7.3–7.4. Pure thermophilic strains got from above step were streaked onto the prepared medium and incubated at room temperature for 48 hours. A change in color of phenol red is used as indicator of the enzyme activity.

Tween 80 agar plate

In order to prepare tween 80 agar plate, peptone 1% (w/v), NaCl (0.5% w/v), $CaCl_2.2H_2O$ (0.01% w/v) and agar (2% w/v) were autoclaved first for 20 minutes. Noted that, tween-80 (1% v/v) was separately sterilized and added into the autoclaved medium, then the mixture was adjusted to obtain pH 6.0. Pure thermophilic strains were streaked onto the prepared medium and incubated at room temperature for 48 hours. Lipolytic activity was detected due to the the appearance of visible precipitate.

Inoculum preparation

Single colony for each lipase producing bacterial colonies were transferred from LB agar plate into 20 ml sterile LB broth medium with pH 7 in 50 ml Erlenmeyer flask and incubated at 65^{o}C, 150 rpm for 18 hours.

Fermentation process

Palm kernel cake was used as the basal medium. 6 g of PKC (moisture content of 70%) was added in the Erlenmeyer flask. 1 M NaOH was used in order to adjust the initial pH to pH 7 before being sterilized at 121°C and 15 psi for 20 min. Four percent (4%) of the inoculum was added into the flask and incubated at 65 ^{o}C for 48 hours.

Enzyme extraction

Extraction method was conducted on the crude enzyme resulted from the fermented material. 50 ml of distilled water was mixed into the fermented substrate and the content was agitated at 150 rpm for two hours at the room temperature. The fermented media then was centrifuged at 8000rpm, 4^{O}C for 20 minutes. Supernatant was collected and used as a lipase assays and biochemical characterization studies.

Lipase assay

A solution of 2.5mmol of p-NPP was prepared in a Tris-HCl buffer. 2.4mL of this solution was added to 0.02mL of crude lipase solution and was incubated at 65 ^{o}C for 15 minutes and cooled for 5 minutes [13] . Absorbance at 410 nm for the solution was recoreded. This is experiment was conducted in triplicate.

RESULTS

Screening of thermophilic bacterial strains

A total of all 53 thermophilic strains survived at temperature 65^{o}C and only 17 strains were identified as pure thermophilic strains; 6 strains from palm kernel cake and 11 strains from compost source as shown in Table 1. No growth was observed above 70^{o}C during isolation of the selected media.

Table 1. Purified thermophilic strains

Source	Strain code
Palm kernel cake	PKC-P13, PKC-P12, PKC-P10, PKC-P52, PKC-P53, PKC-P1.
Compost	PKC-C4, PKC-C6, PKC-C7, PKC-C8, PKC-C9, PKC-C10, PKC-C11, PKC-C12, PKC-C14, PKC-C15, PKC-C16.

Phenol red and Tween-80 agar plate

From the 17 plates, it was observed that only eleven phenol red plates showed changing of color (PKC-C4, PKC-C6, PKC-C7, PKC-C8, PKC-C9, PKC-C10, PKC-C11, PKC-C12, PKC-C16, PKC-P1 and PKC-P13). For tween-80 agar plate test, white precipitate have been observed only on five plates (PKC-C7, PKC-C8, PKC-C12, PKC-C16, and PKC-P13). Table 2 shows the observation of all seventeen bacteria strains on phenol red and tween-80 agar plate test.

Table 2. Qualitative test of lipase producing bacteria

Strain Code	Qualitative test for lipase	
	Phenol red agar plate	Tween-80 agar plate
PKC-P1	Completely change from pink to yellow	No changing occured. This strain give no reaction on this plate
PKC-C4	Slightly change from pink to orange	White precipitate formed
PKC-C6	Completely change from pink to yellow	No changing occured. This strain give no reaction on this plate.
PKC-C7	Completely change from pink to yellow	White precipitate formed
PKC-C8	Slightly change from pink to orange	White precipitate formed
PKC-C9	Slightly change from pink to orange	No changing occured. This strain give no reaction on this plate.
PKC-C10	Slightly change from pink to orange	No changing occured. This strain give no reaction on this plate.
PKC-C11	Change to yellowish-orange	No changing occured. This strain give no reaction on this plate.
PKC-C12	Completely change from pink to yellow	White precipitate formed
PKC-C16	Completely change from pink to yellow	White precipitate formed

PKC-P13	Slightly change from pink to orange	White precipitate formed
PKC-P12	No changing occured.	No changing occured.
PKC-P10	No changing occured.	No changing occured.
PKC-P52	No changing occured.	No changing occured.
PKC-P53	No changing occured.	No changing occured.
PKC-C14	No changing occured.	No changing occured.
PKC-C15	No changing occured.	No changing occured.

Quantitative test of lipase producing bacteria

Enzyme activity of lipase produced by all eleven strains were observed through p-nitrophenylpalmitate (pNPP) assay. From Table 3, it showed that strain PKC-P1 produced highest enzyme activity (11.13 U/g), followed by PKC-P13 and PKC-C9. The lowest enzyme activity was lipase produced by PKC-C10 (0.76Unit/g).

Table 3. Enzyme activity (Unit/g) of lipase produced

Strain Code	Enzyme activity (Unit/g of PKC)
PKC-P13	10.78
PKC-C16	4.45
PKC-C4	4.83
PKC-P1	11.13
PKC-C11	4.14
PKC-C6	7.20
PKC-C10	0.76
PKC-C9	10.20
PKC-C12	9.40
PKC-C7	8.04
PKC-C8	2.67

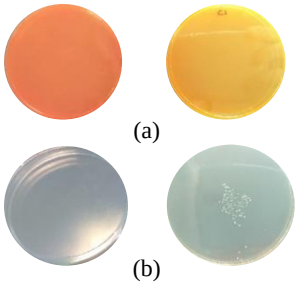
DISCUSSION

Screening of thermophilic bacterial strains

Various types of bacterial strains appeared on the plate considered as thermophilic strains as they can survive at temperature 65^{o}C. All this bacteria strains can grow at high temperature. Besides that, they are also organic solvent tolerant bacteria as they can survive with present of 10% ethanol during cultivation process. All this thermophilic strains was resteaked several times until single colony appeared on the plate. Single colony appeared considered as pure thermophilic bacterial strain.

Phenol red and Tween-80 agar plate

This test was conducted to identify lipase positive strains on phenol red agar plate. The changing of color for phenol red agar plate from pink to yellow shows that pH decreases due to release of fatty acids on lipolysis as shown in Figure 1(a). This situation happened when the strain has potential as lipase producer. Completely change of phenol red agar plate from pink to yellow indicates that strains produced large amount of lipase while slightly change from pink to orange indicates small amount of lipase produced. Besides that, formation of white precipitate is caused by the deposition of insoluble calcium crystal salt formed by the liberation of the fatty acid as the bacteria grow on the Tween-80 agar plates as shown Figure 1(b).



(a)

(b)

Figure 1. (a) Phenol red agar plate test (b) Tween-80 agar plate test

Quantitative test of lipase producing bacteria

This method considered as quantitative screening of lipase producing bacteria. Lipase activity of the isolated bacteria was further tested by solid state fermentation process. Since agricultural waste like PKC is claimed contain substantial amount of fatty acids and nutrients that are able to act as inducers for more economical lipase production [14], this waste was used as a medium for fermentation process. Strain PKC-P1 produced highest enzyme activity of 11.13 U/g shows that this strain has potential as a source of more economical enzyme for biotechnological industry.

CONCLUSION

In this study, purified thermophilic and organic solvent tolerant bacteria was obtained by isolation of bacteria from palm kernel cake and compost source. Thermostable and organic solvent tolerant lipase from both sources was obtained. The

finding showed that the strain PKC-P1 produced highest lipase activity (11.13 U/g). This finding revealed and justifying that local bacterial strains isolated from oil-rich environment has potential as a source of more economical enzyme to be applied in biotechnological industry. Lipases getting more attention for different industrial applications. This current study reports the production of lipases having both characteristics: thermostable and organic solvent tolerant by locally isolated bacteria from palm kernel cake and food compost source. These two characteristics of this enzyme will make it more beneficial and can be applied for many industrial purpose.

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