Biodegradation of Palm Oil by Bacterial Cultures

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Abstract

Soil samples collected from oil-contaminated soil were enriched with palm oil and then cultured and isolated into 34 pure bacterial cultures. They were inoculated on tributyrin agar plates for lipid test and incubated at 37°C for 48 hours. There were 8 bacterial isolates having clear zones surrounding the colony. They investigated the degrading efficiencies of the palm oil in a basal mineral medium (BMM) and synthetic wastewater (SWW) at high lipid concentrations (10% v/v). All isolates were able to degrade palm oil at high concentrations (10% v/v) in both media. In BMM with palm oil, isolates C, D, and B had lipid degrading efficiencies at high levels (76.84%, 73.16%, and 63.68%, respectively). In SWW with palm oil, isolate B had the highest degrading efficiency (76.85%), but the degrading efficiencies of isolates C and D decreased to 47% and 31%, respectively. The efficiency of isolate B was high level in BMM and SWW. Furthermore, the size and clarity of the clear zone on tributyrin agar were related to the lipid degrading efficiency of bacterial strains.

Keywords**:** *Biodegradation, Palm Oil, Wastewater, Bacterial Culture*

1. Introduction

Wastewater containing high amounts of fat and oil is released from the food industry, restaurants, and kitchens, and they are difficult to remove and degrade. In addition, lipids in wastewater are induced in the pipe of the wastewater treatment system. It causes many problems in wastewater plants, such as physical blockage in pumps and filters, the emergence of unpleasant odours, and a reduction in the degrading efficiency of wastewater treatment. (Cammarota & Freire, 2006, Manh, 2008, Phong et al., 2014). Various microorganisms were found to degrade lipids, such as Acinetobacter sp., Aeromonas sp., Alcaligenes sp., Bacillus sp., Burkholderia sp., Enterococcus sp., Pseudomonas sp., Proteus sp., Rhodococcus sp., Staphylococcus sp., and Streptococcus *sp*. They were investigated to be applied in forms of inoculum and extracted enzyme (Ali, 2014, Cammarota & Freire, 2006, Loperena et al, 2009, Muraoka et al, 2008, Phong et al, 2014). Microorganisms produce lipase to catalyze lipids and they are broken down into glycerol and fatty acids (Ali, 2014, Phong et al, 2014).

Muraoka et al, 2008 investigated the ability of the bacteria strain Y1 to degrade salad oil. They reported that the lipid degradation rates at 1, 5, and 10% (w/v) were 83.1%, 30.2%, and 15.4%, respectively. Similar to other studies, most microbes had low lipid degrading ability, especially to eliminate high lipids in wastewater. Microbial strains have been investigated to degrade lipids in wastewater treatment.

2. Objectives

The aims of his study were to (1) isolate pure bacterial cultures from soil enriched with palm oil, (2) identify lipid-degrading bacteria using the tributyrin (TBT) test, and (3) investigate the palm oil degrading efficiency of the bacterial cultures in basal mineral medium (BMM) and synthetic wastewater (SWW), at high lipid concentrations (10% v/v).

3. Materials and Methods

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Enrichment of lipid-degrading bacteria in soil

Soil samples were collected from oil-contaminated soil near the canteen site. The enrichment of lipid degrading bacteria was done as follows: 500 g-soil sample added 3.0 ml-palm oil was incubated in a beaker. An additional amount of 3.0 ml-palm oil was applied to the beaker every week. After 2 months of incubation, a 10 g soil sample was transferred to 100 ml of sterile BMM containing 10 ml palm oil in a 250 ml Erlenmeyer flask. The flasks were shaken at room temperature.

Once every two weeks, 10 ml samples of each flask were transferred to fresh media similar to those described above. After three consecutive transfers, mixed bacterial cultures were collected and isolated using the streak plate technique to become pure bacterial cultures.

Screening tests for lipid-degrading bacteria

All pure bacterial cultures isolated from the soil were streaked on tributyrin (TBT) agar plates and incubated at 37°C for 48 hours. Tributyrin oil forms an opaque suspension in the agar. If a bacterial culture produces lipase and breaks down tributyrin, a clear halo surrounds its colony (called a clear zone). Bacterial cultures occurring in clear zones were investigated for their efficiencies in palm oil degradation.

Efficiencies of palm oil degrading pure cultures

The efficiencies of palm oil degradation in the pure cultures were investigated in both basal mineral medium (BMM) with palm oil and synthetic waste water (SWW) with palm oil. Basal mineral medium (BMM) contained the following ingredients (in grams per liter): K_2HPO_4, 4.8; KH_2PO_4, 1.2; NH_4NO_3, 1.0; $MgSO_4{,}7H_2O$ 0.25; $CaCl_2$, 0.04; and $FeSO_4.7H_2O$, 0.005 (Keprasertsup, 2001). Synthetic wastewater (SWW) is composed of tapioca flour, 2.5; milk powder 2.5; and sugar, 2.5 (in grams per litre) (Chitpirom & Sangaroon, 2012). Both BMM 8 ml and SWW 8 ml, added with palm oil 1 ml, were sterilized. All pure bacterial cultures were inoculated into nutrient broth and shaken at room temperature for 2 days, and the initial cellular density was about $5{\cdot}10^5$ CFU/ml. The pure bacterial cultures with lipid degrading ability (1 ml of inoculum) were inoculated into the BMM with palm oil (9 ml) and into the SWW with palm oil (9 ml). They were tested for lipid degrading ability in BMM with palm oil (10%, v/v) and in SWW with palm oil (10%, v/v). Non-inoculated media served as controls.

The experiments were performed in triplicate using 30 ml test tubes and kept in a rotary shaker at 250 rpm and room temperature (30-35°C). The oil layer in sample tubes was observed every week for one month. After that, oil residues in all samples were extracted using the partition gravimetric method, and their weights were investigated. The efficiency of palm oil degradation was calculated as the percentage of degraded oil (Eaton et al, 1995), as follows:

$$\%\ degraded\ oil = 100 - \left[\left(\frac{weight\ of\ sample\ extracted\ oil}{weight\ of\ control\ extraced\ oil}\right) \times 100\right]$$

The statistical analysis of the results was carried out through a T-test, with confidence levels of 95% and 99%, using the Excel program. The lipid degrading efficiencies of all isolates in BMM were compared with those in SWW. Their efficiencies between isolated strains, including non-inoculum (controls), were also compared.

4. Results and Discussion

4.1 Results

After mixed cultures were isolated into 34 pure bacterial cultures (isolates), all of them were streaked on TBT agar plates. There were 8 isolates (labeled as A-H), occurring clear zones surrounded their colonies. The isolates A, B, E, and F were positive rods, but isolates C and D were negative rods. The isolates G and H were negative cocci.

Table 1: Clear zone characteristics of pure bacterial culture

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Isolate	Width of clear zone (mm)	Clearness
A	2	less turbidity
B	10	transparent
C	4	almost transparent
D	4	almost transparent
E	1	less turbidity
F	1	less turbidity
G	2	less turbidity
H	2	less turbidity

Their size and clarity of clean zones was different, as shown in Table 1. The clear zone of isolate B was the widest (10 mm) and the most transparent. The clear zone of isolates C and D was 4 mm in width and almost transparent. The others (A, E, F, G, and H) were less (2, 1, 1, 2, and 2 mm) and not clear. (Table 1)

Table 2: Weights of oil residue and percentages of lipid degrading efficiency

Isolate	BMM with Oil		SWW with Oil	
	weight of oil residue (*mean*, g)	**%degrading efficiency ($\underline{X} \pm SD$)**	**weight of oil residues (*mean*, g)**	**%degrading efficiency ($\underline{X} \pm SD$)**
A	0.30	52.63±2.05	0.31	53.69±5.65
B	0.23	63.68±4.01	0.16	76.85±11.22
C	0.15	76.84±7.07	0.36	46.80±7.26
D	0.17	73.16±3.57	0.47	31.03±4.13
E	0.53	16.32±1.24	0.55	19.21±9.65
F	0.44	30.00±0.72	0.44	35.47±7.77
G	0.32	50.00±2.11	0.31	53.69±9.47
H	0.46	26.84±5.11	0.46	31.53±7.25
control	0.63	0	0.68	0

The isolates having clear zones were tested for lipid degrading ability in BMM with palm oil (10%, v/v) and in SWW with palm oil (10% v/v). After 30 days, all samples were extracted, and the weight of oil residues was calculated to be a percentage of lipid degrading efficiency. Their lipid degradation efficiencies are shown in Table 2. All isolates were able to degrade palm oil in both media.

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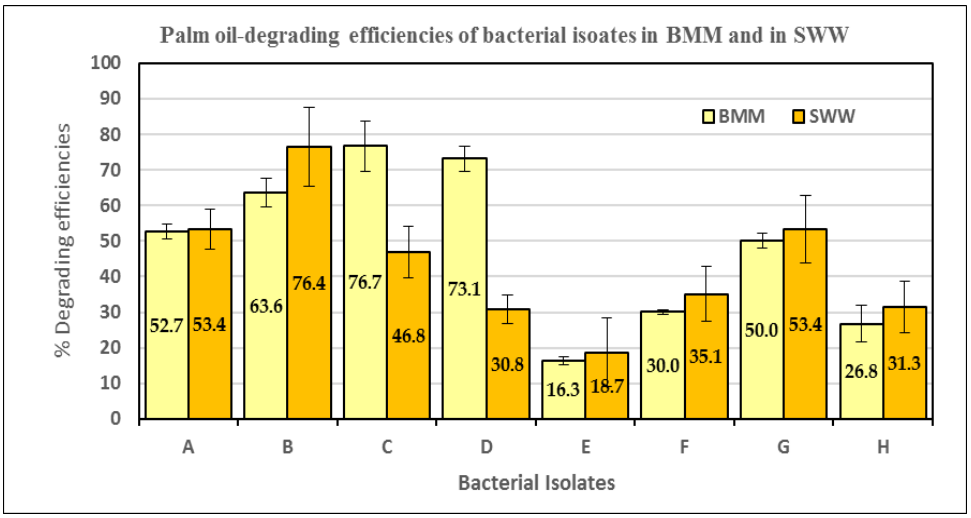

Figure 1: Palm oil degrading efficiencies of bacterial isolates in BMM and SWW

Figure 1 shows that their lipid degrading efficiencies in BMM were compared to those in SWW. In BMM with palm oil, isolate C had the most efficiency (76.84%), followed by isolates D (73.16%) and B (63.68%), respectively. In SWW with palm oil, isolate B had the most efficiency (76.85%), followed by isolates A and G (53.69%). (Table 2 and Figure 1)

Mostly, the bacterial isolates had lipid degrading efficiency in SWW more than that in BMM, except isolates C and D. Although isolates C and D were highly efficient in BMM (77% and 73%), they very decreased in SWW (47% and 31%). (Figure 1)

Table 3: Mean comparison of degrading efficiency analyzed by T-test

BMM&SWW

Isolate	A	B	C	D	E	F	G	H	
BMM&SWW			**	**					

BMM

Isolate	A	B	C	D	E	F	G	H	control
A		**	**	**	**	**		**	**
B			*	*	**	**	**	**	**
C					**	**	**	**	**
D					**	**	**	**	**
E						**	**	**	**
F							**		**
G								**	**
H									**

SWW

Isolate	A	B	C	D	E	F	G	H	control
A		**	*	**	**	**		**	**
B			**	**	**	**	**	**	**

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C	**	**	*	*	**	**
D		*		**		**
E			**	**	**	**
F				**		**
G					**	**
H						**

* Different significantly at a 95% confidence level
** Different significantly at 99% confidence level
Different non-significantly at 95% & 99% confidence levels

The mean comparison of degrading efficiency analyzed by T-test was shown in Table 3. The degrading efficiencies of isolate A, B, E, F, G, and H in SWW were more than that in BMM, but they were insignificant (⟨=0.01). Meanwhile, the efficiencies of isolates C and D in SWW were significantly lower than in that BMM (⟨=0.01) (Figure 1 and Table 3). All the bacterial strains degraded lipids in both media more than their controls significantly (⟨=0.01) (Table 3).

At a 99% confidence level, the degrading efficiencies of isolates B, C and D in BMM were high level and not significantly different. In SWW, the degrading efficiencies of isolate B were more than the others, significantly (⟨=0.01) (Table 3).

4.2 Discussion

Firstly, isolate B, having the widest and transparent clear zone (10 mm), had the highest lipid degrading efficiency. While the other strains (C, D, A, G, H, E, and H) having widths of clear zones were less (4, 4, 2, 2, 2, 1, 1 mm, respectively) and not transparent (Table 1), their lipid degrading efficiency decreased (Figure 1), relatively. The results of TBT-test showed that the size of the clear zone related to the lipid degrading efficiency of bacterial strains. Thus, a Lipase test on the TBT-agar plate might be applied to indicate the lipid degrading efficiency of bacteria.

Secondly, isolates C and D had the highest lipid degrading efficiency in BMM (77% and 73%), but their efficiency became low in SWW (47% and 31%). Other organic compounds, such as glucose, carbohydrate and protein, are probably more suitable for isolating C and d than lipid compounds,

Finally, isolate B should be selected to develop for lipid wastewater treatment. Because of the lipid degrading efficiency of isolate B were high levels in both media. Although isolate B was not the highest degrading strain in BMM (63.68%), its efficiency was the highest in SWW (76.85%) (Table 2 and Figure 1).

5. Conclusion

The results of the TBT-test can indicate the lipid degrading efficiency of bacteria. It helps to reduce the time to identify bacteria that have high lipid degrading efficiency. The isolate B was able to degrade palm oil (10% v/v) in both media (BMM and SWW) at high rates (63.68% and 76.85%). Thus, isolate B should be further investigated for lipid wastewater treatment.

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