Ability of Mangrove Fungi in Biodegradation of Hexadecane

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Ability of Mangrove Fungi in Biodegradation of Hexadecane

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Abstract: Oil pollution, especially in the marine environment, has become a serious environmental problem. Hexadecane (HXD) is a major alkane component and it is present in the aliphatic fragment of crude oil, which can be used by fungi as a sole carbon source. Biosurfactant which is produced by fungi facilitates HXD degradation. This study investigated the ability of mangrove fungi to be used as HXD and produce biosurfactant. The medium used to determine the ability of fungi to use hexadecane is MSM-HXD 2%, whereas Hua medium is used for determining the potential for producing biosurfactants. Biosurfactant production by fungi strains was indicated by oil displacement test, the reduction in surface tension and emulsion index. The results showed that 13 out of 16 species of fungi can use HXD as a sole carbon source. *Inonotus radiatus* LM 3020 is observed to be the most capable isolate to use HXD with a growth ratio of 6.0, and can produce biosurfactant with a positive oil displacement test (ODT) value whose minimum surface tension was 54.01 dyne/cm but the emulsion index was found to be zero.

Key words: Biodegradation, fungi, hexadecane, mangrove.

Introduction

Marine oil pollution has become a serious environmental problem. Hydrocarbons (mainly crude oil, diesel and fuel) are a group of pollutants that are difficult to degrade, non-polar, and have low solubility in water. Fungi can be used as an alternative to overcome hydrocarbon pollution. Many fungi have been known to degrade hydrocarbons. Hexadecane (HXD) is an alkane hydrocarbon with the chemical formula $C_{16}H_{34}$ and is a major alkane component present in the aliphatic fragment of crude oil. HXD can be used as a carbon source by fungi. HXD is often used in many microbial experiments to study the use of hydrocarbons as a carbon source (Dashti et al., 2008; Shiri et al., 2015). It is also used as an indicator of hydrocarbon-degrading potential (Barreto et al., 2010).

Previous research has succeeded in isolating and identifying fungi from mangrove, Wonorejo, Surabaya, Indonesia (Table 1). Isolation is carried out on the soil (Kuswytasari et al., 2011) contaminated by polyethylene and wood (Meiliawati and Kuswytasari, 2013). Some

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of the researchers have found hydrocarbons degrading fungi from soil, such as *Aspergillus niger* (Al-Hawash et al., 2018), *Aspergillus fumigatus, Fusarium solani* and *Penicillium funiculosum* (Al-Jawhari et al., 2014). Hydrocarbons are the main material of plastics (Sarker et al., 2011), so fungi that can degrade plastics can degrade hydrocarbons too. Fungi can also degrade lignin and cellulose present in wood. The ability of fungi to produce nonspecific enzymes in degrading cellulose and lignin demonstrates their ability to degrade compounds with high molecular weight, complex and recalcitrant, including aromatic structures (Potin et al., 2004).

The ability of fungi to degrade hydrocarbons can be seen from its ability to grow on a medium containing HXD (Schoefs et al., 2004). The use of HXD is aided by the presence of biosurfactant, a self-assembling amphiphile molecule. Biosurfactants are an important factor in the use of alkanes or hydrocarbons as a carbon source by microorganisms (Mahjoubi et al., 2013). This research aims to study the growth and the ability of mangrove fungi in using HXD and producing biosurfactant.

Material and Methods

The isolates were obtained from Microbiology and Biotechnology Laboratory, Department of Biology FSAD ITS (Table 1). The isolates were cultured on Potato Dextrose Agar medium.

The growth assay was conducted by comparing the growth rates of fungal strains, as colony diameter, on MSM-HXD medium and control Petri dishes (Al-Jawhari, 2014). MSM contains $Na_2HPO_4.2H_2O$, 3 g/L; KH_2PO_4, 3 g/L; NaCl, 0.5 g/L; NH_4Cl 1 g/L; $MgSO_4.7H_2O$, 0.5 g/L; 0.007 % yeast extract; 0.003% bacto-peptone; 2 % agar and micronutrien $CaCl_2$ 1 mg/L; $FeCl_3.2H_2O$ 1 mg/L (Maddela et al., 2015). The pH of the medium was adjusted to 7.

Sixteen fungi isolates were screened for their biosurfactant activity on a modified Hua's medium (4% glucose as carbon source, 0.4% $NaNO_3$, 0.02% KH_2PO_4, 0.02% $MgSO_4.7H_2O$, 0.1% Yeast extract) (Thaniyavarn et al., 2008) and incubated at 30°C and 200 rpm for 7 days. The culture was harvested by centrifugation at 8,000 rpm for 20 min. The cell-free broth of each strain was measured the biosurfactant activity by the oil displacement test (Soeb et al., 2015), surface tension activity (Lecomte Du Nouy, 1919) and emulsification measurement.

Results and Discussion

Growth Curve of Mangrove Fungi

The growth curves of mangrove fungi on medium with and without hexadecane can be seen in Figure 1. It can be observed that the growth increases during 7 days and is still in the logarithmic phase. Growth patterns in soil fungi showed the same growth patterns in both mediums, except *Trametes polyzona* LM 1020 (Figure 1.1a, 1.1b and 1.2a, 1.2b). On wood fungi, different growth patterns were found in *Inonotus radiatus* LM 3020 (Figure 1.3a and 1.3b). It shows that LM 3020 prefers HXD as a source of growth nutrition.

Isolates that could not grow on both mediums occurred in *Paecilomyces* sp. LM 1031. It could not use peptone, yeast extract or HXD as its nutritional source.

Table 1: Fungal species from mangrove Wonorejo, Surabaya, Indonesia

Code (LM)	*Species*	*Code (LM)*	*Species*
From Soil		**From Wood**	
1003	*Asperrgillus niger*	3001	*Climacodon septentrionalis*
1015	*Chaetomium* sp.	3003	*Climacodon septentrionalis*
1013	*Perenniporia* sp.	3013	*Inonotus hispidus*
1020	*Trametes polyzona*	3020	*Inonotus radiatus*
1021	*Aspergillus terreus*	3023	*Cochliobolus verruculosa*
1025	*Penicillium* sp.	**From Contaminated Soil**	
1031	*Paecilomyces* sp.	1102	*Aspergillus fumigatus*
1041	*Mycelia sterilia*	1105	*Aspergillus terreus*
		1107	*Leptosphaerulina chartarum*

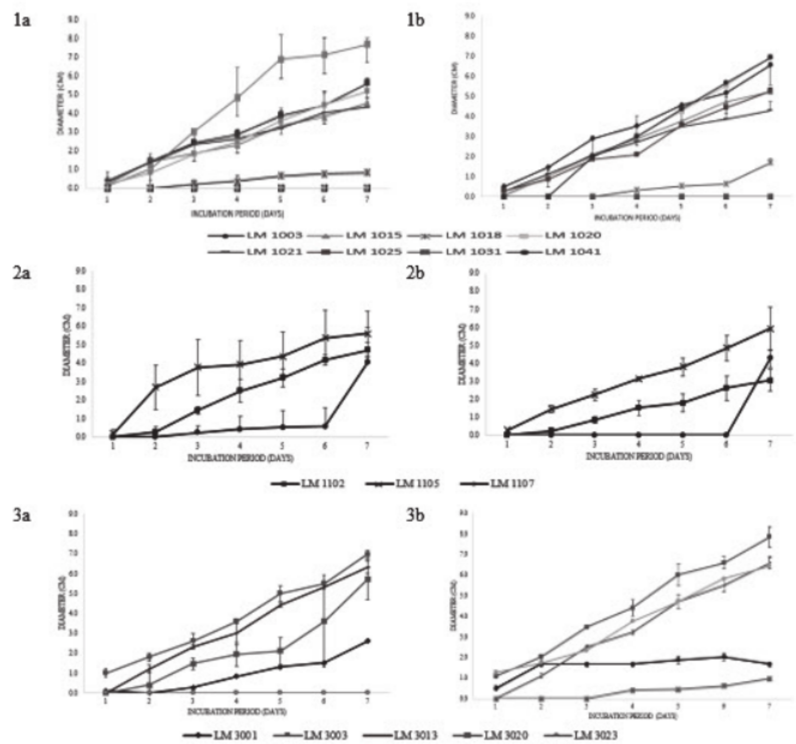

Figure 1: Growth curves of mangrove fungi from soil (1), contaminated soil (2), and wood (3). Fungi were cultured in the MSM-HXD (a) and MSM (b) for 7 days. The data were presented as the mean of three independent experiments.

While *Mycelia sterilia* LM 1041 and *Cochliobolus verruculosa* LM 3023 did not grow only on MSM-HXD medium, but grew on MSM medium. This illustrates that LM 1041 and LM 3023 are inhibited by the presence of HXD.

Fungal growth in each medium is unique. The growth of fungi colonies is greatly influenced by nutrients found in the medium. The composition of the medium can trigger or inhibit the growth of fungi. The growth of fungi in the MSM-HXD medium does not necessarily indicate that the fungi can use HXD as a carbon and energy source because the MSM medium itself contains yeast extract and peptone, which can trigger the growth of the fungal colony.

The Ability of the Fungi to Use HXD

Fungal growth ratio is the percentage value of the ratio among the growth of fungal isolates on MSM-HXD medium with MSM medium. The value of the ratio will indicate the ability of fungi using HXD as an energy and carbon source. The ability of fungi to use HXD in this research are presented in Figure 2. A ratio of 0 indicates that the fungi cannot use HXD as its carbon and energy source. The ratio < 1 indicates that the fungus that grows on the medium uses nutrients derived from the basic medium; in this case, MSM contains peptone and yeast extract. Whereas a ratio of > 1 indicates the ability of fungi to use HXD for energy sources and carbon sources for growth.

Almost all isolates showed an increase in the growth ratio from the first day to a certain day, then the ratio was seen to decrease (Figure 2a, 2b and 2c). According to Grund et al. (1975), HXD use is triggered by its presence in the medium because the genes that regulate it are inducible.

The growth ratio of *Chaetomium* sp. LM 1015 showed a faster growth on medium containing HXD on the first day of incubation and then decreased (Figure 2a). This illustrates that HXD can trigger early growth. This is supported by Meng et al. (2018), which state that

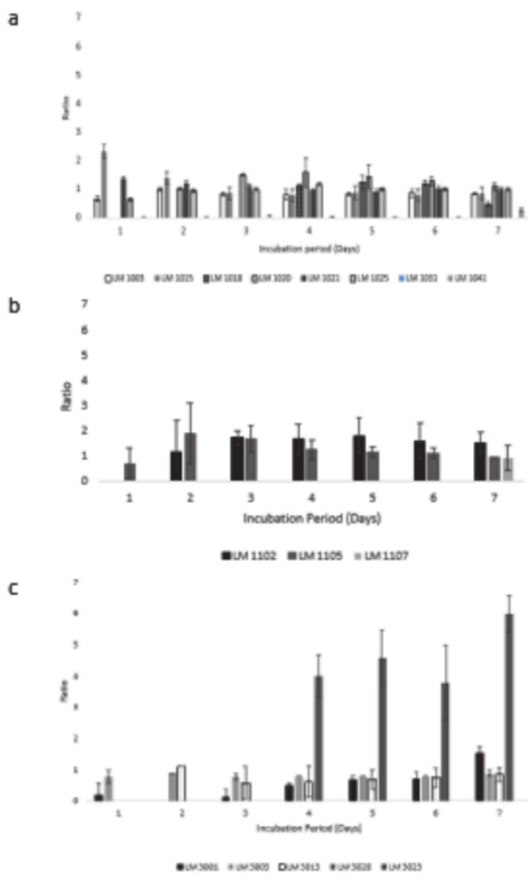

Figure 2: Growth ratio of fungi from (a) soil, (b) contaminated soil and (c) wood. Experiments were conducted in triplicate.

extracellular alkane hydroxylase, an enzyme that plays a role in alkane degradation, was induced in a higher degree in the early incubation time.

Biosurfactant Producer

On the 7th day, a continuous potential test was performed on the isolates with a ratio value > 1 for producing biosurfactants. The result of the test in producing biosurfactant on modified's Hua media containing glucose (Thaniyavarn et al., 2008), showed that all isolates cannot form clear zones in oil displacement tests, but all tested fungi can reduce surface tension (Table 2). Every isolate was able to reduce the surface tension of medium down to 60.33 dyne/cm until 54.01 dyne/cm.

Surface tension can decrease with biosurfactant production. Hakanpaa et al. (2004) stated that biosurfactants are small proteins that play a role in reducing the surface tension of the medium and help the aerial-growth of fungi. Besides, Meng et al. (2018) stated that one-way cells use hydrocarbons is to form aggregates that are supported by the production of biosurfactants.

Production of more biosurfactant leads to lower the surface tension. In addition to a reduction in surface tension, biosurfactants also affect the activity of enzymes that play a role in the use of hexadecane.

Table 2: Potential biosurfactant production from mangrove fungi isolates that can use hexadecane

Isolate	*ODT[a]*	*Mm ST[b] (Dyne/cm)*	*E24(%)[c]*
LM 1020	+	56.86±1.88	2.2
LM 1021	+	54.01±5.56	0.9
LM 1025	+	60.33±0.20	0.62
LM 1102	+	54.51±0.27	–
LM 3001	–	54.97±0.27	–
LM 3020	+	54.01±0.29	–

Besides, the possibility of a negative emulsion index is due to the high pH of the medium. The emulsifier was only active below pH 7. There was a very pronounced loss of activity between pH 6.5 and 7. At pH values greater than 9 or less than 3, the emulsion index was halved and no emulsion formed above pH 11.

Conclusion

Not all mangrove fungi can use hexadecane (HXD) as a source of nutrition and energy. HXD can trigger and inhibit the growth of fungi. All tested mangrove fungi can reduce the surface tension by producing biosurfactants, but not all biosurfactants are emulsifiers. *Inonotus radiatus* LM 3020 is most suitable to be used with HXD for a growth ratio of 6.0 and can produce biosurfactant with a positive ODT value, minimum surface tension 54.01±0.29 dyne/cm; but the emulsion index was found to be zero.

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