Bioremediation of waste lubricating oil by *Serratia* sp. GR2.4

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Abstract

This research aims to isolate and screen the waste lubricating oil degrading-microorganisms from oil contaminated soil that collected from Hat Yai District, Songkhla Province. The isolation and screening of microorganisms showed the existence of 81 isolates (60 bacteria isolates and 21 yeast isolates) capable of degrading the waste lubricating oil (WLO). The most active microorganism in the assimilation of WLO and bio-emulsifier production, GR 2.4 bacterial isolate, when characterized on nutrient agar plates, appeared to have smooth edge and an off-white color. Morphological examination indicated the shape of short rod, gram negative bacteria without endospore. Determination of the nucleotide sequence of the gene encoding 16S rDNA identified the GR 2.4 isolate to be *Serratia* sp. This isolate showed 60.64±0.07 % WLO degrading activity (measured by weight loss method) when grow on basal salt medium (BSM) containing 0.1% WLO as sole carbon source, whereas the control showed 15.00±0.00%. Upon cultivation in the same media without nitrogen source, initial pH 8 or at the optimum temperature, 37 ºC, WLO degrading activity was 61.22 ±0.09%, 63.00±0.00% and 65.85±0.08%, respectively. In addition, *Serratia* sp. GR 2.4 produced bio-emulsifier with xylene with the best emulsification activity (%EA) of 54.55±0.00%

Keywords: bio-emulsifier, emulsification activity, contaminated soil, waste lubricating oil

Introduction

Oil contamination with petroleum hydrocarbons has caused severe environmental and health defects and increasing attention has been paid for developing and implementing innovative technology for cleaning up this contamination (Yeung et al. 1997). Bioremediation methods are currently receiving favorable publicity as promising environmentally friendly treatment technologies for the removal of hydrocarbons (Desai and Banat 1997). Bioremediation can be described as the conversion of chemical compounds by viable organisms, especially microorganisms with novel catabolic functions derived through selections or by the introduction of genes encoding such functions into energy, cell mass and harmless biological waste products. For petroleum hydrocarbons, these biological waste products are primarily CO2, water and methane (Walter et al. 1997).
One of the factors that limit biodegradation of oil pollution in the environment is their limited availability to microorganisms (Providenti et al. 1995). Generally, petroleum hydrocarbon compounds bind to soil components and are difficult to remove or degrade. Biosurfactants (BS) can emulsify hydrocarbons, thus enhancing their water solubility, decreasing surface tension and increasing the displacement of oily substances from soil particles (Banat 1995a; Banat 1995b; Banat et al. 2000). We now report the isolation and screening of waste lubricating oil-degrading microorganisms from oil contaminated soil and their identification. Moreover, selected strain will be screened for bio-emulsifier production.

Methodology

Samples
Microorganisms were isolated from oil contaminated soils, including palm oil plant and garages, in the Southern region of Thailand. Stock culture of the isolates were maintained at 4ºC on plate count agar (PCA) slants and transferred once a month.

Media preparation
Samples were grown on PCA, then, purified colonies were collected on PCA slants. The basal salt medium (BSM) (Katemai et al. 2008) for waste lubrication oil-degradation and production of the biosurfactant contained 3g urea, 0.2g KH_2PO_4, 0.2g $MgSO_4 \cdot 7H_2O$, supplemented with 1g waste lubricating oil per liter of distilled water.

Chemicals
All chemicals are of analytical grade. Waste lubricating oil (WLO) was obtained from a garage in Pha-payom district, Phatthalung Province.

Weight loss method
Waste lubricating oil biodegradation activity was measured by the weight loss method (Shirai et al. 1995) and calculated by using the following equation

$$(\%) \text{ weight loss} = ((A - B)/ A) \times 100 \qquad (1)$$

A = weight of oil (negative control)
B = weight of oil (degraded)

Biosurfactant activity assay
To measure emulsification activity (EA%), 1 ml of xylene was added to 1 ml of supernatant in a test tube. The mixture was mixed by vortex mixer at high speed for 2 min and allowed to stand for 10 min and 24 h (emulsification index: EI). The emulsification activity was calculated by using the following equation (modified from Cooper and Goldenberg 1987).

$$EA (\%) = (\text{height of emulsion formed}/ \text{total height of solution}) \times 100 \qquad (2)$$

Kerosene, xylene and n-hexadecane were used for this study.

Screening of waste lubricating oil-degrading microorganisms from oil contaminated soil
An enrichment culture technique was used for the isolation of microorganisms responsible for the biodegradation of waste lubricating oil. One gram of soil sample was added into BSM containing 0.1% waste lubricating oil as the sole carbon source. The culture was cultivated on a rotary shaker with 200 rpm at 37ºC for 72 h. Finally, growth was measured by OD600nm. Waste lubricating oil biodegradation activity was measured by the weight loss method.

Identification of selected strain by morphology and DNA sequencing
The selected strain which exhibited the highest waste lubricating oil degradation activity was selected and identified based on the morphology by Bergey's Manual of Determinative Bacteriology. In addition, the 16S rDNA fragments of the selected strain were amplified using universal oligonucleotide primers. Sequences of the amplified fragments were analyzed with an ABI PRISMTM 3100 DNA Sequencer (PE Applied Biosystems, Foster City, CA, USA) and a BigDye Terminator Cycle Sequencing kit (Applied Biosystems) according to the manufacturer's instruction manuals. The BLAST program (http://www.ncbi.nlm.nih.gov/BLAST)

Optimization condition of waste lubricating oil degradation
The selected strain was grown in 5 ml of NB (5 g peptone, 3 g yeast extract, 3g malt extract, 10 g glucose, per liter distilled water, pH 7.0) and grow under agitation at 150 rpm and 37ºC for 24 h. The culture was adjusted to obtain OD_{600} of 0.5 and used as the starter culture. Ten percent of starter culture was transferred to 50 ml of the BSM (modified from Katemai et al. 2008) for WLO degradation and the production of the biosurfactant. The optimum condition was established by following: concentration of WLO (0, 1, 5 and 10 g/L), the effect of the nitrogen source (urea, $(NH_4)_2SO_4$ and NH_4Cl at 1g/L), the initial pH of the culture medium (8, 9 or 10), different temperatures (30, 37 and 45ºC). Triplicate samples were taken at 0, 12, 18, 24, 30, 36, 42, 48, 60 and 72 h. Finally, growth was monitored as ΔOD_{600}. The potential of WLO degradation was determined by weight loss (%) and also by %EA.

Statistical analysis
The data were calculated with mean values, and standard deviations (mean±SD) were determined from triplicate determinations.

Results

Screening of waste lubricating oil-degrading microorganisms from oil contaminated soil
Eighty-one isolates (60 bacteria isolates and 21 yeast isolates) were showed to have ability to degrade waste lubricating oil. The most active microorganism in the assimilation of waste lubricating oil was GR 2.4 bacterial isolate. When characterized on NA plates, this isolate appeared to have smooth edge and off-white color. Morphological examination indicated the shape of short rod, gram negative bacteria without endospore. Determination of the nucleotide sequence of the gene encoding 16S rDNA identified the GR 2.4 isolate to be *Serratia* sp.

Optimization condition of waste lubricating oil degradation
When waste lubricating oil was used as carbon source (0.1%), *Serratia* sp. GR 2.4 showed a degrading activity of 65.85% (Fig.1). In addition, *Serratia* sp. GR 2.4 produced bio-emulsifier of kerosene, xylene and n-hexadecane with the emulsification activities (%EA) of 39.17, 54.55 and 41.36%, respectively (Table 1). The characteristic of a good emulsifier should show the emulsification activity and emulsion stability with various hydrocarbons.

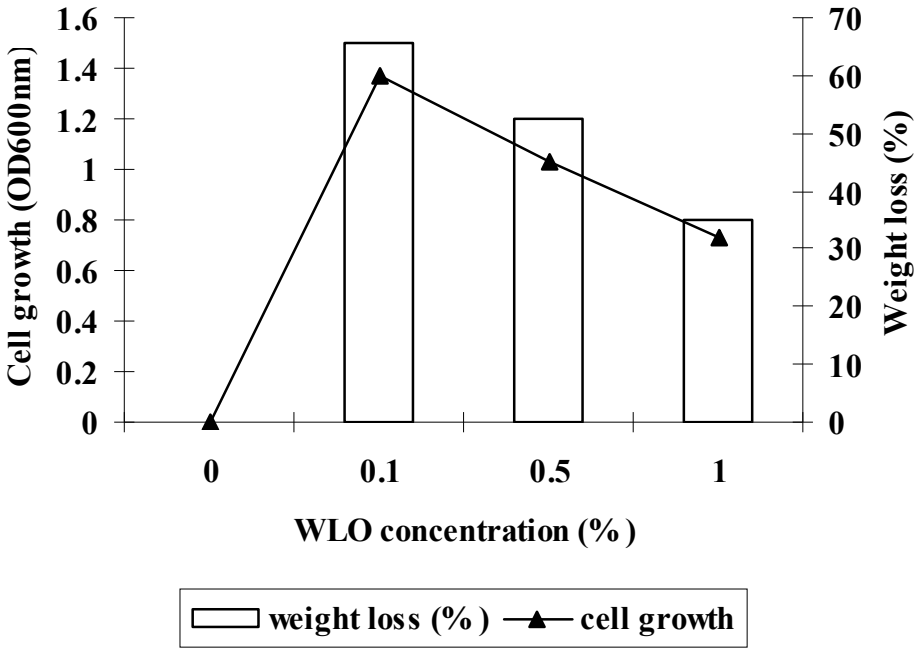

Figure 1. Potential of waste lubricating oil degradation by *Serratia* sp.

Table 1. Emulsification activity from isolate *Serratia* sp.

Hydrocarbons	Emulsification activity (%EA)
Kerosene	39.17±0.00
Xylene	54.55±0.00
n-Hexadecane	41.36±0.00

Conclusion and Discussion

There were 81 isolates (60 bacteria isolates and 21 yeast isolates) capable of degrading the waste lubricating oil. The most active microorganism in both the assimilation of waste lubricating oil and bio-emulsifier production was the GR 2.4 bacterial isolate, when characterized on NA plates, this isolate appeared to have smooth edge and an off-white color, while a morphological examination indicated the shape of short rod, gram negative bacteria without endospore. This agrees with oil-degrading microorganisms previously isolated from enrichment cultures of scale soil (Jirasripongpun 2002), where 26 isolates were selected on their ability to growth on unused lubricating oil, weathered oil and aromatic oil in cultures derived after enrichment. *Nocardia simplex* W9 is the best degrader, among all the isolates, on both used and unused lubricating oil Determination of the 16S rDNA sequence identified the GR 2.4 isolate to be *Serratia* sp. When 0.1% waste lubricating oil was used as carbon source, *Serratia* sp. GR 2.4 showed the highest degrading activity of 65.85%. In addition, *Serratia* sp. GR 2.4 produced a bio-emulsifier with xylene, with the best emulsification activity (%EA) obtained of 54.55%. Kerosene, a mixture of paraffin, naphthalene and aromatic hydrocarbon is more difficult to emulsify compare to xylene and *n*-hexadecane, which consist of 1 benzene ring and long chain hydrocarbon, respectively (Katemai 2008).

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