BIO-EMULSIFIER PRODUCTION BY SELECTED YEAST

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Abstract - The objective of this research was studied the hydrocarbons remediation by microorganisms which isolate and screen the bio-emulsifier producing-microorganisms from oil contaminated soil that collected from Thailand. The isolation and screening of microorganisms showed the most active microorganism in the assimilation of WLO and bio-emulsifier production, WK 12 yeast isolate, when characterized on yeast malt agar plates, appeared to have smooth edge and an off-white color. Determination of the nucleotide sequence of the gene encoding 26S rDNA identified the WK 12 isolate to be Isatchenkiaorientalis. This isolate produced bio-emulsifier with various vegetable oil and hydrocarbons. The isolated yeast strain, WK 12 showed a high emulsification activity (%EA) with various hydrocarbons such asbenzene, xylene, kerosene and n-hexadecane and various vegetable oils such as soy bean oil, palm oil or rice bran oil the result showed the best %EA with rice bran oil (86.67±0.00%). The optimum medium for the growth and production of the emulsifier by I. orientalis WK 12 contained 0.1% waste lubricating oil as a carbon source at 72 hr of cultivation. I. orientalis WK12 was produced the highest emulsifier and showed the Emulsification activity (%EA) and Emulsification Index (%EI) with rice bran oil as 86.67±0.00% and 26.47±0.00%, respectively.

Index Terms - Bio-emulsifier, hydrocarbons, Isatchenkiaorientalis, oil contaminated soil

I. INTRODUCTION

Biosurfactants/ bioemulsifiers are amphiphilic molecules containing hydrophobic and hydrophilic moiety produced by microorganisms, and have the ability to reduce interfacial tension and stabilize emulsion between different fluid phases [2]. Bioemulsifiers are important biological products with a wide range of applications not only in the food, cosmetic, petrochemical industries, agricultural but also environmental which showed unique properties e.g. lower toxicity, higher biodegradability and environmental compatibility compared to their chemical counterparts. Emulsifiers are a large category of compounds considered as surface active agents or surfactants.

An emulsifier acts by reducing the speed of chemical reactions, and enhancing its stability. Bioemulsifiers are known as surface active biomolecule materials, due to their unique features over chemical surfactants, such as non-toxicity, biodegradability, foaming, biocompatibility, efficiency at low concentrations, high selectivity in different pH, temperatures and salinities. Emulsifiers are found in various natural resources and are synthesized by Bacteria, Fungi and Yeast. Bioemulsifiers are amphipathic polymers while biosurfactants are surface active chemicals produced by large number of bacteria, yeast and fungi (LetiziaFracchia et al., 2012).

Bioemulsifiers can emulsify hydrocarbons; thus, enhancing their water solubility, decreasing surface tension and increasing the displacement of oily substances from soil particles [3].

Thus, the objective of this research was to screen bioemulsifier producing- microorganisms from oil contaminated soil and identify them. Moreover, potential of emulsification activity by selected strain was studied.

II. MATERIAL AND METHOD

Isolation and screening of waste lubricating oil-degrading microorganisms

The bacteria strains were isolated from hydrocarbon contaminated soils in the Southern region of Thailand, including palm oil plant and motorcycle garages. Purified colonies were maintained at 4°C on plate count agar. An enrichment culture technique was used for the isolation of microorganisms responsible for the biodegradation of WLO. Five gram of soil sample was added into 50 ml mineral salt medium (MSM) [4]and 10 g/L WLO obtained from garage as sole carbon source. The culture was cultivated on a rotary shaker with 200 rpm at 37°C for three days. Then, the method for screening was done by using serial dilutions of samples, and plated on Yeast Malt agar (YA) medium. Finally, growth was measured by OD660 nm. WLO degradation activity was measured by weight loss method.

Identification of selected strain

The selected strain which exhibited the highest bioemulsification activity was selected and identified based on the 26S rDNA fragments of the selected strain were amplified using universal oligonucleotide primers. Sequences of the amplified fragments were analyzed with an ABI PRISMTM 3100DNA Sequencer (PE Applied Biosystems, Foster City, CA, USA) and a BigDye Terminator Cycle Sequencing kit (Applied Biosystems) based on the manufacturer's instruction manuals. The BLAST program (http://www.ncbi.nlm.nih.gov/BALST) was used to analyze sequence homology.

Potential of bioemulsifierproduction by selected strain

The selected strain was grown into 5 ml of YM

medium and inoculated with shaking at 150 rpm and 30°C for 48 h. The culture was adjusted to obtain OD600 nm as 0.5 and used as the starter culture. Ten percent (10%) of starter culture was transferred to 50 ml. of the basal salt medium (BSM) [7] (Katemai et al. 2013) for the production of the biosurfactant was which contains 3 g urea, 0.2 g KH_2PO_4, 0.2 g $MgSO_47H_2O$ supplemented with 1 g WLO per liter of distilled water in 250 ml flask. The BSM consisting of carbon and nitrogen sources with the proper initial pH and temperature were chosen. Triplicate samples were taken at 0, 18, 24, 48, 60, 72, 96 and 120 h. Finally, growth was measured by OD660 nm. The potential of WLO degradation was determined by weight loss (%) and also Emulsification activity (%EA) and Emulsification Index (%EI).

Bio-emulsification activity assay

The biosurfactant property was analyzed by emulsification activity [11] (Cooper and Goldenberg, 1987). The culture broth was separated by centrifugation at 10,000 rpm at 4°C for 20 min. The supernatant was used in determining the emulsification activity.

The mixture of benzene, xylene, kerosene, n-hexadecane, soy bean oil, palm oil or rice bran oil (1 ml) was added to 1 ml sample supernatant in a vial, then the mixture was vortexed at high speed for 2 min and allowed to stand for 10 min (Emulsification activity; EA) and 24 hour (Emulsification Index; EI) until the solvent and water layers separated, and an emulsified layer appeared between them. The percent of %EA and EI were calculated as in (1).

EA (%) = (height of emulsion formed/total height of solution) x 100

Statistical analysis

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

III. RESULT AND DISCUSSION

Isolation and screening of waste lubricating oil-degrading microorganisms

One hundred-one isolates (50 bacteria isolates and 51 yeast isolates) degrade the waste lubricating oil (Data not shown). The most active microorganism in both the assimilation of waste lubricating oil and bio-emulsifier production was the isolate WK 12. When 1 g/L WLO was used as carbon source, isolateWK 12 showed the highest degrading activity of 60.64±0.07 %. This research to agree with oil-degrading microorganisms which isolated from enrichment cultures of scale soil. The report showed 26 isolates selected on their growth ability on unused lubricating oil, weathered oil and aromatic oil of culture broth derived after enrichment. Nocardia simplex W9 is the best degrader, among all the isolates, on both used and unused lubricating oil [6]. Moreover, Klawech et al., [7] reported that the Bacillus subtilis could utilize waste lubricating oil as sole source of carbon and energy. After testing, it was found that B. subtilis had high potential to degrade waste lubricating oil up to 77.30±0.00%.

Identification of selected strain

When characterized on YM agar plate, appeared to have smooth edge and off-white color. Morphological examination indicated the oval shape of short rod, and produced film. The determination of the nucleotide sequence of the gene encoding 26S rDNA (approx.0.5 kb) analysis of the isolate WK 12, showed 99% identities, homology with Isatchenkiaorientalis WK 12.

Characterization of bio-emulsifier by Isatchenkiaorientalis WK 12

orientalis WK 12 produced bio-emulsifier with hydrocarbons and vegetable oils. The result exhibited the best emulsification activity (%EA) of 54.55±0.00% whereas this yeast can emulsify rice bran oil (Table I).The microorganisms use surface active compounds (biosurfactants / bioemulsifiers) for survival which help them in the aqueous phase to adsorb, emulsify, wet and disperse or solubilize the water-immiscible material [8].

The structure of kerosene is consisted of the mixture of paraffin, naphthalene and aromatic hydrocarbons. So, it was the most difficult to emulsify than xylene, benzene and n-Hexadecane with are consisted of 1 benzene ring and long chain hydrocarbon, respectively [9]. This isolate produced bio-emulsifier with various vegetable oil and hydrocarbons. The isolated yeast strain, WK 12 showed a high emulsification activity (%EA) with various hydrocarbons such as benzene, xylene, kerosene and n-hexadecane and various vegetable oils such as soy bean oil, palm oil or rice bran oil the result showed the best %EA with rice bran oil.

The optimum medium for the growth and production of the emulsifier by I. orientalis WK 12 contained 0.1% waste lubricating oil as a carbon source at 72 hr of cultivation. I. orientalis WK12was produced the highest emulsifier and showed the %EA with rice bran oil (86.67±0.00%%) whereas emusification index (%EI) 26.47±0.00%. This result was similarly with the report ofthe production of a biosurfactant by Candida guilliermondii UCP0992 was studied in a low-cost medium formulated with 2.5 % waste frying oil, 2.5 % corn steep liquor and 4.0 % molasses during 144 h at 28 ºC under 200 rpm. The emulsifying activity against motor oil (up to 90 %) and vegetable oils (30-55 %). (Sarubbo et al., 2016)

TABLE I. EMULSIFICATION ACTIVITY OF CRUDE SUPERNATANT PRODUCED BY I. orientalis WK 12 WITH VARIOUS HYDROCARBONS AND VEGETABLE OILS

Hydrocarbons	Emulsification activity (%)	Emulsification Index (%)
Benzene	51.86±0.00	18.00±0.00
Xylene	54.55±0.00	21.55±0.00
Kerosene	39.17±0.00	0±0.00
n-Hexadecane	41.36±0.00	0±0.00
Soy bean oil	67.65±0.00	23.87±0.00
Palm oil	54.59±0.00	20.09±0.00
Rice bran oil	86.67±0.00	26.47±0.00

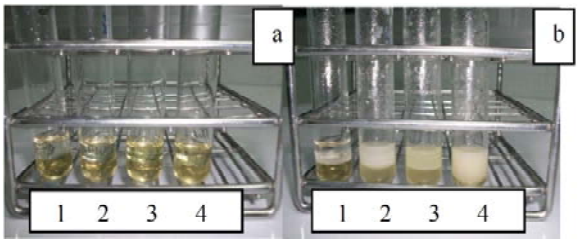


Fig.I Bio-Emulsifier production by IssatchenkiaorientalisWK 12, before (a) and after (b) ; 1 : kerosene, 2: soy bean oil, 3: palm oil and 4: rice bran oil

CONCLUSION

The isolation and screening of hydrocarbon degrading-microorganisms from oil contaminated soil that collected from Thailand. The result showed the existence of 101 isolates (50 bacteria isolates and 51 yeast isolates) capable of degrading the waste lubricating oil (WLO). The most active microorganism in the assimilation of WLO and bio-emulsifier production, WK 12 yeast 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 26S rDNA identified the WK 12 isolate to be I. orientalis WK 12.This isolate showed 60.64±0.07 % WLO degrading activity (measured by weight loss method) when grow on basal salt medium (BSM) containing 1.0% WLO as sole carbon source, whereas the control showed 15.00±0.00%. In addition, I.orientalis WK 12 produced bio-emulsifier with rice bran oil with the best emulsification activity (%EA) of 86.67±0.00%and also can emulsify xylene as a hydrocarbon with 54.55±0.00%. Thus, when application by added of the crude bio-emulsifier to the oil contaminated water or soil could promote the biodegradation of the hydrocarbons.

ACKNOWLEDGMENT

The authors wish to thank the Biology Laboratory of the Department of Applied Biology, Faculty of Sciences and Liberal Arts, Rajamankala University of Technology Isan, NakhonRatchasima, Thailand.

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