The Journal of Microbiology, September 1999, p. 128-135
Vol. 37, No. 3

Identification and Characterization of an Oil-degrading Yeast, *Yarrowia lipolytica* 180

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(Received July 28, 1999 / Accepted August 26, 1999)

Among oil-degrading microorganisms isolated from oil-polluted industrial areas, one yeast strain showed high degradation activity of aliphatic hydrocarbons. From the analyses of 18S rRNA sequences, fatty acid, coenzyme Q system, G+C content of DNA, and biochemical characteristics, the strain was identified as *Yarrowia lipolytica* 180. *Y. lipolytica* 180 degraded 94% of aliphatic hydrocarbons in minimal salts medium containing 0.2% (v/v) of Arabian light crude oil within 3 days at 25°C. Optimal growth conditions for temperature, pH, NaCl concentration, and crude oil concentration were 30°C, pH 5-7, 1%, and 2% (v/v), respectively. *Y. lipolytica* 180 reduced surface tension when cultured on hydrocarbon substrates (1%, v/v), and the measured values of the surface tension were in the range of 51 to 57 dynes/cm. Both the cell free culture broth and cell debris of *Y. lipolytica* 180 were capable of emulsifying 2% (v/v) crude oil by itself. They were also capable of degrading crude oil (2%). The strain showed a cell surface hydrophobicity higher than 90%, which did not require hydrocarbon substrates for its induction. These results suggest that *Y. lipolytica* has high oil-degrading activity through its high emulsifying activity and cell hydrophobicity, and further indicate that the cell surface is responsible for the metabolism of aliphatic hydrocarbons.

Key words: *Yarrowia lipolytica* 180, hydrocarbon degradation, cell hydrophobicity, bioemulsification

Degradation of petroleum hydrocarbons by microorganisms has been extensively studied since ZoBell's work in 1946 (34). It has been reported that hundreds of species of microorganisms including bacteria, actinomycetes, cyanobacteria, and fungi are involved in hydrocarbon degradation (2, 34). In particular, yeasts are well known to utilize alkane, alkene, thiophene, and polyaromatic hydrocarbons, and have been studied for the production of single cell protein and emulsifying agents for increased oil recovery from oil tanks for several decades (17).

The growth of microorganisms on hydrocarbons requires the transport of substrate from the oil phase into cells. The processes by which alkanes are transported to cells and subsequently assimilated by cells are still not fully understood. Oil-degrading yeasts are known to produce emulsifying substances to form micelles or microdroplets of oil. *Yarrowia lipolytica*, which was previously classified as *Candida lipolytica*, has been known to produce extracellular emulsifying substances (21, 24) under the growth on hexadecane. In many instances biosurfactants can stimulate the biodegradation of hydrocarbons, especially in the early phase of hydrocarbon assimilation (10, 14, 22, 33). However, biosurfactants can also inhibit the biodegradation genus specifically (10) or substrate specifically (9). Besides the production of biosurfactants, cell hydrophobicity can be considered one of the important factors controlling hydrocarbon assimilation. Cells with higher hydrophobicity have a greater chance to adhere to oil droplets than those with lower hydrophobicity. Microbial adhesion to hydrocarbons was the subject of a pioneering study by Mudd in 1924 (23) and more extensive ones over the last 20 years (8, 23, 26, 29).

This study deals with the identification and characterization of an *n*-alkane utilizing yeast, *Yarrowia lipolytica*. The oil degradation activity, emulsifying activity, and cell hydrophobicity of the strain were also investigated in their relation to *n*-alkane utilization.

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Materials and Methods

Isolation of crude oil-degrading microorganisms

To isolate microorganisms with high oil-degrading activity, sediment and soil samples were collected from

oil polluted coastal and industrial areas. Samples were diluted with sterilized aged sea water (ASW) / distilled water (DW) mixture (3:1, v/v) and then spread on the mineral salts agar medium (NH_4Cl, 0.6 g; K_2HPO_4, 0.26 g; ASW, 750 ml; DW, 250 ml; agar, 15 g, final pH 7.5) containing Arabian light crude oil (3). Plates were incubated at 25°C for a week and pure strains were isolated on YM agar medium (yeast extract, 3 g; malt extract, 3 g; bactopeptone, 5 g; dextrose, 10 g; ASW, 750 ml; DW, 250 ml; agar, 15 g, final pH 7.2).

Among the isolated oil-degrading microorganisms one was selected for this study. The strain was a yeast strain isolated from oil-contaminated soil sampled in the Panweol industrial region, Republic of Korea.

Identification of the yeast strain

Morphological characteristics of the isolated strain was observed on YM agar medium and the formation of ascospores was checked after 2 weeks of incubation on the same medium. Physiological and biochemical characteristics were tested using API 20 C AUX and Biolog YT Kit. Coenzyme Q was extracted by the method of Yamada *et al.* (31) and analysed by HPTLC (Merck, 10 × 10 cm) using a solvent system of acetone-acetonitrile (80:20). The G+C content of DNA was analysed following the method by Tamaoka and Komagata (28). DNA was hydrolyzed to nucleosides using nuclease PI and bacterial alkaline phosphatase, and then analysed using reversed-phase HPLC. To analyse the fatty acid composition of the cell, the isolated strain was cultured in SDA (Sabraud Dextrose Agar, Difco) medium and incubated at 28°C for 24 h. Fatty acid was extracted from 40~50 mg of cell mass obtained during the latent log phase as described by Lee *et al.* (16). The extracted fatty acid was analysed by the Microbial Identification System, which consisted of a fused silica capillary column (25 m × 0.2 mm) and gas liquid chromatography (Hewlett-Packard 5890A). The temperature of the column increased from 170°C to 270°C at a rate of 5°C/min, and temperatures in the injector and the detector were 250°C and 300°C, respectively. The flow rate of the carrier gas, H_2 (ultra high purity), was 30 ml/min. The volume of the sample was 2.0 µl.

PCR amplification and determination of partial 18S rRNA gene sequences

Partial 18S rRNA gene was amplified from genomic DNA, prepared by using a Wizard genomic DNA kit (Promega, Madison, WI) according to the manufacturer's instructions. 18S rDNA primers used for amplification were modified P108 (5'-CTGGTTGATCCTGCCAGT-3'; nucleotides 4 to 21 *Saccharomyces cerevisiae* 18S rRNA gene) and M2130 (5'-TAAATCCAAGAATTTCACC-3'; nucleotides 918 to 900) (11). The reaction mixtures for PCR contained 1 × PCR buffer, deoxynucleotide triphosphates at a concentration of 200 µM, 1.5 mM $MgCl_2$, each primer at a concentration of 0.2 µM, 100 ng DNA template, and 2.5 U of *Taq* DNA polymerase (Promega) in a final volume of 100 µl. DNA amplification was performed using a model 2400 thermal cycler (PE Applied Biosystems, Inc., Foster City, CA) with 5 min initial denaturation at 94°C, and 35°C cycles consisting of denaturation (1 min at 94°C), annealing (1 min at 55°C), and extension (2 min at 72°C), and final extension at 72°C for 7 min. The amplified DNA was visualized by electrophoresis of aliquots of PCR mixtures (5 µl) in 0.8% agarose in 1 × TAE (40 mM Tris, 20 mM acetic acid, 1 mM EDTA) buffer. The PCR products were purified with a Wizard PCR Preps kit (Promega) and cloned into pGEM-T Easy Vector (Promega). Recombinant plasmid DNA was prepared using a Wizard Plus SV miniprep kit (Promega). Sequencing of the plasmid was performed using a Big-Dye terminator cycle sequencing kit (PE Applied Biosystems) and an Applied Biosystems model 377 automatic DNA sequencer (PE Applied Biosystems).

Analysis of sequences

Analyses of the 18S rRNA gene sequences were performed using SIMILARITY_RANK from the ribosomal database project (RDP) (18) and Basic Local Alignment Search Tool (BLAST) (1). These analyses were used to determine partial 18S rRNA sequences to estimate the degree of similarity to other 18S rRNA gene sequences. The partial 18S rRNA gene sequences were manually aligned to 18S rRNA sequence data from the RDP. The Phylogenetic Inference Package (PHYLIP), version 3.57c (6) was used to further analyze the sequence data, DNADIST performed with Jukes-Cantor option, was employed to determine sequence similarities, and FITCH was used to create a phylogenetic tree. Sequences of the following organisms were utilized in the phylogenetic analysis and were obtained from the GenBank: *Candida albicans* ATCC 18804 (GenBank accession no. M55626), *Candida glabrata* str. CBS 138 (X51831), *Candida krusei* ATCC 6258 (M60305), *Debaryomyces hansenii* NRRL Y-7426 (X62649), *Dekkera bruxellensis* MUCL 27700 (X58052), *Galactomyces geotrichum* MUCL 28959 (X69842), *Pichia anomala* MUCL 28639 (X58054), *Pichia membranifaciens* MUCL 30004 (X58055), *Rhodotorula glutinis* MUCL 30253 (X69853), *Rhodotorula gramnis* (X83827), *Rhodotorula mucilaginosa* (X84326), *Saccharomyces cerevisiae* (V01335), *Yarrowia lipolytica* ATCC 18942 (M60312), and *Zygosaccharomyces rouxii* MUCL 30254 (X58057).

Growth conditions for the yeast strain

To determine the effect of environmental factors such as temperature and pH on the cell growth, the

cells were inoculated into 100 ml of YM broth and then incubated under various conditions. The growth rate was obtained by measuring optical density at 660 nm using Spectronic 21D (Milton Roy). To determine the optimum concentration of oil for growth, yeast cells were inoculated into mineral salts medium containing various concentrations of oil. After incubation at 25°C for 3 days with shaking at 120 rpm, the number of colonies formed on YM agar medium were estimated.

Measurement of oil-degrading activity

To estimate the oil-degrading activity, seed culture was prepared by inoculating one loop of microbial cells into 10 ml of mineral salts medium containing 0.2% (v/v) of Arabian light crude oil. After growth in a rotary shaker (120 rpm) at 25°C for one day, 1 ml of the seed culture broth was transferred into a 250 ml Erlenmeyer flask containing 50 ml of mineral salts medium supplemented with 0.2% (v/v) Arabian light crude oil. The culture was incubated in a rotary shaker (120 rpm) at 25°C for 3 days and the amount of degraded oil was determined. A flask under the same conditions without microorganisms served as the abiotic control.

Extraction and analysis of crude oil after biodegradation

After incubation, the residual oil in the culture medium was extracted twice using the same volume of chloroform with the addition of squalene (n-C_{30}, 10% of added crude oil) as an internal standard. The extract was treated with anhydrous Na_2SO_4 (10 g) and Cu powder (10 mg) to remove water and sulfur, respectively. The solvent was evaporated and the residue was redissolved in 1 ml of chloroform. The prepared sample was introduced into a silica-alumina column (30). After two successive elutions with 25 ml of hexane and 25 ml of chloroform, the aliphatic fraction and aromatic fraction were separated. The aliphatic fraction was analyzed using a gas chromatograph (Hewlett-Packard, HP 5890) equipped with a methyl silicon capillary column (SPB-1, Supelco). The GC conditions were: carrier gas (N_2) flow rate, 45 ml/min; injector (splitless) temperature, 280°C; oven temperature, from 100°C to 280°C at a rate of 8°C/min; detector temperature, 260°C.

Measurement of surface tension

Production of the extracellular emulsifying agent of the yeast was examined. Aliphatic hydrocarbons from octane to squalene and some organic nutrients were used as substrates. Surface tension was measured according to the method by Zajic *et al.* (32). Cells in mineral salts medium (20 ml) containing each carbon source (1%, v/v for liquid substrates and w/v for solid substrates) were cultured in a rotary shaker (120 rpm) at 25°C for 3 days. The culture broth was centrifuged at 12,000 rpm for 15 min. The aqueous phase was taken and then filtered through a 0.2 μm membrane filter (Green filter). Surface tension of the filtrate was measured using a ring tensiomat (Surface Tensiomat 21, Fisher Scientifics) and the mean value of eight measurements was calculated.

Measurement of hydrophobicity

Hydrophobicity of the yeast cells was measured using the method of Rosenberg (26). After the culture broth was centrifuged as described for the determination of surface tension, the recovered cells were washed twice with buffer solution (K_2HPO_4, 16.9 g; KH_2PO_4, 7.3 g; urea, 18.0 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; DW 1 L, pH 7.0) and resuspended in 4.0 ml of the same buffer solution to obtain an absorbance of 1.0 at 400 nm. After addition of 1.0 ml of hexadecane, the solution was vortexed for 60 sec and the aqueous phase was obtained after standing for 30 min. Light absorbance of the aqueous phase was measured at 400 nm. Hydrophobicity was expressed as the percentage of adherence to hexadecane which was calculated using the following equation:

$$\% \text{ Hydrophobicity} = 100 \times (1 - A_{400} \text{ of aqueous phase} / A_{400} \text{ of the cell suspension})$$

Preparation of cell free culture broth and cell debris of *Y. lipolytica* 180

For the preparation of cell free culture broth (CFCB) and cell debris (CD), *Y. lipolytica* 180 was cultured in mineral salts medium containing 1% (v/v) of heptadecane for 4 days at 25°C with shaking at 120 rpm. Cells were removed by centrifugation at 9,000 rpm for 20 min and the supernatant was filtered through a membrane filter (0.2 μm, Green filter). Fifty milliliter aliquots of the supernatant were transferred to 250 ml Erlenmeyer flasks and crude oil was added to obtain a concentration of 2% (v/v). Cell pellets were washed twice with Ringer solution (pH 7.0) and resuspended in 250 ml of 0.25 M sucrose-phosphate buffer solution. The suspension was treated with a Bead beater homogenizer (Biospec Products) in the presence of 0.5 μm glass beads for cell breakage. After washing the treated cells twice with Ringer solution at 5,000 rpm for 15 min, the pellets were weighed and resuspended in 50 ml of sterile ASW. The suspension was transferred to 50 ml of mineral salts medium with 2% (v/v) crude oil to obtain final cell debris concentrations of 0.024%, 0.12%, and 0.24% (wet w/v). The prepared CFCB and CD solutions were incubated at 25°C for 3 days with shaking at 120 rpm to determine the oil-emulsifying activity.

Results and Discussion

Isolation of crude oil-degrading microorganism

A total of 78 microorganisms, which showed growth on Arabian light crude oil, were isolated from oil polluted coastal areas and industrial regions in Republic of Korea. Among these strains, a yeast strain showed a 94% degradation rate of saturated aliphatic components in crude oil within 3 days of incubation at 25°C. Fig. 1 shows the changes in the peaks of aliphatic hydrocarbon compounds in crude oil before and after cultivation with the isolated yeast strain. Most of the aliphatic hydrocarbon peaks disappeared compared to the control, and the ratios of heptadecane to pristane and octadecane to phytane drastically decreased from 2.6 to 0.2 and from 3.5 to 0, respectively. These results imply that the isolated yeast strain is very active for the biodegradation of aliphatic hydrocarbon compounds in crude oil.

Identification of the oil-degrading yeast strain

For identification of the isolated yeast strain, analyses of fatty acid, coenzyme Q system, G+C content of DNA, and physiological characteristics were examined (Table 1). The strain formed white creamy colonies on YM agar medium and cells were divided by budding. One to four round ascospores were formed in an ascus after 2 weeks of incubation indicating that the strain belongs to the typical ascomycetes yeast. As a general characteristic for yeasts, the isolated yeast strain had a coenzyme Q-9 system (data not shown)

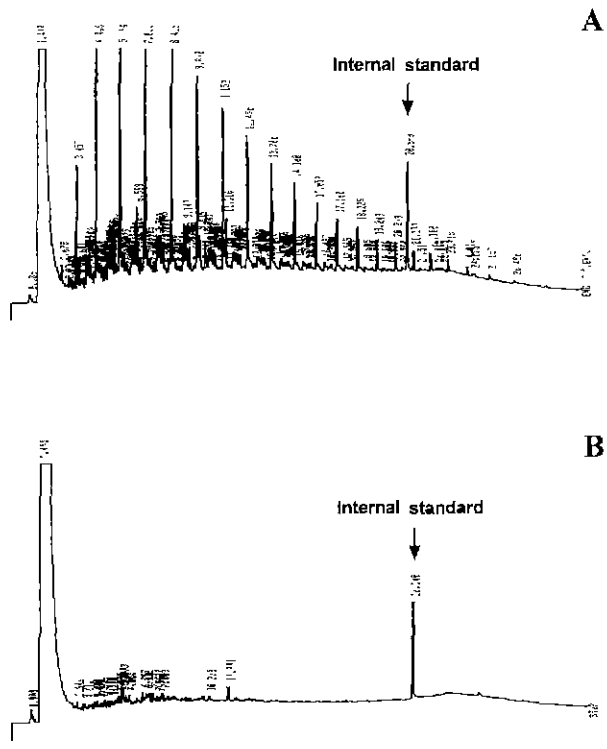

Fig. 1. Gas chromatographic tracings of residual Arabian light crude oil in the control flask (A), and in the flask inoculated with *Y. lipolytica* 180 (B), after 3 days of incubation at 25°C and 120 rpm.

and the G+C content was estimated as 49.6%. The strain can utilize glycerol, erythritol, D-mannitol, D-gluconate, DL-lactate, and N-acetyl-glucosamine. Sor-

Table 1. Morphological and biochemical characteristics of *Y. lipolytica* 180

Characteristics		Characteristics	
Colony color	white to creamy	Growth on	
Reproduction	by budding	Maltose	–
Ascospore	1-4, *round-shape*	Trehalose	–
Coenzyme Q	Q 9	α-Methyl-D-glucoside	–
Mol % of G+C	49.6%	Cellobiose	–
Cellular fatty acid		Melibiose	–
C18:2 cis9,12/C18:0	38.1%	Lactose	–
C18:1 cis9(w9)/C18:1(w8)	31.4%	*Raffinose*	–
C16:0	14.0%	Melezitose	–
C16:1 cis9(w7)	9.4%	Glycerol	+
C18:0	2.1%	Erythritol	++
Fermentation	none	D-Mannitol	+
Urea hydrolysis	+	*myo-Inositol*	–
Growth on		2-Keto-gluconate	–
D-Galactose	–	D-Gluconate	+
L-Sorbose	–	DL-Lactate	+
D-Glucosamine	–	D-Glucuronate	–
D-Ribose	–	N-Acetyl-glucosamine	++
D-Xylose	–	Sorbitol	w
L-Arabinose	–	Palatinose	–
L-Rhamnose	–	Levulinate	–
Sucrose	–		

–: no growth: w: slow growth; +: medium growth; ++: very high growth.

bitol, however, was weakly utilized for growth. Fermentation of various carbon sources was not observed and urease was produced by the strain. API data results showed a high confidence level (% id= 99.9) to *Yarrowia lipolytica* after 24 h and 48 h of growth, and Biolog data analysis matched that of *Yarrowia lipolytica* with a confidence level of % id=0.566 (<0.75) and % id=0.948 at 24 h and 48 h of growth, respectively. From these results, the strain was classified as *Yarrowia lipolytica* with a confidence level of 99.9% and designated as *Y. lipolytica* 180, hereafter.

Yarrowia sp. which was classified as *Candida* sp. previously, has been commonly found as fungal oil-degraders in natural environments. According to reports by Floodgate (7) and Bossert and Bartha (4), *Candida* sp. has been known as one of the major genera of hydrocarbonoclastic fungi in marine, brackish water, and soil environments. The history of hydrocarbon fermentation using yeasts in the production of SCP (Single Cell Protein) was characterized by intense activities in basic research and development in many industrialized nations.

Analyses of 18S rRNA gene sequences

The partial 18S rDNA sequence (approximately 771 bases, positions 22 to 899, *S. cerevisiae* numbering) was obtained for the strain. The SIMILARITY_RANK option from the RDP and BLAST analysis was used to identify the most similar sequences available in the database. The strain closely resembled *Yarrowia lipolytica* (99.7% similarity in 771 bases) and the intergeneric phylogenetic tree was constructed (Fig. 2). The partial sequence of the 18S rRNA gene of *Y. lipolytica* 180 has been submitted to GeneBank database under accession number AF156969.

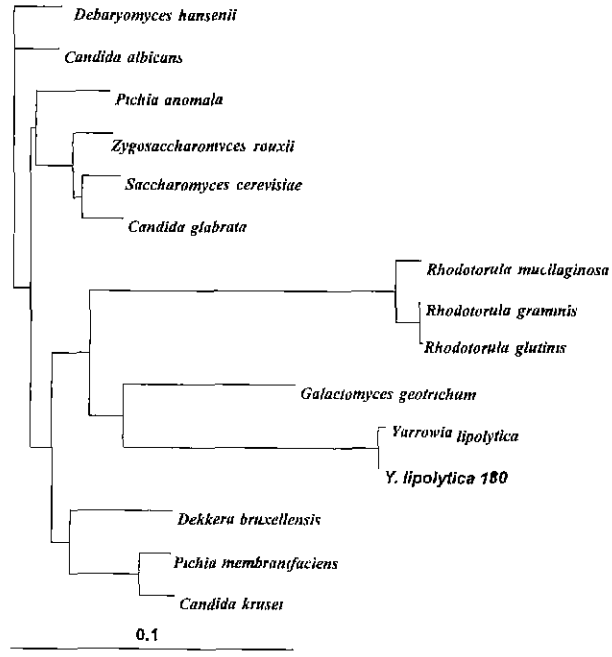

Fig. 2. Phylogenetic tree based on partial 18S rRNA gene sequence data (771 bases) showing the location of *Y. lipolytica* 180. The scale bar represents 0.1 substitution per base position.

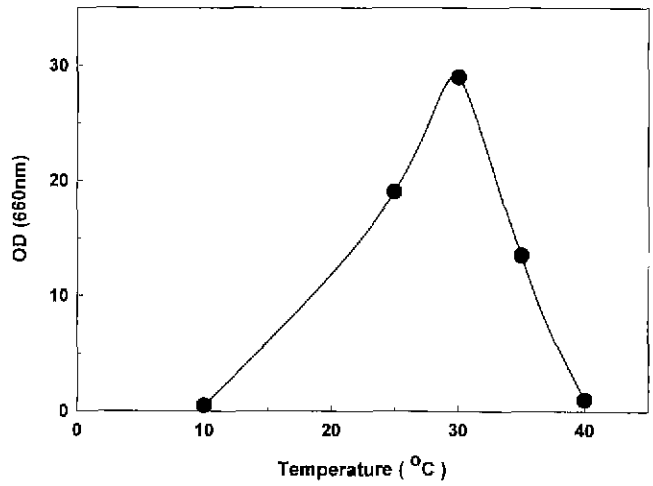

Fig. 3. Effect of temperature on cell growth of *Y. lipolytica* 180.

Effects of temperature, pH, NaCl, and crude oil concentrations on cell growth

Cell growth was maximam at 30°C and the strain did not show any active growth at temperatures higher than 40°C or lower than 10°C within 24 h (Fig. 3). These results indicated that *Y. lipolytica* 180 was a typical mesophilic yeast. *Y. lipolytica* 180 showed growth in a wide pH range, from 3 to 9, with highest growth in the pH range of 5 to 7 (data not shown).

The growth of *Y. lipolytica* 180 on various con-

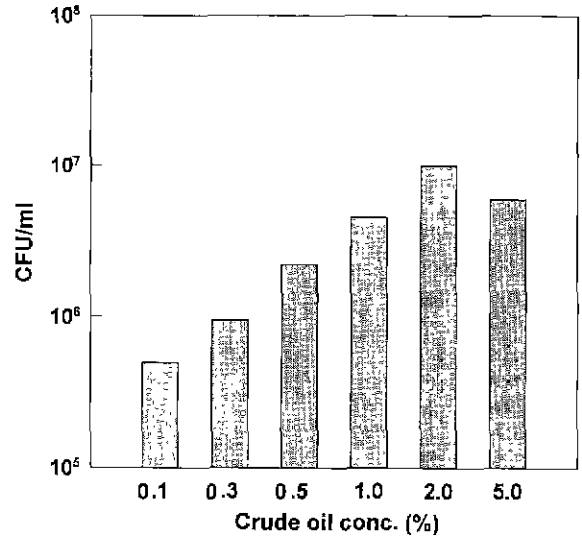

Fig. 4. The effect of crude oil concentration on cell growth of *Y. lipolytica* 180.

centrations of crude oil after 3 days incubation was compared (Fig. 4). The cell number gradually increased as the oil concentration increased up to 2% and maintained at similar levels thereafter. From these results it can be concluded that the yeast strain can be applied to highly oil-contaminated environments in a wide range of temperature and pH without any retardation of growth due to the toxicity of crude oil.

Measurement of surface tension

Growth on hydrocarbon substrates with limited solubility poses unique transport problems for microbes. In many instances, biosurfactants are known to stimulate biodegradation of hydrocarbons through improved dispersion or increased surface area for microbial attachment (10, 14, 22, 33). However, biosurfactants can also inhibit biodegradation and may interfere with the interaction between biosurfactant-dispersed substrates and microbial cells (9, 25).

Cell growth and surface tension were measured after *Y. lipolytica* 180 was incubated at 25°C for 3 days in mineral salts medium containing various carbon sources and the results are presented in Table 2. A decrease in the surface tension of the medium implies the enhancement of emulsifying activity resulting from biosurfactant production during cultivation. Cells grown on petroleum compounds showed a lower surface tension value than cells grown on water soluble organic substrates such as glucose, yeast extract, and bactopeptone. The viable counts of cells grown on glucose was comparable to those on decane or hexadecane. However, cells grown on glucose showed much lower emulsification activity compared to the activities of cells grown on decane or hexadecane. This result is similar to the observation reported by Cirigliano and Carman (5) with *C. lipolytica* producing an inducible extracellular emulsifier named liposan. Among the hydrocarbon compounds, octane and squalene which did not serve as growth substrates showed a higher surface tension value compared to other hydrocarbon compounds. From these results it could be concluded that the production of an emulsifying substance was inducible in the presence of utilizable hydrocarbons by *Y. lipolytica* 180.

Table 2. Effect of carbon sources on cell growth and surface tension value after 3 days of incubation at 25°C

Carbon Source (1%)	Viable count[1] (× 10^6 CFU/ml)	Surface tension[2] (Dynes/cm)
Glucose	20.0	70.9±1.4
Yeast Extract	8.0	70.8±1.7
Bacto Peptone	8.5	67.3±2.1
Methanol	2.3	67.3±2.1
Ethanol	1.3	55.9±0.3
Pristane	4.3	51.4±1.1
Paraffin oil	5.3	57.3±1.4
Kerosene	7.7	55.3±0.8
Octane	2.7	69.9±1.0
Decane	22.0	55.6±2.5
Dodecane	43.5	53.1±0.3
Tridecane	44.0	54.9±2.0
Tetradecane	15.0	55.5±1.8
Hexadecane	25.0	55.9±1.2
Heptadecane	80.0	53.2±1.3
Squalene	1.2	67.6±2.6
Crude oil	4.6	55.3±1.0

[1] Inoculum size of *Y. lipolytica* 180 was 1.9×10^6 CFU/ and viable counts in the control experiment after 3 days was 1.1×10^6 CFU/ml.

[2] Surface tension value of sterilized minimal salts medium represented 75.4±0.0 dynes/cm.

Effect of substrates on cell hydrophobicity

To evaluate the effect of substrates on cell hydrophobicity of *Y. lipolytica* 180, changes in cell numbers, pH and hydrophobicity under the growth on crude oil (1%, v/v) or glucose (1%, w/v) were observed (Fig. 5). Hydrophobicity indicates a potential of cells adhering to insoluble hydrocarbon substrates (21). Hydrophobicity of cells is important in hydrocarbon degradation, and it has been known that cells with higher

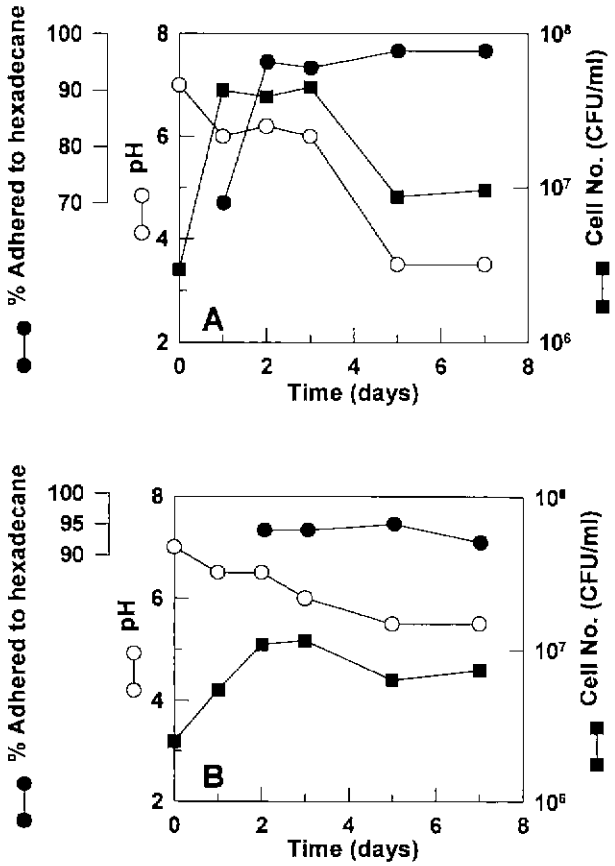

Fig. 5. Effects of glucose (A) and crude oil (B) on pH, hydrophobicity, and cell growth of *Y. lipolytica* 180.

hydrophobicity have a potential to utilize hydrocarbons more effectively than cells with lower hydrophobicity (20, 27). Both *Y. lipolytica* 180 cells grown on crude oil and glucose showed hydrophobicity greater than 90%. These results indicate that the hydrophobicity of *Y. lipolytica* 180 is not induced by the presence of hydrocarbon substrates, however, the strain itself has a high adhering tendency to hydrocarbons. These results contrast other studies which reported that *C. tropicalis* cells grown on alkane showed higher hydrophobicity than cells grown on glucose (12, 13).

Emulsifying activity of cell free culture broth and cell debris of *Y. lipolytica* 180

Both the CFCB and CD of *Y. lipolytica* 180 showed strong emulsification activities as shown in Table 3. CD and CFCB at concentrations higher than 0.12% degraded as much as 34% of added oil (2%, v/v) in the absence of living cells. These results indicated that some components in the cell wall or membrane as well as certain extracellular materials released from the cells of *Y. lipolytica* 180 had the ability for emulsification as well as transformation and/or degradation of crude oil. It was reported that the cell wall of *Candida rugosa* and *C. lipolytica* played an important role in the incorporation of hydrocarbons into the cells, whereas cell wall of *C. tropicalis* did not take any part in the process of *n*-alkane oxidation (19). Mineki *et al.* (19) showed that spheroplasts of *C. lipolytica* maintained the respiration rate of glucose in the range of 32% to 34%, however, they did not show any respiration activity of *n*-decane.

From these results, it can be concluded that *Y. lipolytica* 180 can easily uptake and degrade hydrocarbons using its high emulsifying activity through which oils can be dispersed as fine emulsions in the water phase. Then the emulsified oils can be adsorbed to the hydrophobic cell surfaces for transportation into cells for further metabolism. The great ability of oil utilization of *Y. lipolytica* 180 seems to be closely associated to cell surfaces and this ability shows a high potential for the application in bioremediation of oil-spilled areas where oil exists in a biphasic suspension. Ko *et al.* (15) observed that the oil biodegradation rates of crude oil was decreased by mixed cultures of petroleum-degraders having different modes of hydrocarbon uptake such as emulsifying activity or cell hydrophobicity. However, it is still unclear how the emulsifying activity and cell hydrophobicity can cooperate within a cell having different modes of hydrocarbon uptake.

Table 3. Oil-degrading activity and emulsifying activity of the cell free culture broth (CFCB) and the cell debris (CD) of *Y. lipolytica* 180

Culture condition	% Degraded[1]	Emulsifying activity[2]
CFCB alone	27	+++
CD (0.024%)	1	+++
CD (0.12%)	34	+++
CD (0.24%)	34	+++
MSM alone	0	–

[1] % Degraded=100×(amount of remained oil in test flask/amount of remained oil in control flask)

[2] Emulsifying activity: +++, Excellent; ++, Very good; +, Good; ±, Poor; –, None

None of m/o, CB or CFCB was introduced into MSM in control flasks.

Acknowledgments

This study was performed under G7 project granted by the Korea Ministry of Environment and Ministry of Science and Technology.

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