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Crude Oil and n-Octadecane Degradation under Saline Conditions by *Fusarium* sp., F092

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ABSTRACT

Biodegradation of crude oil-A and -C and n-octadecane, by the *Fusarium* sp. F092 was investigated under saline conditions. In liquid saline culture, less crude oil-C (56%) than crude oil-A (89%) was degrade which was increased by the addition of optimum surfactant T_{80} (78%) or T_{40} (67%), or Mn^{2+} (67%). In sea sand contaminated with crude oil-C, the degradation was higher than in soil which indicated that F092 was suitable to degrade oil under saline conditions. The metabolites of the aliphatic fraction with n-octadecane were identified to form six carboxylic acid groups, where the dioxygenase and monooxygenase inhibitor influenced the degradation of n-octadecane. This suggested that F092 could initially convert n-octadecane to form octadecyl peroxides by a dioxygenase and was further catalyzed to produce carboxylic acid. F092 is a potential degrader for bioremediation in crude oil-contaminated saline environments.

Key words: Biodegradation, saline, crude oil, n-octadecane, *Fusarium* sp., F092

INTRODUCTION

Crude oil or petroleum is a viscous liquid containing a variety of compounds consisting mainly of carbon and hydrogen. The worldwide production of crude oil is more than three billion tons per year (Harayama *et al*., 1999) and the consumption of petroleum is increasingly related with the huge growth of human populations around the world. Crude oils are typically transported long distances by sea to be refined (Head and Swannell, 1999; Prince and Walters, 2007), but more than 8.8 million tons per-year (Head and Swannell, 1999) enter the marine environment through (1) human activities such as drilling and accidents on offshore rigs, transportation accidents, ruptured pipelines and routine washing of storage tankers and (2) natural seepage (Harayama *et al*., 1999; Kvenvolden and Cooper, 2003; Minai-Tehrani *et al*., 2006a; Chaineau *et al*., 2005; Elshafie *et al*., 2007; Hasanuzzaman *et al*., 2007). This has caused environmental problems in oceans and on coasts.

Petroleum hydrocarbons themselves contain saturates or aliphatics, aromatics, asphaltenes and resins (Atlas, 1981; Harayama *et al*., 1999; Venosa and Zhu, 2003; Prince and Walters, 2007; Jain *et al*., 2011). The relative distribution of these fractions depends on many factors: the source,

age, geological history, migration and alteration of crude oil (Singh, 2006). These components have raised public concern due to their potential for carcinogenic, mutagenic, or toxic effects and changes in composition can lead to variations in overall physical properties and chemical toxicity (Yang *et al*., 2009). The aliphatic fraction is usually the largest followed by the aromatics and its removal from contaminated ocean and coastal areas has become an environmental priority. The use of microorganisms to reduce petroleum through bioremediation has been shown to be a viable, relatively low cost, low-tech approach and is more widespread than both chemical and physical treatments (Bogan and Lamar, 1996; Kang and Oulman, 1996; Vidali, 2001; Erdogan and Karaca, 2011). However, the water or soil around oil fields is saline and it is more difficult to obtain a powerful degrading microorganism because the rate of degradation decreases with increasing salinity and pH (Venosa and Zhu, 2003). Consequently, bioremediation of petroleum hydrocarbons in marine environments has been little attempted.

Numerous researchers have examined the ability of bacteria to degrade petroleum in saline conditions (Mille *et al*., 1991; Thavasi *et al*., 2007; Feijoo-Siota *et al*., 2008; Hao and Lu, 2009; Kumar *et al*., 2009; Sarma and Sarma, 2010), but few studies of fungi have been reported. Microbial degraders have been shown to act by degrading the short-chain aliphatics or lower-molecular-weight aromatics of oil and other fractions, resins and asphaltenes, considered more recalcitrant only showed low rates of biodegradation (Atlas, 1981; Lal and Khanna, 1996). However, the aliphatic and Polycyclic Aromatic Hydrocarbons (PAHs) with high carbon numbers are considered poorly degradable due to their low solubility and high hydrophobicity. A surfactant may be useful for enhancing crude oil degradation at saline sites because it has the ability to increase dissolution into the aqueous phase and change the affinity between the microbial cell and hydrocarbon by increasing cell surface hydrophobicity (Paria, 2008). *Fusarium* sp., F092 was isolated based on the ability to degrade chrysene under saline conditions (Hidayat *et al*., 2012) and can degrade the aliphatic fraction in crude oil (Hidayat and Tachibana, 2012). However, whether it degrades all fractions and aliphatic degradative pathways in crude oil are not yet clearly understood. The present study was carried to (a) investigate the ability of *Fusarium* sp. F092 to degrade crude oil in both liquid-saline and sea sand, (b) evaluate the effect of Mn^{2+}, Tween 40 (T_{40}) and Tween 80 (T_{80}) on bioavailability for crude oil degradation and (c) identify metabolites of aliphatics of petroleum using a representative compound, n-octadecane.

MATERIALS AND METHODS

This research was conducted at the Faculty of Agriculture, Ehime University, Japan in 2011.

Chemicals: Crude oil-C was obtained from Taiyo Petroleum Co. Ltd., (Japan). Crude oil-A was collected from sea water polluted by an oil spill in Kure city, Hiroshima, Japan. C-200 silica gel and other chemicals were purchased from Wako. Co. Ltd., (Japan). Piperonyl butoxide and n-octadecane were purchased from TCI. Co. Ltd., (Japan). The material for synthetic sea water was obtained from Delphis (Japan).

Degradation of crude oil: Biodegradation of crude oil was conducted in liquid, soil and sea sand cultures. Crude oil was added to Potato Glucose (PG) liquid medium (Atlas, 2004), as the best culture medium for the isolate F092, containing (per-liter of H_2O) potato extract 500 g, glucose 20 g, yeast extract 5 g and artificial sea water 35 g, to a final concentration of 1000 mg L^{-1}. The medium was stood overnight on a clean bench for evaporation of the solvent solution in crude oil. Three 5 mm diameter fungal disks punched out with a cork-borer from an active growing fungus

on agar plates were inoculated into the culture. The culture was incubated at 25°C and the degradation was analyzed at 15, 30 and 60 days. The effect of Mn^{2+}, surfactant Tween 40 (T_{40}) and Tween 80 (T_{80}) on the degradation of crude oil-C was studied. A negative control was also prepared to confirm the loss of crude oil during the incubation and quantification process. In all experiments, the cultures were grown in triplicate.

Crude oil also was added to both soil and sea sand to a final concentration of 1000 mg L^{-1}. The soil was collected from the surface layer (0-20 cm), pH 6.16 (in water with the ratio 1:5) and had no history of waste disposal. The soil of microcosms was air-dried, passed through a 3 mm sieve and homogenized. The sea sand was taken 20 m from the shoreline in the surface layer (0-20 cm), pH 8.25 (in water, 1:5) and had any history of waste disposal. Prior to use, samples were autoclaved (121°C for 180 min) to eliminate microorganisms. A fungus pre-grown in PG liquid medium for 7 days was applied to both soil and sea sand. The culture was incubated and degradation was analyzed as previously explained.

After a fixed time, Total Petroleum Hydrocarbon (TPH) concentrations were determined by extraction and purification using several solvents by the modified method described by Chaineau *et al.* (2005). Briefly, the culture was extracted three times using n-hexane, dichloromethane (CH_2Cl_2) and chloroform ($CHCl_3$) with 100 mL of each solvent. The extracts were analyzed gravimetrically, yielding aliphatic, aromatic, Nitrogen-Sulfur-Oxygen (NSO) and asphaltene fractions. n-Hexane was used to separate the fractions. The n-hexane-soluble fraction was further separated to obtain aliphatic, aromatic and NSO fractions by purification on a chromatography column filled with 5 g of C-200 silica gel and anhydrous Na_2SO_4 eluted with 100 mL of n-hexane, 100 mL of toluene and 200 mL of a combination of $CHCl_3$ and methanol at a ratio of 1:1 (v/v). The n-hexane-insoluble fraction consisted of asphaltenes and some organic compounds. The weight of each residual fraction was recorded. The aliphatic and aromatic fractions were analyzed by gas chromatography (GC-FID Shimadzu 2014) with a TC-5 capillary column (length: 30 m, id: 0.24 mm). The carrier gas was helium delivered at a constant rate of 1.5 mL min^{-1} with a column pressure of 100 kPa and interface temperature of 280°C. The temperature program was started at 60°C and increased at 10°C min^{-1} to 280°C where it was maintained for 10-20 min to allow late eluting compounds to exit the column. The injection volume was 2 μL and the injector temperature was maintained at 280°C.

Degradation of n-octadecane: The degradation of n-octadecane (initial concentration, 0.5 mM) was investigated. Three disks (5 mm diameter) obtained with a cork-borer from a fungus actively growing on agar were placed into each flask culture containing PG medium. The inoculated flasks were pre-incubated for several days to obtain similar radial growth and minimize growth variation. This was followed by the addition of n-octadecane solubilized in dimethylformamide (DMF), T_{80} and water. After a fixed time (15, 30 and 60 days), the culture flask was acidified with 5 mL of 1 N HCl. The culture was blended at 10000 rpm for 10 min and extraction was conducted with 3 volumes (40 mL) of ethyl acetate. The ethyl acetate extract was dried over anhydrous Na_2SO_4 and evaporated dry in a vacuum at 40°C. The concentrations of compounds were quantified by gas chromatography (GC-FID Shimadzu, 2014) with the same methods mentioned above. The monooxygenase and dioxygenase-based inhibition of n-octadecane degradation was carried out as previously reported (Tsai and Li, 2007; Mori *et al.*, 2009). The cultures were incubated for 15 days and degradation and the formation of metabolite products were estimated.

In order to identify the molecular structure of metabolites, the dried extracts were methylated with trimethylsilylation (TMS), n,O-bis-trimethylsilyl acetamide (40 μL) and trimethylchlorosilane

(40 µL) in pyridine (80 µL), prior to the GC-MS analysis. The methylated sample and authentic standard were analyzed by gas chromatography with a mass spectrometer (GC-MS Shimadzu QP-2010) equipped with a TC-1 column (30 m, id: 0.24 mm). The carrier gas, helium, was delivered at a constant rate of 1.5 mL min^{-1}, with a column pressure of 100 kPa and interface temperature of 280°C. The temperature program was 60°C and increased at 10°C min^{-1} to 280°C where it was maintained for 10-20 min to allow late eluting compounds to exit the column. The injection volume was 1 µL and the injector temperature was maintained at 280°C. The conditions for GC-MS consisted of a scan interval of 1.3 eV (per-second) and a mass range of 40-700.

Statistical analysis: All results are presented as Mean±SD of triplicates. Data were analyzed for significant differences between treatments using a one-way analysis of variance (ANOVA). To establish if differences ($p<0.05$) were significant between individual treatments, a T test was applied. All analyses were done using SPSS version 15 for windows.

RESULTS

Degradation of crude oil: Crude oil-A contained mostly the aliphatic fraction (75%) followed by the aromatic (9%), asphaltene (13%) and NSO (3%) fractions. The aliphatic fraction was composed of C_{12}-C_{20} including linear and branched hydrocarbons. The degradation of crude oil-A was evaluated by inoculation of *Fusarium* sp., F092 into saline liquid culture. This strain degraded 89% of TPHs (from 1000-108 mg L^{-1}) at 60 days: mostly aliphatics followed by the aromatic, NSO and asphaltene fractions (Fig. 1).

Crude oil-C was also composed mostly of the aliphatic fraction (44%) followed by the aromatic (31%), NSO (8%) and asphaltene (17%) fractions. The degradation of crude oil-C was performed in saline liquid culture with F092 as the degrader. This strain degraded crude oil-C from 1000 to 554 mg L^{-1}, to 551 and to 439 mg L^{-1} (Fig. 1). The degradation was 56% at 60 days as a result of the degradation of aliphatic and aromatic fractions. The degradation of TPHs was 78 and 67% on addition of 0.5% of T_{80} and T_{40} at 60 days. The effect of T_{80} and T_{40} on crude oil-C degradation

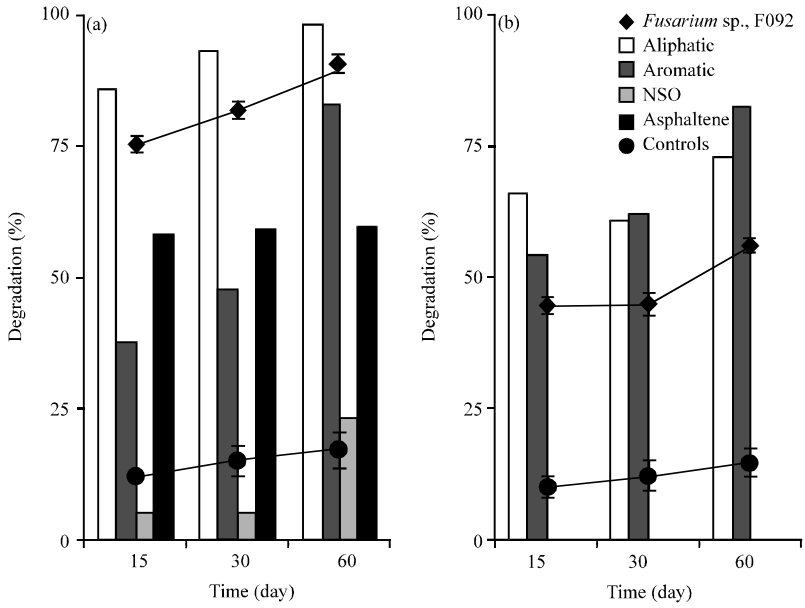

Fig. 1(a-b): Degradation of crude (a) Oil-A and (b) Oil-C by *Fusarium* sp. F092

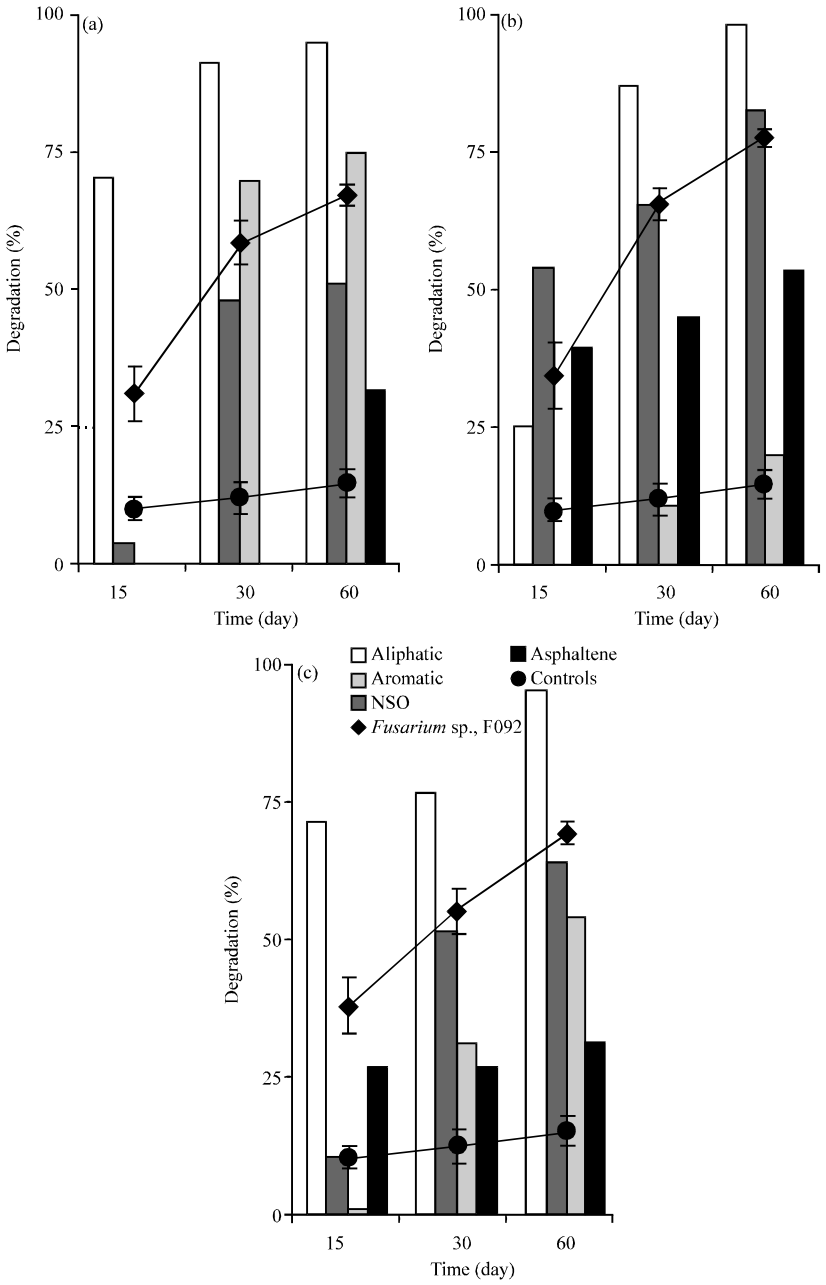

Fig. 2(a-c): (a) Effect of Mn^{2+}, (b) T80 and (c) T40 on degradation of crude oil-C by *Fusarium* sp., F092

resulted in an increase in the aliphatic fraction (35 and 27%), NSO fraction (20 and 52%) and asphaltene fraction (53 and 30%) while the increase in the aromatic fraction was only 1% on addition of T_{80}.

The effect of Mn^{2+} on the degradation of crude oil-C by F092 was also investigated, given that this strain could secrete ligninolytic enzymes. The degradation of TPH was 67% at 60 days when the culture contained 100 mg L^{-1} of Mn^{2+}. It increased in the aliphatic fraction (23%), NSO fraction (75%) and asphaltene fraction (31%), but not the aromatic fraction (Fig. 2). The capacity of F092

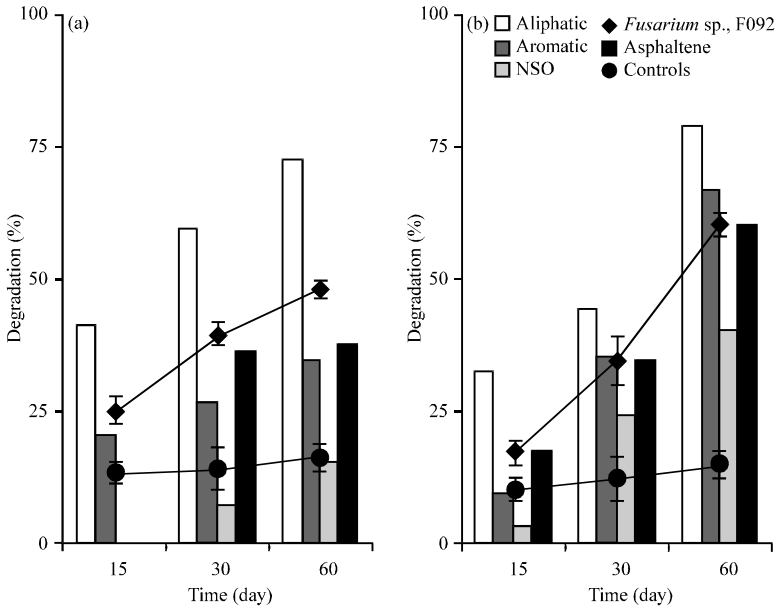

Fig. 3(a-b): Degradation of crude oil-C by *Fusarium* sp. F092 in (a) Soil and (b) Sea sand

to degrade crude oil-C was also investigated in both soil and sea sand cultures. In the sea sand contaminated with crude oil-C (Fig. 3), the degradation of TPH was 60% as a result of degradation of the aliphatic (78%), aromatic (67%), NSO (40%) and asphaltene (60%) fractions. The ability of F092 to degrade crude oil in sea sand was higher than in soil ($p<0.05$) at 60 days.

Degradation of n-octadecane: *Fusarium* sp., F092 degraded 89% of n-octadecane (from about 125-13 mg L^{-1}) at 60 days (Table 1). The metabolites produced during the degradation of n-octadecane were also investigated in saline liquid culture. Based on GC-MS, six of the major metabolites were detected after trimethylsilylation (Table 2). The first metabolite had a retention time (Rt) of 19.367, a molecular ion [M^+] at m/z 356 and fragmentation ions at m/z 341 (M^+ -CH_3) and 283 (M^+ -$Si(CH_3)_3$), identical to octadecanoic acid. The second metabolite had a Rt of 18.458, a molecular ion [M^+] at m/z 342 and fragmentation ions at m/z 327 (M^+ -CH_3) and 269 (M^+ -$Si(CH_3)_3$), identical to heptadecanoic acid. The third metabolite had a Rt of 17.508, a molecular ion [M^+] at m/z 328 and fragmentation ions at m/z 313 (M^+ -CH_3) and 255 (M^+ -$Si(CH_3)_3$), identical to hexadecanoic acid. The fourth metabolite had a Rt of 16.508, a molecular ion [M^+] at m/z 314 and fragmentation ions at m/z 299 (M^+ -CH_3) and 241 (M^+ -$Si(CH_3)_3$), identical to pentadecanoic acid. The fifth metabolite had a Rt of 15.467, a molecular ion [M^+] at m/z 300 and fragmentation ions at m/z 285 (M^+ -CH_3) and 227 (M^+ -$Si(CH_3)_3$), identical to tetradecanoic acid. The sixth metabolite had a Rt of 13.250, a molecular ion [M^+] at m/z 272 and fragmentation ions at m/z 257 (M^+ -CH_3) and 199 (M^+ -$Si(CH_3)_3$), identical to that of authentic dodecanoic acid. After 15 days' incubation, three of six metabolites were detected and the major metabolite was hexadecanoic acid (47%) followed by octadecanoic acid (18%) and tetradecanoic acid (1%) (Table 1). All metabolites could be detected after 30 days incubation and the major metabolite was octadecanoic acid (41%). At the end of the incubation, heptadecanoic and pentadecanoic acid had disappeared and concentrations of other metabolites were lower than previously but not for dodecanoic acid (Table 1).

Table 1: Degradation by *Fusarium* sp., F092 and metabolites of n-octadecane

				Metabolite product													
		Target (C18H38)		Total		Dodecanoic acid		Tetradecanoic acid		Pentadecanoic acid		Hexadecanoic acid		Heptadecanoic acid		Octadecanoic acid	
Incubation (d)	Treatments (mM)	Recovery (mg L^{-1})	Degradation (%)	(mg L^{-1})	(%)	(mg L^{-1})	(%)	(mg L^{-1})	(%)	(mg L^{-1})	(%)	(mg L^{-1})	(%)	(mg L^{-1})	(%)	(mg L^{-1})	(%)
15	Control	41.4±1.9	67.0±1.7	83.9	67.1	-	-	1.3	1.0	-	-	59.9	47.8	-	-	22.8	18.2
	PB																
	0.1	44.7±0.9	64.8±0.7	99.3	69.0	-	-	7.5	5.2	-	-	75.7	52.5	2.9	2.0	13.3	9.2
	0.5	43.3±5.4	66.0±4.2	96.4	67.1	-	-	3.5	2.4	-	-	78.3	54.5	2.7	1.9	11.9	8.3
	1.0	36.7±2.1	71.1±1.6	90.1	64.7	-	-	6.9	5.0	-	-	70.1	50.4	2.4	1.7	10.7	7.7
	2.0	46.3±2.1	63.6±1.6	104.4	75.0	-	-	6.8	5.0	-	-	86.6	62.2	2.2	1.6	8.7	6.3
	Ag NO_3																
	0.1	55.5±1.9	56.3±1.5	83.1	59.6	-	-	-	-	-	-	-	-	2.0	1.4	81.1	58.2
	0.5	65.2±3.8	48.7±3.0	86.3	62.0	-	-	-	-	-	-	-	-	0.6	0.4	85.6	61.6
	1.0	88.2±1.9	30.6±1.5	47.9	34.6	-	-	-	-	-	-	-	-	-	-	47.9	34.6
	2.0	96.3±1.9	24.2±1.5	34.2	24.5	-	-	-	-	-	-	-	-	-	-	34.2	24.5
30	Control	31.5±7.4	75.0±6.0	94.2	75.0	0.4	0.3	2.7	2.2	0.9	0.7	36.6	29.1	1.2	0.9	52.5	41.7
60	Control	13.9±2.1	88.9±1.6	35.5	28.2	0.9	0.8	1.9	1.5	-	-	24.7	19.7	-	-	7.9	6.3

Initial concentration of n-octadecane: 0.5 mM, PB: Piperonyl butoxide, $AgNO_3$: Silver nitrate, -: Undetectable

Table 2: GC-MS analysis of n-octadecane metabolites produced by *Fusarium* sp., F092

Retention time (min)	Principal ions and relative abundance (% base peak)	Identification or possible structure
19.367	356 (M^+, 4) 341 (M^+ -CH_3, 93); 283 (M^+ -$Si(CH_3)_3$, 1); 257 (4); 201 (10); 145 (30); 129 (40); 117 (100); 73 (62)	Octadecanoic acid
18.458	342 (M^+, 2) 327 (M^+ -CH_3, 62); 269 (M^+ -$Si(CH_3)_3$, 0.5); 243 (2); 201 (7); 145 (25); 129 (39); 117 (100); 73 (71)	Heptadecanoic acid
17.508	328 (M^+, 2) 313 (M^+ -CH_3, 80); 255 (M^+ -$Si(CH_3)_3$, 0.5); 243 (2); 201 (8); 145 (26); 129 (38); 117 (100); 73 (70)	Hexadecanoic acid
16.508	314 (M^+, 1) 299 (M^+ -CH_3, 68); 241 (M^+ -$Si(CH_3)_3$, 0.6); 201 (6); 145 (22); 129 (37); 117 (100); 73 (74)	Pentadecanoic acid
15.467	300 (M^+, 2) 285 (M^+ -CH_3, 93); 227 (M^+ -$Si(CH_3)_3$, 0.7); 201 (7); 145 (21); 129 (38); 117 (100); 73 (71)	Tetradecanoic acid
13.250	272 (M^+, 2) 257 (M^+ -CH_3, 94); 199 (M^+ -$Si(CH_3)_3$, 0.5); 201 (5); 145 (18); 129 (36); 117 (100); 73 (82)	Dodecanoic acid

When various Piperonyl Butoxide (PB) concentrations (0.1-2 mM) were applied to the cultures, n-octadecane degradation and metabolites were not significantly different ($p<0.05$) compared to the control (Table 1). On addition of 0.1-2 mM silver nitrate ($AgNO_3$), n-octadecane degradation was significantly different ($p<0.05$) compared to the control and the formation of metabolic products was inhibited (Table 1).

DISCUSSION

In this study, the use of *Fusarium* sp. F092 to degrade crude oil-A and -C under saline conditions was investigated. The results showed that crude oil-A was broken down easily than crude oil-C. Efforts to biodegrade crude oil-C should be made with several treatments to enhance the yield. Surfactants can be used to improve the bioavailability of crude oil through microbial conversion (Mulligan, 2005; Minai-Tehrani *et al*., 2006b). Generally, when the culture contained surfactant, the degradation of crude oil-C increased. The degradation of the aliphatic fraction made the greatest contribution to the degradation of TPHs. In this case, the aliphatic fraction was almost completely degraded (98 and 92%) by addition of T_{80} and T_{40} (Fig. 3). Effects of T_{80} and T_{40} were quite different. This was caused by their differences in chemical structure and properties, such as hydrophobic tail length, the branched hydrophobic chain and unsaturated or saturated hydrophobic chains that strongly influence the solubilization of surfactants (Paria, 2008).

The degradation of TPH also increased when the culture was treated with Mn^{2+}. The Mn^{2+} oxidized the substrate during the degradative process (Blanchette, 1984). The increase was also caused by Manganese Peroxidase (MnP). Mn^{2+} is a co-factor of this enzyme (Kerem and Hadar, 1995) which might preferentially degrade a substrate. Subsequently, this strain was found to be suitable for degrading oil under saline conditions and consistent with the results obtained in the sea sand. The complete degradation of all fractions was difficult to achieve due to the complex structure of crude oil. Most individual microorganisms prefer to degrade only a certain fraction of crude oil (Amund and Adebiyi, 1991; Olfat, 2005). However, F092 has the ability to degrade all fractions under saline conditions although it could be made more effective by adding surfactant or Mn^{2+}.

Aliphatic compounds, a major group in crude oil, are rapidly degraded by F092. In most cases, aerobic biodegradation of aliphatic hydrocarbons begins with the addition of one or both atoms of

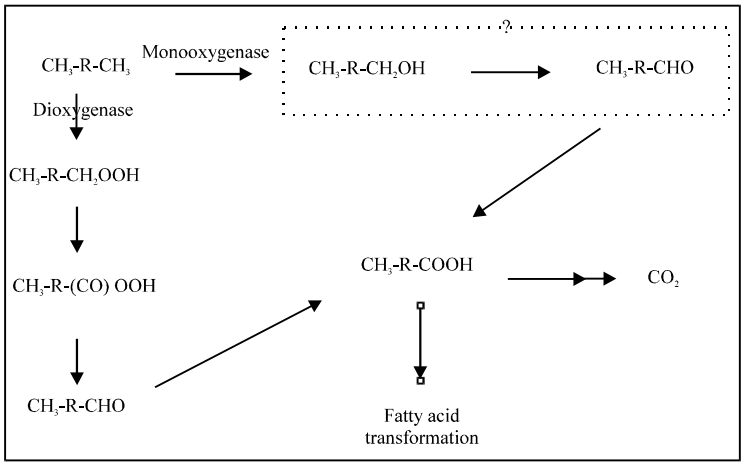

Fig. 4: Proposed aliphatic degradative pathway for *Fusarium* sp., F092

diatomic oxygen by enzymes known as monooxygenases or dioxygenases (Prince and Walters, 2007). The formation of hydroxylated products indicated that octadecanal might be influenced by monooxygenases. n-Octadecane monooxygenase produces octadecanol and then octadecanal and octadecanoic acid. In this study, PB was chosen as the monooxygenase inhibitor for fungal degradation (Mori *et al*., 2009) and its effect on the degradation of n-octadecane and its metabolite was evaluated. The results showed that degradation was not significantly different ($p<0.05$) compared to the control. This revealed that the degradation of n-octadecane by F092 indeed occurred via oxidation and not by a monooxygenase.

Aliphatic degradation also occurred alkyl hydroperoxidases, a process which might be mediated by dioxygenases. The route of n-octadecane`s degradation was initially to form octadecyl hydroperoxides and then octadecanal and finally a fatty acid. These were taken out via fatty acid metabolism in cellular lipids (Hadibarata *et al*., 2007) or via., β-oxidation to generate acetyl-CoA (Zhang *et al*., 2011). Furthermore, 1,2-dioxygenase was also detected as a major enzyme, followed by 2,3-dioxygenase, manganese peroxidase (MnP) and laccases secreted by F092. The maximum activity of 1,2- and 2,3-dioxygenase reached 289.5 and 49.7 U L^{-1} while that of manganese peroxidase and laccases was 69.2 and 48.8 U L^{-1}, respectively. In order to make sure that the initial degradation was catalyzed by dioxygenase, silver nitrate ($AgNO_3$) was chosen as the dioxygenase inhibitor for fungal degradation (Tsai and Li, 2007) and the effect of $AgNO_3$ on degradation of n-octadecane and its metabolite was evaluated. n-Octadecane`s degradation was significantly different ($p<0.05$) compared to the control and the formation of metabolic products was inhibited, indicating that a dioxygenase secreted by F092 was involved in the initial oxidation of aliphatic compounds to form alkyl hydroperoxides. Based on these results, six of the major metabolites of n-octadecane, enzymatic activities and addition of the dioxygenase inhibitor, the aliphatic degradative pathways by F092 were purposed (Fig. 4). This strain could be converted from n-octadecane to octadecyl hydroperoxides by dioxygenase at the terminal position under saline conditions and further oxidized to produce n-octadecanal and octadecanoic acid. This proposed aliphatic degradative pathway was first reported by Finnerty (1977), followed by Maeng *et al*. (1996a, b). The results obtained in our study are the first evidence that *Fusarium* sp. F092 has the ability to convert aliphatic fractions to form alkyl hydroperoxide via a terminal oxidation pathway involving dioxygenase.

CONCLUSION

Fusarium sp., F092 metabolized crude oil and n-octadecane, a representative compound of aliphatic fractions, under saline conditions converting them to carbon dioxide or secondary metabolites. This strain had the capacity to degrade all fractions of crude oil under saline conditions and proved to be more effective in the presence of a surfactant or Mn^{2+}, making it a potential degrader for bioremediation in crude oil-contaminated saline environments.

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