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Whole Cell-derived Fatty Acid Profiles of *Pseudomonas* sp. JS150 during Naphthalene Degradation

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Abstract

Changes in cellular fatty acid composition during naphthalene degradation, at the concentrations of 0.5 g l^{-1} or 1.0 g l^{-1}, by *Pseudomonas* sp. JS150 were investigated. In response to naphthalene exposure an increase in saturated/unsaturated ratio was observed. Additionally, the dynamic changes involved alterations in the contents of hydroxy, cyclopropane and branched fatty acids. Among the classes of fatty acids tested the most noticeable changes in the abundance of cyclopropane fatty acids were observed. Since day 4 of incubation these fatty acids were not dectected in bacterial cells growing on naphthalene. In contrast, markedly increased in the percentage of hydroxy fatty acids over time was observed. However, the proportions of saturated straight-chain and branched fatty acids did not change such significantly.

Key words: *Pseudomonas* sp. JS150, naphthalene degradation, fatty acid composition

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are an important class of environmental contaminants because many of them are toxic, mutagenic and resist biodegradation. The simplest of that class is naphthalene, a common component of industrial products and waste materials. Naphthalene is a dicyclic aromatic compound with molecular mass of 129.19, boiling point 218°C, melting point of 80.5°C, solubility (at 20°C) of 32 mg l^{-1} and specific gravity of 1.145. It is widely distributed in the environment because it is used as the starting material for the synthesis of moth repellent, soil fumigant, naphthylamines, anthranilic and phtalic acids, and syntetic resins (Vuchetich *et al*., 1996; Smith *et al*., 1997; Stohs *et al*., 2002). The fate of naphthalene is of great interest because its expossure might cause toxic effects on skin, lungs, eyes, kidney, liver and brain of animals and humans. Toxic manifestations depend on naphthalene dose, route of expossure and species involved (Stohs *et al*., 2002).

Bacterial degradation represents a significant way for the removal of naphthalene (PAHs) from the environment. Numerous strains of microorganisms that are capable of degrading naphthalene have been isolated and identified. Considerable attention has focused on the metabolic pathways and their genetic regulation by gram-negative bacteria, particularly of the genera *Pseudomonas* (Fuenmayor *et al*., 1998; Filonov *et al*., 1999; Kozlova *et al*., 2004) and gram-positive bacteria of the genera *Rhodococcus* (Kulakov *et al*., 1998; Di Gennaro *et al*., 2001). The metabolism of naphthalene under aerobic conditions is different in gram-positive and gram-negative bacteria. It is documented that naphthalene degradation in gram-negative bacteria proceeds through the formation of 1,2-dihydroxynaphthalene which is then dehydrogenated to the corresponding 1,2-dihydroxy derivative and further transformed into salicylic acid. The next step is salicylate oxidation to catechol, which can undergo either *ortho* or *meta* fission depending upon bacterial metabolism (Rossello-Mora *et al*., 1994; Mrozik *et al*., 2003). However, in naphthalene metabolism by gram-positive bacteria such as *Rhodococcus*, salicylate is converted to gentisic acid (Grund *et al*., 1992, Di Gennaro *et al*., 2001). A different naphthalene degradation pathway has been recently described in the thermophilic bacterium *Bacillus thermoleovorans*. Apart from typical metabolites known from mesophiles, intermediates such as 2,3-dihydroxynaphthalene, 2-carboxycinnamic acid, phthalic acid and benzoic acid in the pathway of this bacterium were identified (Annweiler *et al*., 2000).

In the presence of naphthalene and other aromatic compounds crucial changes in the fatty acid composition of bacterial membrane lipids have been observed. Aromatic compounds disturb of membrane integrity and permeability, inhibit bacterial growth, respiration, nutrients transport or even cell death may occur. The toxic effects of these chemicals are based on their lipophilic properties, since they interact preferentially with cell membrane and change its fluidity (Sikkema *et al*., 1994; 1995; Mrozik *et al.,* 2004b). Bacteria withstand these changes by altering the fatty acid composition and the degree of saturation of their membrane lipids. The saturation degree of fatty acids is known as a major adaptive response of the cells to keep the fluidity of their membranes at a constant value. This parameter changes when bacteria grow thus it can be a potential marker of toxicity only in living cells (Diefenbach *et al*., 1992; Loffhagen *et al*., 1995). Another fundamental mechanism enabling bacteria to adapt to presence of aromatic compounds is isomerization of *cis* to *trans* unsaturated fatty acids. This is a short-term response that does not depend on growth. Therefore, this parameter is a second potential indicator of the acute toxicity of these compounds (Heipieper *et al*., 1995; Loffhagen *et al*., 2001; Heipieper *et al*., 2003). For decreasing the deleterious effect of aromatic compounds on membrane, bacteria can also change the proportion between *iso* and *anteiso* branched fatty acids, content of cyclopropane fatty acids and membrane proteins, and the average acyl chains length (Heipieper *et al*., 1994; Sajbidor, 1997; Denich *et al*., 2003). These tolerance mechanisms enabling bacteria to stabilize of membrane fluidity and reduce the accumulation of toxic compounds in the membrane. Molecular and biochemical investigations performed to characterize these adaptive response systems were conducted using many strains of the genera *Pseudomonas*, including the major representatives *P. putida* and *P. aeruginosa,* and *Vibrio* and series of phenolic compounds such as phenol, *p*-cresol, toluene (Guckert *et al*., 1986; Heipieper *et al*., 1992; Weber *et al*., 1994; Mrozik *et al*., 2004a). However, there is still a little information about the effect of naphthalene on cellular fatty acids of these microorganisms.

The aim of this study was to determine the changes in whole cell-derived fatty acids in *Pseudomonas* sp. JS150 during naphthalene degradation.

Experimental

Materials and Methods

Strain and growth conditions. *Pseudomonas* sp. JS150 strain was kindly provided by Dr J. Spain from Air Force Civil Engeenering Support Agency, Tyndall Air Force Base, Florida, USA. *Pseudomonas* sp. JS150 is a nonencapsulated mutant of strain JS1 obtained after ethyl methanesulphonate mutagenesis (Haigler *et al*., 1992). Bacteria were grown in Kojima *et al*., (1962) minimal medium containing: 3.78 g of $Na_2HPO_4 \times 12H_2O$; 0.5 g of KH_2PO_4; 5.0 g of NH_4Cl; 0.2 g of $MgSO_4 \times 7H_2O$ and 0.1 g of yeast extract in 1000 ml of deionized water. Naphthalene was added at concentrations of 0.5 g l^{-1} or 1.0 g l^{-1} as a sole carbon and energy source. Due to the low solubility of naphthalene in water it was dissolved in N,N-dimethylformamide (DMF) before addition to the medium. The final pH of the medium was 7.2–7.3. To show the impact of naphthalene on fatty acid composition, bacteria were also cultivated in medium without aromatic substrate. In this case sodium citrate at the concentration of 0.5 g l^{-1} was used. Naphthalene-dosed and control cultures were further incubated in the dark at 30°C with shaking at 125 rpm. Samples of the cultures were withdrawn periodically for analysis of cell density (OD) at 600 nm and naphthalene removal.

Determination of naphthalene. For determination of naphthalene concentration triplicate samples of 10 ml cells culture were melted with 10 ml of hexane using magnetic stirrer for 15 min. After separation of hexane fraction, the lower phase was washed twice with 2 ml of hexane and hexane fractions were combined. The organic phase was filtrated through anhydrous sodium sulphate. Hexane was vacuum evaporated to the volume of 2 ml and each sample was transferred into GC vials and analysed by GC chromatography (Perkin Elmer) equipped with flame ionization detector and a capillary column (phenyl-methyl-polysiloxane 25 m × 0.25 mm in diameter) and helium as a carrier gas. Concentration of naphthalene was determined on the 4, 7 and 14 of incubation and calculated by comparison of the peak height of standard with the tested samples.

Enzyme assay. For preparation of cell extract and enzyme activity assay the method of Feist and Hegeman (1969) was used. Enzyme activity was expresses as μmol of 2-hydroxymuconic semialdehyde formed per mg of protein per min. The protein content of the cell extract was estimated by the method of Bradford (1976) with bovine albumin as a standard. Catechol 2,3-dioxygenase activities were measured on 4, 7 and 14 day of culturing.

Isolation and identification of fatty acids. The whole cell-derived fatty acids were extracted and determined on the 4, 7 and 14 day of incubation. Cellular fatty acids were extracted from both cells growing on naphthalene and sodium citrate. Bacteria were harvested by centrifugation (8000 g) at 4°C for 30 min. The cell pellets obtained from medium amended with naphthalene were washed with 1.0 ml of DMF to remove undegraded naphthalene and finally were washed twice with 0.85% NaCl to remove residue of the culture medium. To decrease the humidity of bacterial cell, pellets were left through 2h at room temperature. Next 55 mg of bacterial biomass was transferred in duplicate to reaction tubes (Pyrex) and 1 ml of first reagent (150 g NaOH in 1 litre of 50% methanol) for saponification was added. Samples were incubated for 30 min at 100°C in water bath. To methylate liberated fatty acids, 2 ml of reagent II (6N HCl in aqueous methanol) was added to each tube and incubated again for 10 min at 80°C in water bath. Fatty acid methyl esters (FAMEs) were extracted from the aqueous phase by addition of 1.15 ml of reagent III (hexane/methyl tert-butyl ether, 1:1, v/v) to each tube. Then samples were rotated end-over-end for 10 min. After removing aqueous (lower) phase, 3 ml of 1.2% NaOH in H_2O was added and the tubes were again rotated for 5 min (Sasser, 1990). Finally, the organic (upper) phase containing FAME was transferred to a gas chromatography vial (Hewlett-Packard). Fatty acids were analysed by gas chromatography (Hewlett-Packard 6890, USA) using capillary column Ultra 2-HP (cross-linked 5% phenyl-methyl silicone 25 m, 0.22 mm ID, thickness 0.33 μm) and hydrogen as a carrier gas. FAME were detected by a flame ionization detector (FID) and identified by MIS (Microbial Identification System) software, using the aerobe method and TSBA library version 3.9 (MIDI, USA).

Fatty acids were designed by the number of carbon atoms, followed by a colon, the number of double bonds and then by a position of the first double bond from the methyl (ω) end of the molecule. The prefixes *c* or *t* indicate *cis* or *tran*s configuration of the double bond, *cy* – cyclopropane fatty acids, Me – the position of the methyl group from the acid end, and -OH indicates the position of the hydroxyl group from the acid end of the molecule. Branched fatty acids are designed as *iso* and *anteiso*, if the methyl branch is one or two carbon from the ω end of acyl chain.

Results

Cell growth and naphthalene biodegradation. *Pseudomonas* sp. JS150 was grown on naphthalene at the concentration of 0.5 g l^{-1} or 1.0 g l^{-1} as a sole carbon and energy source. The highest optical densities (OD) were observed on 4 day of incubation reaching the value 0.839 and 0.901 for the concentrations 0.5 g l^{-1} and 1.0 g l^{-1}, respectively. These OD values were equivalent of bacterial cell numbers 5.6×10^8 and 4.1×10^9. After 4 days OD started to decrease and at the end of experimental period showed 0.496 and 0.399 for the lower and the higher dose of naphthalene.The increasing number of bacteria during the first 4 days of the incubation was accompanied with the highest degradation rate of naphthalene by strain used. In that time *Pseudomonas* sp. JS150 metabolized 60% and 66% of total naphthalene added to the medium at the concentrations of 0.5 g l^{-1} and 1.0 g l^{-1}, respectively. In succesive days naphthalene was degraded much slowly. Cell growth and biodegradation rate of substrate by *Pseudomonas* JS150 is presented in Figure 1.

The biodegradation ability of strain tested was correlated with an induction of catabolic enzymes involved in naphthalene metabolism. Catechol 2,3-dioxygenase activities in cell-free extracts are shown in Table I. The highest enzyme activities were observed for both naphthalene doses used on 4 day and showed value 0.70 and 0.63 μM min^{-1} mg^{-1} of protein. Then the activity of this enzyme was decreasing over time. When naphthalene was served as a substrate *Pseudomonas* sp. JS150 did not induce catechol 1,2-dioxygenase indicating that naphthalene degradation by this strain procceded *via meta* metabolic pathway.

Changes in fatty acid composition. To determine the effect of naphthalene on whole cell-derived fatty acid profiles of *Pseudomonas* sp. JS150 cultured on naphthalene and sodium citrate were compared. Table II contains the percentage of total fatty acids and shows the compositional changes during naphthalene degradation by bacteria tested. For the interpretation of naphthalene impact on bacteria, identified fatty acids were grouped into two major classes. The first class included saturated fatty acids. It was additionaly divided into four sub-classes: straight-chain, hydroxy, cyclopropane and branched fatty acids. The second class comprises unsaturated fatty acids.

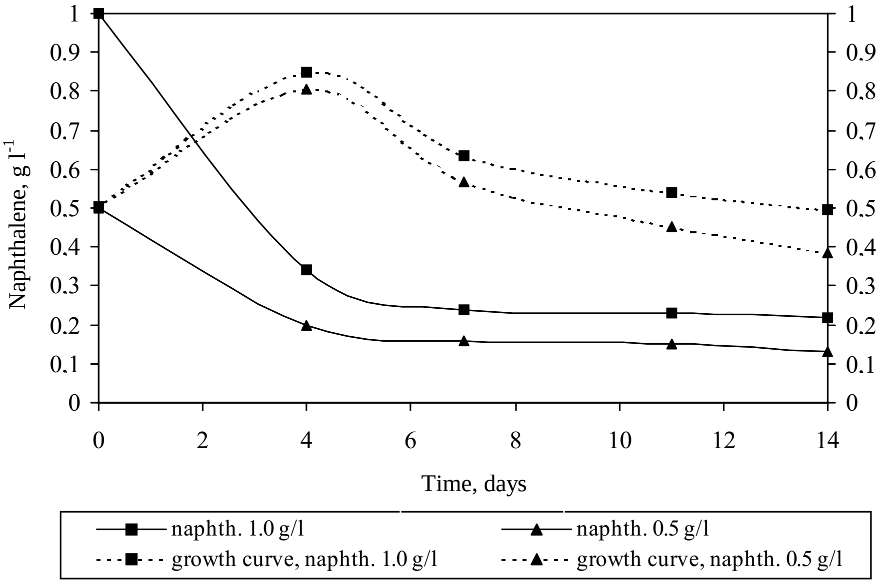

Fig. 1. Naphthalene degradation and growth curve of *Pseudomonas* sp. JS150.

Table I
Enzyme activities in cell-free extracts of *Pseudomonas* sp. JS150 grown on different naphthalene concentrations

Bacterial strain	Naphthalene ($g\ l^{-1}$)	Catechol 2,3-dioxygenase activity, $\mu M\ min^{-1}\ mg^{-1}$ of protein		
		4 day	7 day	14 day
Pseudomonas sp.	0.50	0.70 ± 0.06	0.68 ± 0.05	0.45 ± 0.05
JS150	1.00	0.63 ± 0.02	0.58 ± 0.04	0.31 ± 0.01

Number of replicates, n = 3

As indicated in Table II the remarkable differences in contents of saturated fatty acids on day 14 were observed. For bacteria grown on naphthalene at the concentration of 0.5 $g\ l^{-1}$ the content of this class of fatty acids was the lowest (80.0% of total saturated fatty acids) as compared to control (88.26%) and naphthalene treatment samples determined on 4 (83.55%) and 7 (84.30%) day. In contrast, in bacteria grown at the dose of 1.0 $g\ l^{-1}$naphthalene the amount of saturated fatty acids was the highest (93.47%). As a consequence the changes in the saturated/unsaturated ratio were found. In the presence of lower naphthalene concentration this ratio was lower in comparison with control samples. It reached the value 5.85, 5.37 and 5.59 for 4, 7 and 14 day, respectively, whereas in control it was 7.52. In opposite, the saturated/unsaturated ratio significantly increased on day 7 (11.46) and 14 (14.31), when bacteria cultivated with 1.0 $g\ l^{-1}$ of naphthalene.

Naphthalene treatment caused changes in the distribution of straight-chain, hydroxy, cyclopropane and branched fatty acids in *Pseudomonas* sp. JS150. In a case of straight-chain fatty acids no significant differences in the amount of these fatty acids during naphthalene degradation were observed, with one exception. On the last sampling time in bacteria grown on the medium with 0.5 $g\ l^{-1}$ of naphthalene the content of straight-chain saturated fatty acids was markedly lower (13.56%) as compared to the control (18.78%). In turn, essential changes in the distribution of hydroxy fatty acids over experimental period were detected. The addition of both concentrations of naphthalene increased the amounts of these fatty acids. The highest abundance was found at the naphthalene concentration of 0.5 $g\ l^{-1}$ on 14 day and was 4 times higher than in the control, represented up 30.43% of the total saturated fatty acids. In remaining samples, the contents of hydroxy fatty acids were about 2–2.5-fold higher than in the control. In contrast, as a response to naphthalene exposure, in *Pseudomonas* sp. JS150 during the first 4 days of the incubation the amount of cyclopropane fatty acid 17:0 *cy* decreased as compared to the control. Interestingly, on the second and third sampling times this fatty acid either on the lower or higher naphthalene concentration was not detected. Similar trend was observed in a case of 18:0, 17:0 *anteiso*, 13:0 *iso* and one of unsataturated fatty acid MIDI-undistinguishable

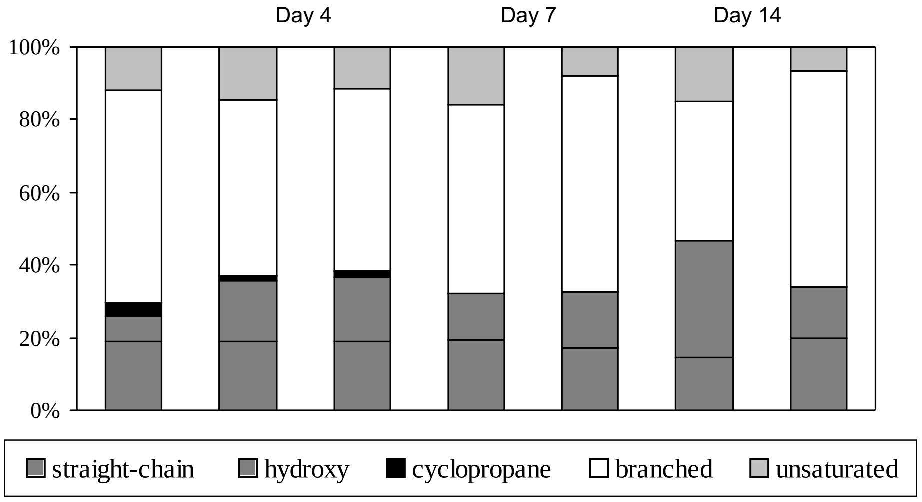

Fig. 2. Proportions of fatty acids in *Pseudomonas* sp. JS150 growing on citrate (control) and naphthalene at the concentration 0.5 $g\ l^{-1}$ (A) or 1.0 $g\ l^{-1}$ (B) during naphthalene degradation.
Class of hydroxy fatty acids contains additionally the branched hydroxy fatty acids. Methylated fatty acid 16:0 10 Me is included to branched fatty acids.

Table II
Percentage of total fatty acids from *Pseudomonas* sp. JS150 grown in the presence of citrate (0.5 g l^{-1}) or different naphthalene concentrations during 14 days of incubation

Fatty acids	% of total fatty acids						
		4 day		7 day		14 day	
	Citrate g l^{-1}	Naphthalene g l^{-1}					
		0.5	1.0	0.5	1.0	0.5	1.0
Saturated							
10:0	0.00	0.60	0.57	1.35	0.00	1.43	1.90
10:0 *iso*	0.00	0.00	0.00	0.00	0.00	0.00	9.30
10:0 3OH	0.00	0.39	0.37	0.00	0.00	1.50	0.00
11:0 *iso*	0.00	3.11	3.02	7.00	4.99	6.84	0.00
11:0 *iso* 3OH	0.00	2.96	3.06	0.00	5.57	6.59	0.00
12:0 3OH	2.95	5.66	5.54	0.00	0.00	12.16	0.00
12:0 *iso* 3OH	0.46	0.43	0.42	0.00	0.00	0.00	14.13
13:0 2OH	1.10	1.40	1.41	0.00	0.00	0.00	0.00
13:0 *iso*	0.00	0.78	0.58	0.00	0.00	0.00	0.00
13:0 *iso* 3OH	2.88	5.42	6.39	12.69	8.96	10.18	0.00
14:0	3.46	2.79	3.03	3.74	3.34	1.95	3.70
14:0 *iso*	1.73	1.22	1.36	0.00	0.00	0.00	0.00
15:0	1.13	0.62	0.56	0.00	0.00	0.00	0.00
15:0 *iso*	26.59	23.46	23.29	20.69	28.12	17.10	30.94
15:0 *anteiso*	19.36	12.76	15.33	14.51	13.84	7.57	10.52
16:0	13.63	13.69	13.27	14.37	13.36	10.18	14.07
16:0 *iso*	3.52	2.00	2.80	3.00	3.20	2.14	5.22
16:0 *anteiso*	0.00	0.00	0.42	0.00	0.00	0.00	0.00
16:0 10Me	4.00	0.00	0.00	3.95	4.02	0.00	0.00
17:0 *iso*	3.00	3.37	2.86	3.00	3.10	2.36	3.41
17:0 *anteiso*	0.58	0.61	0.85	0.00	0.00	0.00	0.00
17:0 *cy*	3.31	1.24	1.68	0.00	0.00	0.00	0.00
18:0	0.56	1.04	1.44	0.00	0.00	0.00	0.00
19:0 *iso*	0.00	0.00	0.00	0.00	0.00	0.00	0.28
Unsaturated							
15:1 *iso*	0.75	0.75	0.80	0.00	0.00	0.00	0.00
16:1 ω7*c*	5.72	4.20	3.02	5.38	5.14	4.33	4.40
16:1 ω9*c*	2.73	2.35	2.10	2.32	2.44	1.49	0.00
17:1 ω9*c*	0.00	0.00	3.51	0.00	0.00	2.54	0.00
17:1 *iso*	0.00	4.13	0.00	5.12	0.00	3.35	0.00
18:1 ω9*c*	1.67	1.85	1.49	1.88	0.00	1.40	2.00
18:1 2OH	0.00	0.00	0.00	0.00	0.14	0.00	0.13
18:1 ω7*c*/ω9*t*/ω12*t*	0.87	1.01	0.26	1.00	0.00	1.20	0.00
Other	0.00	2.16	0.00	0.00	3.78	5.61	0.00
Sat./unsat. ratio	7.52	5.85	7.84	5.37	11.46	5.59	14.31

Abbreviations: ω – methyl end of fatty acid, *c* or *t* indicate *cis* or *tran*s configuration of the double bond, *cy* – cyclopropane fatty acid, Me – the position of the methyl group from the acid end, -OH indicates the position of hydroxyl group from the acid end, *iso* and *anteiso* – branched fatty acids.

18:1ω7*c*/ω9*t*/ω12*t*. However, the last fatty acid was not detected only when bacteria grown on the medium amended with 1.0 g l^{-1} of naphthalene on 7 and 14 day of the incubation (Fig. 2, Table II).

The impact of naphthalene on whole cell-derived fatty acids comprised also changes in the contents of branched fatty acids including *iso*, *anteiso* and methyl-fatty acids. During degradation of 0.5 g l^{-1} naphthalene

Pseudomonas sp. JS150 decreased the abundance of branched fatty acids over time, whereas in the presence of 1.0 g l^{-1} of substrate used the abundance of these fatty acids was similar to those observed in the control. On day 4, the percentage of branched fatty acids isolated from bacteria grown on 0.5 g l^{-1} of naphthalene was 47.31% and declined to 36.01% on day 14, whereas in the control reached value 58.78%. (Fig. 2).

The another reaction of tested strain to naphthalene stress was forming new fatty acids which were not found in the bacteria growing on citrate. During the period of the incubation *Pseudomonas* sp. JS150, depending on the sampling day and naphthalene concentrations, formed from 3 to 6 of new fatty acids. They were mainly represented by saturated short-chain 10:0, 10:0 *iso*, 10:0 3OH, 11:0 *iso* and 11:0 *iso* 3OH and 13:0 *iso*, as well as by unsaturated fatty acids such as 17:1ω9*c*, 17:1 *iso* and 18:1 2OH. Surpraisingly, particulary high content (9.3%) of 10:0 *iso* fatty acid was detected only on 14 day in the presence of the higher naphthalene concentration. In turn, fatty acid 17:1 *iso* was present on each sampling day but only in bacteria cultured on the lower naphthalene doses. In the presence of 1.0 g l^{-1} of naphthalene *Pseudomonas* sp. JS150 did not form this fatty acid (Table II).

Discussion

Our results confirmed the ability of *Pseudomonas* sp. JS150 for biodegradation of naphthalene, well-known from previous studies (Haigler *et al.*, 1992). This genetic modified strain was able to metabolize not only naphthalene but also a range of aromatic compounds such as toluen, benzen, chlorobenzene, phenol, benzoate and salicylate. However, this strain did not metabolize of naphthalene so effectively and fast as compared to other wild-type strains from genus *Pseudomonas*. For example, *P. vesicularis* and *P. stutzeri* were more efficient degraders and utilized 0.5 g l^{-1} of naphthalene within 11 and 14 days of the incubation, respectively (Mrozik *et al.*, 2004c), whereas *Pseudomonas* sp. JS150 characterized by much slower rate of naphthalene degradation and after 14 days of the incubation in the medium there was still about 26% of naphthalene added. Interestingly, *Pseudomonas* sp. JS150 degraded the higher dose of naphthalene more quickly and after 14-days experiment only 22% of naphthalene added was present in the culture medium. The biodegradation studies indicated that in *Pseudomonas* sp. JS150 naphthalene induced catechol 2,3-dioxygenase what evidenced that degradation of naphthalene proceeded *via meta* metabolic pathway (Dagley, 1971; Wiliams and Sayers, 1994).

The obtained results shown that *Pseudomonas* sp. JS150 underwent crucial changes in cell-derived fatty acids when grown on naphthalene as a sole carbon and energy source. Structural changes involved alterations in distribution of the separated classes of fatty acids. Under naphthalene treatment the saturated/unsaturated ratio depended on the dose of aromatic substrate. During degradation of 0.5 g l^{-1} of naphthalene this ratio was at the same level and was slightly lower as compared to control. In turn, this ratio in the presence of 1.0 g l^{-1} of naphthalene increased and at the end of the experiment was 2-fold higher in comparison with the control indicating an increase in degree of saturation of membrane fatty acids. The similar correlation between an increase in degree of membrane saturation and tolerance towards the toxic compounds has been observed in phenol-degrading strain *Pseudomonas putida* P8 (Heipieper *et al.*, 1992), in *Rhodoccocus* sp. 33 in the presence of benzene (Gutierrez *et al.*, 1999) and *Ralstonia eutropha* H850 in the presence of biphenyl (Kim *et al.*, 2001). The increasing degree of membrane saturation is a major adaptive mechanism to the presence of many toxic substances, that enable bacterial cells to survive under aromatic hydrocarbons stress (Sikkema *et al.*, 1994; 1995). Considering our results it may be suggested that naphthalene at the lower concentration did not influence on degree of fatty acid saturation indicating that this naphthalene dose was too low for *Pseudomonas* sp. JS150 to induce essential changes in the proportional contents of saturated and unsaturated fatty acids.

Another mechanism for bacterial cells to adapt to membrane active compounds is to alter cyclopropane fatty acid composition of their membranes (Grogan and Cronan, 1997). In *Pseudomonas* sp. JS150 only one 17:0 *cy* fatty acid was detected and its amount significantly decreased at the begining of the experiment and was not found to the end of the incubation period. Similarly, the decrease in the cyclopropane fatty acid percentage was observed in our previous studies conducted with *P. vesicularis* and *P. stutzeri*. However, 17:0 *cy* was detected on each sampling days. The low level and the disappearance of cyclopropane fatty acids might indicate that cyclopropane ring were cleaved by a separate cellular system activated by enzymes involved in naphthalene biodegradation.

Naphthalene exposure influenced also on the composition and content of branched fatty acids. In the presence of the lower naphthalene concentration the content of these fatty acids visibly decreased whereas in the presence of the higher dose of naphthalene did not change significantly. These obtained results for *Pseudomonas* sp. JS150 were different in comparison with our previous experiments when *P. stutzeri*, *P. vesicularis* and *P. putida* were used. In these strains during naphthalene treatment the content of branched fatty acids markedly increased. It should be point out that *Pseudomonas* sp. JS150, in contrast to other strains of *Pseudomonas*, under control conditions synthesized significant amounts of branched fatty acids. They constituted 62% of total saturated fatty acids and were mainly represented by two fatty acids 15:0 *iso* and 15:0 *anteiso*. In contrast, for example in *P. vesicularis* grown on glucose branched fatty acids constituted only 10% of the total saturated fatty acids (Mrozik *et al*., 2004c). An increase in branched fatty acids contents was also reported by Tsitko *et al.* (1999), who studied the impact of different aromatic hydrocarbons on cellular fatty acid composition of *Rhodococcus opacus*. The high proportion of branched fatty acids might be related with genetic manipulation of *Pseudomonas* sp. JS150 towards the resistance to aromatic compounds. Probably the high amount of branched fatty acids, found in this strain, is sufficient to keep the proper membrane stability, both under control and naphthalene stress conditions.

Another reaction of *Pseudomonas* JS150 to naphthalene toxicity, in comparison with wild-strains from the genus *Pseudomonas*, were chnages in the percentage of hydroxy fatty acids. During naphthalene degradation by strain tested the content of this class of fatty acids increased average from 2 to 4-fold, while for example, in *P. vesicularis* the abundance of hydroxy fatty acids decreased upon naphthalene exposure (Mrozik *et al*., 2004c). This mechanism is not understood yet and seems to be an attribute of an individual strain. Similarly, a role of new synthesized fatty acids in the protection on the bacterial cell against naphthalene tolerance requires further explanations.

A detailed understanding of all mechanisms responsible for physiological changes in bacterial cells during hydrocarbons degradation may be useful for the application of these bacteria in field studies on bioremediation of contaminated sites.

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