APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Feb. 1999, p. 853–855 Vol. 65, No. 2
0099-2240/99/$04.00+0
Copyright © 1999, American Society for Microbiology. All Rights Reserved.

Effect of Aromatic Compounds on Cellular Fatty Acid Composition of *Rhodococcus opacus*

IRINA V. TSITKO,[1,2]* GENNADI M. ZAITSEV,[3] ANATOLI G. LOBANOK,[2] AND MIRJA S. SALKINOJA-SALONEN[1]

Department of Applied Chemistry and Microbiology, University of Helsinki, FIN-00014, Helsinki,[1] and Juvegroup Oy, 96400 Rovaniemi,[3] Finland, and Institute of Microbiology, Belarus Academy of Sciences, 220141 Minsk, Belarus[2]

Received 19 November 1998/Accepted 24 November 1998

In cells of *Rhodococcus opacus* GM-14, GM-29, and 1CP, the contents of branched (10-methyl) fatty acids increased from 3% to 15 to 34% of the total fatty acids when the cells were grown on benzene, phenol, 4-chlorophenol, chlorobenzene, or toluene as the sole source of carbon and energy, in comparison with cells grown on fructose. In addition, the content of *trans*-hexadecenoic acid increased from 5% to 8 to 18% with phenol or chlorophenol as the carbon source. The 10-methyl branched fatty acid content of *R. opacus* GM-14 cells increased in a dose-related manner following exposure to phenol or toluene when toluene was not utilized as the growth substrate. The results suggest that 10-methyl branched fatty acids may participate in the adaptation of *R. opacus* to lipophilic aromatic compounds.

Aromatic hydrophobic compounds are toxic to bacteria due to their high partition into the membrane (15, 16). A change in the degree of saturation of cellular fatty acids is a well-known reaction of bacteria to the presence of membrane-active compounds (2, 8). Another adaptation response is the isomerization of *cis* unsaturated fatty acids to the *trans* form, which has been described for several *Pseudomonas* strains (4, 5, 11, 20).

The influence of organic solvents on the cellular fatty acid composition described above has been shown for gram-negative bacteria. The effect of aromatic compounds on the fatty acid composition of nocardioform bacteria, particularly those that are able to utilize high concentrations of such compounds, has not been studied intensively.

We studied the response of the cellular fatty acid composition of *Rhodococcus* strains to the presence of aromatic compounds.

Three bacterial strains were used in this study. *Rhodococcus opacus* GM-14 grew in mineral medium on benzene or chlorobenzene as the sole carbon and energy source when substrates were added in the liquid phase (21). New isolate GM-29 was obtained from an enrichment culture with toluene as the sole carbon source. It grew in saturated aqueous solutions of toluene and benzene when the substrates were added at amounts of up to 7 g liter^{-1}. Strain 1CP, which degrades 4-chlorophenol and 2,4-dichlorophenol, was isolated by Gorlatov et al. (3) in 1994 and, based on phenotypic characteristics, was identified as *R. erythropolis*. As determined by 16S rRNA gene sequences strains GM-29 and 1CP belong to the species *R. opacus*. Data on 16S rRNA gene sequences are available from the EMBL database. The accession numbers are Y11892 and Y11893 for strains GM-29 and 1CP, respectively (14).

Bacteria were cultivated in 1-liter flasks with 200 ml of mineral KSN medium (21) on a gyratory shaker at 28°C. Aromatic compounds were added directly to the culture medium. The cells were grown to early stationary phase, harvested by filtration (Supor-450; 0.2-μm pore size), and washed twice with mineral medium. The fatty acids were isolated from 50 to 60 mg of wet cells by direct saponification. Fatty acid methyl esters (FAME) were analyzed by gas chromatography (GC)-mass spectrometry with an HP 6890A gas chromatograph equipped with an HP 5972A mass selective detector (Hewlett-Packard Co., Palo Alto, Calif.) and an HP-Ultra 2 cross-linked 5% phenyl methyl silicone capillary column (25 m by 0.2 mm; 0.33 μm). The oven temperature was programmed with injection and a 1-min hold at 80°C, followed by an increase to 160°C at 60°C min^{-1}, a hold at 160°C for 28 min, and an increase at 5°C min^{-1} to 230°C. Individual FAME were identified by comparing their mass spectra with those in the Wiley 138K mass spectral database. The *cis* and *trans* isomers of hexadecenoic and octadecenoic acids were verified by comparison with the retention times of authentic standards from the Even Unsaturated Fatty Acid Methyl Esters Kit (Analabs, North Haven, Conn.). The fatty acid content of cells was calculated as the average of three independent cultivations; the standard deviation was less than 7%.

All three strains changed their fatty acid composition when grown on aromatic compounds as the sole sources of carbon and energy, in comparison to cells grown on fructose (Table 1).

Hexadecanoic acid was the predominant saturated fatty acid, making up 30 to 32% of the total in fructose-grown cells and 20 to 27% of the total in cells grown on the aromatic substrates. The amounts of straight-chain saturated fatty acids in cells of GM-29, 1CP, and GM-14 grown on fructose ranged from 43 to 49% of the whole-cell fatty acids. Cells of strains GM-14 and GM-29 grown on benzene, chlorobenzene, or toluene, but not those grown on phenol or 4-chlorophenol, contained >60% straight-chain saturated fatty acids. In comparison with cells grown on fructose, those grown on the aromatic substrates had two- to threefold increased contents of odd-number carbon chain saturated fatty acids, mainly $C_{15:0}$ and $C_{17:0}$.

The most dramatic growth substrate-dependent change was the 3- to 10-fold increase of the branched-chain (10-methyl) fatty acids in cells grown at the expense of benzene derivatives. In strain 1CP, the amount of 10-methyl branched fatty acids increased from 3.1% in cells grown on fructose to 24.8 or 34.3% in cells grown on KSN medium with phenol or 4-chlorophenol as the carbon source, respectively. In all strains, the increase in the amount of 10-methyl branched fatty acids was

* Corresponding author. Mailing address: Division of Microbiology, Department of Applied Chemistry and Microbiology, P.O. Box 56, FIN-00014, Helsinki, Finland. Phone: 358 9 708 59324. Fax: 358 9 708 59322. E-mail: Irina.Tsitko@Helsinki.Fi.

TABLE 1. Whole-cell fatty acid compositions of *R. opacus* GM-14, GM-29, and 1CP grown in KSN medium with different compounds as the sole carbon sources

Fatty acid(s)	Relative content (%) of fatty acid(s) in cells of strain:											
	GM-14					GM-29				1CP		
	Fructose[a]	Phenol[b]	4-Chloro-phenol[c]	Benzene[a]	Chloro-benzene[b]	Fructose[a]	Phenol[b]	Benzene[a]	Toluene[a]	Fructose[a]	Phenol[b]	4-Chloro-phenol[c]
9:0-13:0	0.4	1.1	0.8	2.4	4.1	0.7	0.8	3.7	3.6	0.8	0.7	0.6
14:0	2.6	2.9	2.7	5.8	4.8	3.5	2.5	4.8	6.4	3.0	1.9	2.1
15:0	5.9	12.2	9.4	18.3	16.6	3.2	10.5	18.7	11.0	3.6	5.2	7.2
16:0	30.1	23.1	23.1	27.2	21.6	31.9	25.0	20.9	30.8	31.7	19.7	20.7
17:0	4.8	7.4	7.1	10.5	8.6	1.8	8.6	8.5	6.5	3.6	5.0	7.0
18:0	4.4	2.5	2.8	4.0	3.6	1.7	2.7	2.6	2.4	3.5	1.3	2.1
19:0	<0.5	0.8	1.6	2.7	1.7	<0.5	0.8	1.6	1.3	<0.5	1.3	1.3
Minor saturated	<0.5	ND[d]	1.0	1.7	1.6	ND	<0.5	1.4	1.0	ND	0.9	1.8
Sum of straight saturated	48.8	49.9	48.5	72.6	62.6	42.9	51.1	62.1	63.0	46.5	35.9	42.8
16:1 (ω 7c)	7.9	2.3	0.9	1.0	0.9	15.5	3.9	1.7	4.5	8.8	1.3	1.4
16:1 (ω 7t)	4.8	8.3	10.8	2.4	3.7	5.2	5.3	4.3	2.9	4.8	17.5	10.4
17:1 (ω 8c)	13.1	9.8	4.2	2.4	2.4	8.5	7.6	5.0	5.2	8.9	5.2	1.9
17:1 (ω 5c)	ND	0.8	1.8	0.1	0.9	ND	0.6	0.9	<0.5	ND	2.9	2.8
18:1 (ω 9c)	20.0	7.3	4.8	2.7	3.6	22.5	7.9	2.2	6.5	25.0	4.9	2.2
19:1	0.8	2.2	1.8	3.7	5.9	<0.5	2.0	3.7	2.9	0.7	1.2	1.3
Minor unsaturated	1.7	3.5	3.2	2.1	2.7	1.3	1.9	2.7	3.1	2.1	2.3	2.9
Sum of unsaturated	48.8	34.2	27.6	14.3	20.1	54.2	29.5	20.4	25.5	50.3	35.3	22.9
10-Me-16:0	<0.5	1.2	0.8	1.6	1.9	0.5	3.0	2.6	1.7	0.4	0.3	3.2
10-Me-17:0	0.9	6.4	6.4	3.9	4.1	0.5	6.9	7.7	3.5	0.8	7.1	8.5
10-Me-18:0	1.3	6.9	14.2	5.2	8.3	1.9	9.3	5.5	5.3	2.0	18.5	17.5
10-Me-19:0	ND	0.9	2.8	1.9	2.8	ND	<0.5	1.7	1.0	ND	2.9	5.1
Sum of branched chain	2.4	15.4	24.1	12.7	17.1	2.9	19.6	17.6	11.5	3.2	28.8	34.3
Total	100	99.5	100	99.6	99.8	100	100	100	100	100	100	100

[a] The substrate concentration was 1.0 g liter^{-1}.
[b] The substrate concentration was 0.5 g liter^{-1}.
[c] The substrate concentration was 0.1 g liter^{-1}.
[d] ND, not detectable.

greatest for 10-methyl-octadecanoic acid, and it occurred at the expense of unsaturated rather than saturated fatty acids.

Cells of *R. opacus* GM-14, GM-29, and 1CP contained a significant amount (5%) of *trans*-hexadecenoic acid (16:1 ω 7t). The content of the *trans* isomer was higher (8 to 18%) in cells of strains GM-14 and 1CP grown on phenol and 4-chlorophenol. Isomerization of the *cis* to the *trans* form as a response to exposure to toxic compounds has been described for *Pseudomonas putida*, which synthesizes fatty acids by the anaerobic pathway commonly utilized by gram-negative bacteria (4, 5, 11, 20). Moreover, the occurrence of *trans* fatty acid isomers was reported for a few gram-negative genera and *Bacillus cereus*, which use the anaerobic and the aerobic routes for fatty acid biosynthesis (9, 12).

The dynamic changes in strain GM-14 fatty acid composition in response to exposure to toxic aromatic compounds are shown as an example in Fig. 1. The strain was grown in mineral medium containing fructose (1.0 g liter^{-1}). Phenol or toluene was added in increasing concentrations to exponentially growing cultures (optical density at 540 nm [OD_{540}], $\approx$0.3). The cultures were then grown for two generations and harvested.

Strain GM-14 responded to increasing concentrations of phenol in the medium by replacing *cis*-unsaturated fatty acids with 10-methyl branched saturated fatty acids (Fig. 1A). There was little change in the proportions of saturated straight-chain fatty acids at phenol concentrations permissive for cell growth. While growing on fructose, the cells also used phenol. However, when phenol was added to the medium at concentrations of 0.5 to 1.25 g liter^{-1}, the amount of phenol utilized by the cells remained constant at 0.3 to 0.4 g liter^{-1}. This was verified by GC analysis.

The effect of toluene on the fatty acid composition of *R. opacus* GM-14 is shown in Fig. 1B. We chose this strain for display because it was not able to metabolize toluene (21) and the fatty acid changes therefore should reflect the response to the toxicity of the solvent. The cellular fatty acid composition changed with toluene in the growth medium similarly as with phenol: a dose-related increase in the cellular content of 10-methyl fatty acids was observed.

In summary, our study showed major changes in the whole-cell fatty acid compositions associated with the adaptation of *R. opacus* to the presence of aromatic solvents. Compared with cells grown in mineral medium on fructose, the cellular contents of 10-methyl branched fatty acids of the three *R. opacus* strains were 3- to 10-fold higher during growth on toxic aromatic compounds as sole carbon sources. Moreover, dose-related increases in the levels of cellular 10-methyl branched fatty acids were observed as a response to an increasing con-

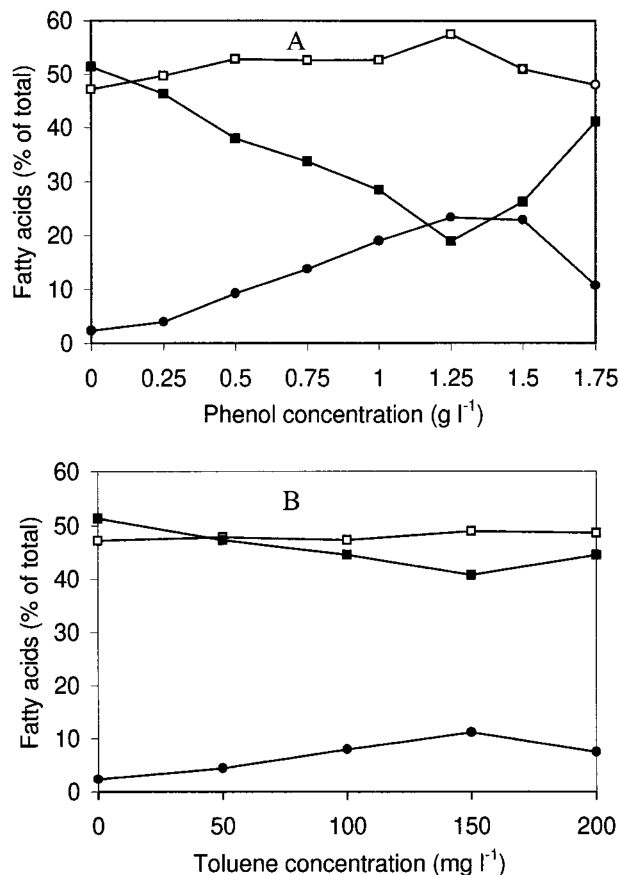

FIG. 1. Effects of different concentrations of phenol (A) and toluene (B) on the cellular fatty acid composition of *R. opacus* GM-14. Cells were grown in KSN medium containing fructose and various concentrations of phenol or toluene. Phenol or toluene added to an exponentially growing culture with an OD_{540} of ≈0.3. The biomass was allowed to grow for two doublings of the OD_{540} before harvesting. Symbols: ■, unsaturated fatty acids; □, straight-chain saturated fatty acids; ●, 10-methyl branched fatty acids.

centration of phenol or toluene in the medium, independently of the ability to use toluene.

Pimelobacter sp. has been reported to increase the content of 10-methylheptanoic and 10-methyloctanoic acids while growing on pyridine as the sole carbon source (13). An increase of cellular 10-methyl branched fatty acids was also observed as a response to an increased temperature in *Mycobacterium phlei* (18, 19).

The physiological role of 10-methyl branched fatty acids that occur in bacteria belonging to the genera *Nocardia*, *Gordona*, *Rhodococcus*, *Mycobacterium*, *Dietzia*, and *Tsukamurella* is unresolved, and the localization of 10-methyl branched fatty acids in the lipids of *Rhodococcus* has not been described. Mycobacterial lipids have been studied intensively, and there is evidence that 10-methyl octadecanoic (tuberculostearic), palmitic, and stearic acids are located in cell envelope lipids, mainly lipoarabinomannan and ornithine-amide lipid (1, 7, 10, 17). It is known that lipoarabinomannan is one of the major components of the cell envelope, and it traverses the cell wall of a mycobacterium. Moreover, tuberculostearic acid and palmitate are major acyl groups of the phosphatidylinositol moiety which anchors lipoarabinomannan to the cytoplasmic membrane (6, 7). Based on this, we suggest that an increasing amount of lipoarabinomannan may be involved in the protection of actinomycete cells against disruption of the membrane-cell wall structure.

We thank Raimo Mikkola for advice about GC-mass spectrometry. We are grateful to L. A. Golovleva for donation of *R. erythropolis* 1CP.

This work was financially supported by the Academy of Finland (M.S.S.) and the Helsinki University Fund for Center of Excellence (M.S.S.).

REFERENCES

1. **Daffé, M., and P. Draper.** 1998. The envelope layers of mycobacteria with reference to their pathogenicity. Adv. Microb Physiol. **39:**131–203.
2. **Diefenbach, R., H.-J. Heipieper, and H. Keweloh.** 1992. The conversion of *cis* into *trans* unsaturated fatty acids in *Pseudomonas putida* P8: evidence for a role in the regulation of membrane fluidity. Appl. Microbiol. Biotechnol. **38:**382–387.
3. **Gorlatov, S. N., O. V. Mal'tseva, V. I. Shevchenko, and L. A. Golovleva.** 1989. Degradation of chlorophenols by a culture of *Rhodococcus erythropolis*. Mikrobiologiya **58:**802–806. (In Russian.)
4. **Heipieper, H.-J., and J. A. M. de Bont.** 1994. Adaptation of *Pseudomonas putida* S12 to ethanol and toluene at the level of fatty acid composition of membranes. Appl. Environ. Microbiol. **60:**4440–4444.
5. **Heipieper, H.-J., R. Diefenbach, and H. Keweloh.** 1992. Conversion of *cis* unsaturated fatty acids to *trans*, a possible mechanism for the protection of phenol-degrading *Pseudomonas putida* P8 from substrate toxicity. Appl. Environ. Microbiol. **58:**1847–1852.
6. **Hunter, S. W. H., and P. J. Brennan.** 1990. Evidence for the presence of a phosphatidylinositol anchor on the lipoarabinomannan and lipomannan of *Mycobacterium tuberculosis*. J. Biol. Chem. **265:**9272–9279.
7. **Hunter, S. W. H., H. Gaylord, and P. J. Brennan.** 1986. Structure and antigenicity of the phosphorylated lipopolysaccharide antigens from the leprosy and tubercle bacilli. J. Biol. Chem. **261:**12345–12351.
8. **Keweloh, H., R. Diefenbach, and H. J. Rehm.** 1991. Increase of phenol tolerance of *Escherichia coli* by alterations of the fatty acid composition of the membrane lipids. Arch. Microbiol. **157:**49–53.
9. **Keweloh, H., and H. J. Heipieper.** 1996. *Trans* unsaturated fatty acids in bacteria. Lipids **31:**129–137.
10. **Lanéelle, M.-A., D. Promé, G. Lanéelle, and J.-C. Promé.** 1990. Ornithine lipid of *Mycobacterium tuberculosis*: its distribution in some slow- and fast-growing mycobacteria. J. Gen. Microbiol. **136:**773–778.
11. **Pinkart, H. C., J. W. Wolfram, R. Rogers, and D. C. White.** 1996. Cell envelope changes in solvent-tolerant and solvent-sensitive *Pseudomonas putida* strains following exposure to *o*-xylene. Appl. Environ. Microbiol. **62:**1129–1132.
12. **Pirttijärvi, T. S. M., T. H. Graeffe, and M. S. Salkinoja-Salonen.** 1996. Bacterial contaminants in liquid packaging boards: assessment of potential for food spoilage. J. Appl. Bacteriol. **81:**445–458.
13. **Rhee, S.-K., G. M. Lee, Y. B. Kimand, and S.-T. Lee.** 1996. Effect of pyridine on the fatty acid composition of *Pimelobacter* sp. FEMS Microbiol. Lett. **141:**139–143.
14. **Salkinoja-Salonen, M. S.** 1997. Direct submission to EMBL database.
15. **Sikkema, J., J. A. M. de Bont, and B. Poolman.** 1994. Interactions of cyclic hydrocarbons with biological membranes. J. Biol. Chem. **269:**8022–8028.
16. **Sikkema, J., J. A. M. de Bont, and B. Poolman.** 1995. Mechanisms of membrane toxicity of hydrocarbons. Microbiol. Rev. **59:**201–222.
17. **Sugden, E. A., B. S. Samagh, D. R. Bundle, and J. R. Duncan.** 1987. Lipoarabinomannan and lipid-free arabinomannan antigens of *Mycobacterium paratuberculosis*. Infect. Immun. **55;**762–770.
18. **Suutari, M., and S. Laakso.** 1993. Effect of growth temperature on the fatty acid composition of *Mycobacterium phlei*. Arch. Microbiol. **159:**119–123.
19. **Toriyama, S., I. Yano, M. Masui, E. Kusunose, M. Kusunose, and N. Akimori.** 1980. Regulation of cell wall mycolic acid biosynthesis in acid-fast bacteria. I. Temperature-induced changes in mycolic acid molecular species and related compounds in *Mycobacterium phlei*. J. Biochem. **88:**211–221.
20. **Weber, F. J., S. Isken, and J. A. M. de Bont.** 1994. *Cis*/*trans* isomerization of fatty acids as a defense mechanism of *Pseudomonas putida* strains to toxic concentrations of toluene. Microbiology **140:**2013–2017.
21. **Zaitsev, G. M., J. S. Uotila, I. V. Tsitko, A. G. Lobanok, and M. S. Salkinoja-Salonen.** 1995. Utilization of halogenated benzenes, phenols, and benzoates by *Rhodococcus opacus* GM-14. Appl. Environ. Microbiol. **61:**4191–4201.