

The Effect of Alkylresorcinol on Lipid Metabolism in *Azotobacter chroococcum*

Joanna Rejman^a and Arkadiusz Kozubek^{b,*}

^a Institute of the Experimental Treatment of Cystic Fibrosis, H. S. Raffaele, 20132 Milano, Italy

^b Institute of Biochemistry and Molecular Biology, University of Wrocław, Przybyszewskiego 63/77, 51-148 Wrocław, Poland. E-mail: kozubek@ibmb.uni.wroc.pl

* Author for correspondence and reprint requests

Z. Naturforsch. **59c**, 393–398 (2004); received November 12, 2003/March 16, 2004

We studied the effect of exogenous alkylresorcinols on the lipid metabolism of *Azotobacter chroococcum*. We observed that when 5-*n*-pentadecylresorcinol was present in the growth medium, the more endogenous alkylresorcinols were synthesized. Concurrently, a drop in the amount of phospholipids was observed. These changes were associated with increasing numbers of dormant cysts, while the number of vegetative cells diminished. The chemical nature of the alkylresorcinols synthesized by *Azotobacter chroococcum* was dependent on the duration of exposure of the bacteria to exogenous alkylresorcinols. When the exposure time was prolonged to four days, 5-*n*-nonadecylresorcinol (C 19:0) was substituted by 5-*n*-heneicosylresorcinol (C 21:0) and 5-*n*-tricosylresorcinol (C 23:0). Two fluorescent membrane probes, NBD-PE and TMA-DPH, further revealed that the presence of alkylresorcinols in the lipid bilayer restrains the phospholipid rotational motion.

Key words: Phenolic Lipids, Alkylresorcinols, *Azotobacter*

Introduction

Alkylresorcinols are long-chain homologues of orcinol (1,3-dihydroxy-5-methylbenzene). The natural occurrence of these compounds or their derivatives has been demonstrated in several plant families: Anacardiaceae, Ginkgoaceae, Gramineae, Proteaceae, Myrsinaceae and Leguminosae (reviewed by Kozubek and Tyman, 1999). The presence of alkylresorcinols has also been reported in bacteria. The homologue 5-*n*-nonadecylresorcinol (C₁₉) was isolated for the first time from cysts of *Azotobacter vinelandii*, a gram negative, free-living, nitrogen-fixing bacterium that lives in the soil (Reusch and Sadoff, 1979, 1981, 1983; Segura *et al.*, 2003). Another member of this family, *Azotobacter chroococcum*, has been reported to be a rich source of alkylresorcinol homologues with side chains varying from C₁₃ to C₂₇ (Batrakov *et al.*, 1977; Kozubek *et al.*, 1996). Interestingly, these compounds have also been isolated from the culture broth of *Pseudomonas carboxydoflava* (Osipov *et al.*, 1985) and *Streptomyces* (Aoyagi *et al.*,

1971; Kumagai *et al.*, 1971; Yamada *et al.*, 1995). Remarkably, the alkyl chains of bacterial resorcinols thus far isolated and identified were found to be saturated.

The precise physiological role of alkylresorcinols in bacteria remains to be explained. Nevertheless, their occurrence seems to be essential for some bacteria. It has been speculated that their amphiphilic nature allows them to be incorporated into the cell membrane, thus significantly changing its properties (Su *et al.*, 1981). If vegetative cells of *Azotobacter vinelandii* undergo differentiation to dormant cysts, their membrane phospholipids are substituted by 5-*n*-alkylresorcinols and 6-*n*-alkylpyrones (Reusch and Sadoff, 1981). Since these resting cells are far more resistant to various chemical and physical factors than vegetative cells, it is conceivable that they serve as endogenous anabiotic compounds (Bitkov *et al.*, 1992; Nenashev *et al.*, 1989, 1994). This hypothesis could be further supported by the observation that these compounds are also secreted into the growth media by several bacterial strains (Osipov *et al.*, 1985; Tsuge *et al.*, 1992).

The purpose of our work was to examine the effect of exogenous alkylresorcinols on the synthesis of lipids in *Azotobacter chroococcum*.

Abbreviations: NBD-PE, *N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine; TMA-DPH, 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene-*p*-toluenesulfonate; MLV, multilamellar vesicles.

Materials and Methods

Microorganisms

In our studies we utilized *Azotobacter chroococcum* Az 12 strain. It was a kind gift from Stanislaw Pietr from the Department of Agricultural Microbiology, Agricultural University, Wrocław.

Growth of bacteria

The bacteria were cultivated on Burk's nitrogen-free broth supplemented with glucose (1%). Erlenmeyer flasks (300 ml) containing 100-ml cultures were placed in a rotary shaker (150 rpm, amplitude 4 cm) and incubated at 30 °C.

Extraction of lipids

Bacteria were collected by centrifugation at 4 °C, washed with 0.1 M MgSO₄ and subsequently freeze-dried. Lipids were extracted from the dry material with a chloroform/methanol mixture (2:1, v/v). To separate solids from extracts, the suspension was centrifuged. The solvent was removed first in a vacuum rotary evaporator then in a vacuum desiccator. Extracted lipids were dissolved in a chloroform/methanol solution (2:1, v/v) and stored under nitrogen at -20 °C.

The effect of alkylresorcinols present in growth medium on lipids synthesis in *Azotobacter chroococcum* Az 12

Bacteria were cultivated in the presence of 5-*n*-pentadecylresorcinol (0.5 mg/ml) for 1, 2, 3 and 4 d. Subsequently, they were centrifuged and suspended in fresh medium without resorcinolic lipids. This procedure was repeated five times to make sure that all unabsorbed resorcinols were removed from medium. The entire time of incubation was 96 h thus bacteria were further cultivated for an appropriate number of days (3, 2 or 1). Control bacteria were grown for 96 h in medium without alkylresorcinols.

Quantitative determinations

The amount of resorcinols was determined by the method described by Tluscik *et al.* (1981). Total phosphorus amount was determined by the method of Rouser *et al.* (1966). To determine the amount of protein we employed the method of Lowry *et al.* (1955).

Chromatography

Resorcinolic lipids were separated from lipid extracts by column chromatography (1 × 10 cm) on Silica Gel Si60 (Merck, Darmstadt, Germany) suspended in chloroform. The sample dissolved in chloroform (1 mg/ml) was applied to the column. Fractions were eluted with chloroform/ethyl acetate (9:1, v/v).

Thin-layer chromatography on Silica Gel Si60 RP-18 plastic sheets (Merck) was employed to analyze the composition of each fraction. To detect alkylresorcinols, the plates developed in methanol/water (95:5, v/v) were sprayed with 0.5% Fast Blue B × BF₄ solution (Chemapol, Prague, Czech Republic) in 5% acetic acid.

Liposome preparation

Liposomes were formed by mixing lipids [phosphatidylethanolamine and phosphatidylglycerol, 4:1 (Avanti Polar Lipids, Alabaster, AL, USA)] in a chloroform/methanol solution with methanol solutions of NBD-PE (Molecular Probes) or TMA-DPH (Molecular Probes) (0.5 mol%) and dried under a stream of nitrogen gas. The remnants of solvents were removed by additional drying *in vacuo* for 2 h. The lipid film was subsequently resuspended in Tris (Tris-hydroxymethyl-amino-methane)-HCl buffer (5 ml, 10 mM, pH 7.4; Sigma, Poznan, Poland) by intense vortexing, resulting in the formation of multilamellar vesicles (MLVs). This suspension was sonicated in a bath type sonicator until clarity.

Fluorescence measurements

Each sample contained Tris-HCl buffer (2.7 ml, 10 mM, pH 7.4), liposomes (containing 1 mg of phospholipid) and a methanolic solution of all studied resorcinols. Subsequently, fluorescence was measured with a spectrofluorimeter (SMF 25, Kontron, Milan, Italy). NBD-PE λ_{ex} 460 nm, λ_{em} 534 nm; TMA-DPH λ_{ex} 355 nm, λ_{em} 450 nm.

All other chemicals of the best available purity were from ALCHEM (Wrocław, Poland).

Results

The effect of exogenous alkylresorcinols on lipid synthesis in *Azotobacter chroococcum* Az 12

To investigate the effect of exogenous alkylresorcinols on lipid synthesis in *Azotobacter chroococcum* Az 12 the bacteria were cultivated in me-

dium containing 5-*n*-pentadecylresorcinol for 1, 2, 3 and 4 d. Subsequently, the cultures were thoroughly washed and re-suspended in fresh medium without resorcinol.

The entire time of incubation was 96 h; therefore the bacteria were further cultivated for an appropriate number of days (3, 2 or 1). Control bacteria were grown for 96 h in medium without alkylresorcinols. Table I presents the amount of phospholipids and alkylresorcinols in *Azotobacter chroococcum* Az 12 cells following this treatment. The amount of phospholipids per mg of lyophilized bacterial cells decreased with increasing exposure time to 5-*n*-pentadecylresorcinol in the growth medium. When this compound was present in the medium for 4 d, the amount of phospholipids was reduced by almost 40%, relative to control cells. In contrast, the amount of alkylresorcinols increased during the course of the experiment. Relative to control cells, the amount of resorcinolic lipids doubled already after 1 d incubation with 5-*n*-pentadecylresorcinol present in medium. 4 d of incubation of *Azotobacter chroococcum* Az 12 in the presence of 5-*n*-pentadecylresorcinol resulted in a 15-fold increase of the amount of endogenous resorcinols. Interestingly, the amount of alkylresorcinols increased more strongly than the amount of phospholipids decreased.

The data presented in Table I indicate also that the longer the time of incubation with 5-*n*-pentadecylresorcinol, the lower the number of vegetative bacterial cells in culture will be. When this compound was present in growth medium for 4 d, the amount of cells decreased by 25%. The number of vegetative cells which differentiated to metabolically dormant cysts progressively in-

creased with extending the time of 5-*n*-pentadecylresorcinol presence in the incubation broth. The largest number of cysts (25%) was determined when *Azotobacter chroococcum* cells were exposed to 5-*n*-pentadecylresorcinol for 4 d.

In conclusion, the presence of alkylresorcinols in growth medium significantly influences physiological activities of *Azotobacter chroococcum*. The degree of these changes correlates with the time of exposure to these compounds. The drop in the amount of phospholipids is correlated with an increase in the amount of alkylresorcinols synthesized and the number of dormant cysts present in culture.

Chromatographic analysis of alkylresorcinols extracted from Azotobacter chroococcum Az 12

The chromatographic analysis of alkylresorcinols extracted from *Azotobacter chroococcum* Az 12 is presented in Fig. 1. 5-*n*-Nonadecylresorcinol (C 19:0) was the major homologue isolated from *Azotobacter chroococcum* cells exposed to exogenous alkylresorcinol (C 15:0) for 1 and 2 d (lane 2 and 3). Extending the time of incubation to 3 d resulted in the occurrence of alkylresorcinols with longer side chains (C 21:0 and C 23:0). However, the most prominent homologue was still 5-*n*-nonadecylresorcinol (lane 4). The most pronounced change in the character of extracted homologues was observed while exogenous alkylresorcinols were present in growth medium for 4 d. In these cells the amount of 5-*n*-nonadecylresorcinol was significantly reduced, while the presence of homologues with longer side chains (C 21:0 and C 23:0) was greatly enhanced.

Table I. The effect of exogenous alkylresorcinols on the number of dormant and vegetative cells and lipid synthesis in *Azotobacter chroococcum* Az 12.

The bacteria were first cultivated in medium containing 5-*n*-pentadecylresorcinol for 1, 2, 3 or 4 d. After intense washing the cells were re-suspended in fresh medium without resorcinols. The entire time of incubation was 96 h. Therefore the bacteria were further cultivated for an appropriate number of days (3, 2 or 1). Control bacteria were growing for 4 d in medium without alkylresorcinols. Three independent experiments were carried out in duplicate.

	Number of bacteria (in 1 μ l after 96 h)	Percent of cysts (after 96 h)	Cell/cyst ratio	Phospholipids [μ g/mg of cells ^a]	Alkylresorcinols [μ g/mg of cells ^a]	Phospholipids/ alkylresorcinols ratio
Control	600 \pm 10	4 \pm 1	150	80 \pm 4	4 \pm 1	20
1 d	560 \pm 15	5 \pm 2	112	68 \pm 3	10 \pm 1	6.8
2 d	500 \pm 5	9 \pm 1	55	62 \pm 5	11 \pm 2	5.6
4 d	450 \pm 10	25 \pm 3	18	48 \pm 1	60 \pm 5	0.8

^a Dry weight.

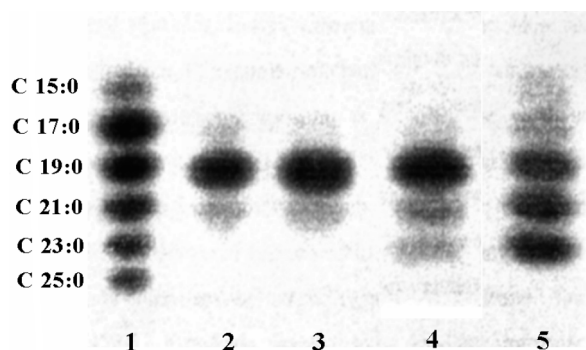


Fig. 1. Chromatographic analysis of 5-*n*-alkylresorcinols extracted from *Azotobacter chroococcum* Az 12. The lipids were extracted from bacteria and separated by column chromatography as described in Materials and Methods. Thin-layer chromatography on Silica Gel Si60 RP-18 plastic sheets was employed to analyze the composition of each fraction. The plates were developed in methanol/water (95:5, v/v) and sprayed with 0.5% Fast Blue B solution in 5% acetic acid.

(1) Alkylresorcinols standard mixture; (2) alkylresorcinols extracted from lyophilized *Azotobacter chroococcum* after incubation with 5-*n*-pentadecylresorcinol for 1 d; (3) alkylresorcinols extracted after incubation with 5-*n*-pentadecylresorcinol for 2 d; (4) alkylresorcinols extracted after incubation with 5-*n*-pentadecylresorcinol for 3 d; (5) alkylresorcinols extracted after incubation with 5-*n*-pentadecylresorcinol for 4 d.

The chromatographic pattern of alkylresorcinols extracted from the lyophilized control bacteria was identical to that presented for *Azotobacter chroococcum* incubated with 5-*n*-pentadecylresorcinol for 1 d (lane 2). The intensity of the spots visualized with Fast Blue B (reacting with the ring structure) reflects the relative amount of each homologue in the sample.

These results demonstrate that 5-*n*-pentadecylresorcinol added to media promotes the synthesis of endogenous alkylresorcinols. The alkyl chain length of the newly synthesized homologues increased with increasing time of incubation of the cells with 5-*n*-pentadecylresorcinol.

The effect of alkylresorcinols on liposome membrane properties

It has been suggested that alkylresorcinols may incorporate into the plasma membrane and consequently change its properties (Bitkov *et al.*, 1990, 1992; Kozubek *et al.*, 1988, 1992). To examine this possibility, we employed fluorescent probes (NBD-PE and TMA-DPH) incorporated into liposomes containing alkylresorcinols (5-*n*-pentadecylresorcinol or 5-*n*-heneicosylresorcinol, 15 μ M). It is generally assumed that TMA-DPH resides in

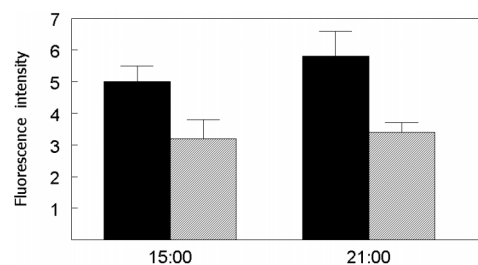


Fig. 2. The effect of alkylresorcinols on liposome membrane properties fluorescence intensity. Each sample contained Tris-HCl buffer (2.7 ml, 10 mM, pH 7.4), liposomes (containing 1 mg of phospholipid, preparation as described in Materials and Methods) and a methanolic solution of all studied resorcinols. Subsequently, fluorescence was measured with a spectrofluorimeter.

the center of the lipid bilayer parallel to the lipid acyl chain axis. Its fluorescence polarization is high in the absence of rotational motion and is very sensitive to reorientation of the long axis resulting from interactions with surrounding lipids. On the other hand, the NBD-PE localizes in the head-group region of the lipid bilayer, therefore it is suitable to monitor changes in this region of the plasma membrane. To mimic the natural phospholipid composition of prokaryotic organisms (Reusch and Sadoff, 1979, 1981, 1983; Segura *et al.*, 2003; Su *et al.*, 1981), liposomes were formed from a mixture of phosphatidylethanolamine and phosphatidylglycerol (4:1, v/v). Fig. 2 shows the changes in fluorescence intensity caused by the presence of alkylresorcinols in the lipid bilayer. The data indicate that these changes, for both homologues, are most pronounced in the region monitored by TMA-DPH, in the center of the plasma membrane. Although, 5-*n*-heneicosylresorcinol had a stronger effect on lipid order in that plasma membrane region.

These results are further supported by the results of fluorescence polarization of TMA-DPH and NBD-PE studies as a function of 5-*n*-heneicosylresorcinol concentration, indicating a decrease of phospholipids rotational motion in both bilayer regions (data not shown).

Discussion

Our data reveal that exogenous resorcinols strongly influence the lipid metabolism of *Azotobacter chroococcum*. Apparently, the exogenous alkylresorcinols cause metabolic changes in *Azotobacter chroococcum* cells, which cause the inhibi-

tion of phospholipid synthesis. As a result, the bacteria stop dividing and switch to the synthesis of longer alkylresorcinol homologues that subsequently switch on the process of encystment. Recent data presented by Segura *et al.* (2003) indicating that in *Azotobacter vinelandii* strains impaired in poly-beta-hydroxybutyrate synthesis the production of alkylresorcinols is significantly enhanced. As the common metabolic source for both beta-hydroxybutyrate synthesis (one of the ketone bodies) and of alkylresorcinols is the acetyl-CoA the data may suggest the feedback and the regulatory activities of 5-*n*-alkylresorcinols in the process of encystment. For this purpose the cells may use two alternative pathways. In the first one most of phospholipids and glyceride's acyl chains are degraded completely to acetyl-CoA from which, consequently, both polyhydroxybutyrate and alkylresorcinols are synthesised *de novo*. In an alternative pathway, already suggested earlier (Kozubek and Tyman, 1999) and confirmed in the recent work of Suzuki *et al.* (2003), only a part of the acyl chains is degraded and used for the synthesis of the 1,3-dihydroxybenzene ring structure. The remaining chains are attached to these rings leading to the formation of the 5-*n*-alkylresorcinol structure. Our data further demonstrate that alkylresorcinols restrain the rotational motion of the phospholipids in the membrane bilayer that may result from the interaction between both types of the molecules, as suggested previously for nonbacterial (liposomal) and bacterial membranes (Bitkov *et al.*, 1992; Kozubek *et al.*, 1988). The introduction of resorcinols with one saturated alkyl chain and a relatively large head group, able to form hydrogen bonds, is an efficient way to reduce membrane fluidity. The synthesis of alkylresorcinols with longer side chains (observed after 4 d) is the next step in

this process. Each additional -CH₂- increases the energy of interaction between neighbouring acyl chains by approx. 2.1 kJ/mol.

On the other hand, gradual introduction of exogenous alkylresorcinols into the plasma membrane causes changes in membrane fluidity that may restrict the functioning of membrane-associated proteins (Kozubek *et al.*, 1992). This may include enzymes involved in the synthesis of bacterial phospholipids, which are also associated with the plasma membrane (Bell *et al.*, 1971; Cronan and Vagelos, 1972; Tsuge *et al.*, 1992). This inactivation restrains the synthesis of phospholipids, required for generating new cells. They will stop dividing and start to differentiate into metabolically dormant cysts. This process in bacteria from genus *Azotobacter* is combined with excessive synthesis of alkylresorcinols. During the process of encystment, the length of the side chain of the synthesized alkylresorcinols increases, rendering the plasma membrane more rigid and resistant to various physical and chemical agents. Recently Segura *et al.* (2003) demonstrated that synthesis of alkylresorcinols in *Azotobacter vinelandii* involves utilisation of the same acetyl-CoA pool as those used for the synthesis of polyhydroxybutyrate (PHB). The inhibitory properties of other long-chain alkylresorcinol homologues against triglyceride synthesis has been also shown for animal adipocytes (Rejman and Kozubek, 2004). These data together with our observations suggest that alkylresorcinols may act as a trigger of the bacterial metabolism from the vegetative state to the formation of cysts.

Acknowledgement

The work was supported by the University of Wroclaw research grants.

- Aoyagi T., Yagisawa M., Kumagai M., Hamada M., Okami Y., Takeuchi T., and Umezawa H. (1971), An enzyme inhibitor, panosialin, produced by *Streptomyces*. I. Biological activity, isolation and characterization of panosialin. *J. Antibiot.* **24**, 855–860.
- Batrakov S. G., Pridachina N. N., Kryglyak E. B., and Novogrudskaya E. D. (1977), Phenolic lipid from *Azotobacter chroococcum*. *Khim. Prirod. Soed. (USSR)* **4**, 494–499.
- Bell R. M., Mavis R. D., Osborn M. J., and Vagelos P. R. (1971), Enzymes of phospholipid metabolism: Location in the cytoplasmic and outer membrane of the cell envelope of *Escherichia coli* and *Salmonella typhimurium*. *Biochim. Biophys. Acta* **249**, 628–635.
- Bitkov V. V., Nenashev V. A., Khashaev Z. H., Pridachina N. N., and Batrakov S. G. (1990), Long-chain 5-*n*-alkylresorcinols as the regulators of structure in lipid membranes. *Biol. Membr.* **7**, 135–140.
- Bitkov V. V., Nenashev V. A., Pridachina N. N., and Batrakov S. G. (1992), Membrane-structuring properties of bacterial long-chain alkylresorcinols. *Biochim. Biophys. Acta* **1108**, 224–232.
- Cronan J. E. and Vagelos P. R. (1972), Metabolism and function of membrane phospholipids of *Escherichia coli*. *Biochim. Biophys. Acta* **265**, 25–60.
- Kozubek A. and Tyman J. H. P. (1999), Resorcinolic lipids, the natural non-isoprenoid phenolic amphiphiles and their biological activity. *Chem. Rev.* **99**, 1–26.
- Kozubek A., Jezierski A., and Sikorski A. F. (1988), The effect of nonadec(en)ylresorcinol on the fluidity of liposome and erythrocyte membranes. *Biochim. Biophys. Acta* **944**, 465–472.
- Kozubek A., Nietubyc M., and Sikorski A. F. (1992), Modulation of the activities of membrane enzymes by cereal grain resorcinolic lipids. *Z. Naturforsch.* **47c**, 41–46.
- Kozubek A., Pietr S., and Czerwonka A. (1996), Alkylresorcinols are abundant lipid components in *Azotobacter* and *Pseudomonas* strains. *J. Bacteriol.* **178**, 4027–4030.
- Kumagai M., Suhara Y., Aoyagi T., and Umezawa H. (1971), Enzyme inhibitor, panosialin, produced by *Streptomyces*. II. Chemistry of panosialin, 5-alkylbenzene-1,3-disulfates. *J. Antibiot.* **24**, 870–875.
- Lowry O. H., Rosenbrough N. J., Farr A. L., and Randall R. J. (1955), Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**, 265–275.
- Nenashev V. A., Pridachina N. N., Pronevich L. A., and Batrakov S. G. (1989), 5-Alkyl(C19–25)resorcinols as regulators of the oxidation of succinate and NAD-dependent substrates by mitochondria. *Biokhimiya* **54**, 784–787.
- Nenashev V. A., Pridachina N. N., Elregistan G. I., Zolotareva I. N., and Batrakov S. G. (1994), Effect of autoregulators of anabiosis of some microorganisms on respiration of rat liver mitochondria. *Biokhimiya* **59**, 11–15.
- Osipov G. A., El-Registan G. I., Svetlichny V. A., Kozlova A. N., Duda V. I., Kaprelyants A. S., and Pomazanov V. V. (1985), The chemical nature of the autoregulator factor d1 in *Pseudomonas carboxydoflava*. *Mikrobiologiya* **54**, 186–190.
- Rejman J. and Kozubek A. (2004), Inhibitory effect of natural phenolic lipids upon NAD-dependent dehydrogenases and on triglyceride accumulation in 3T3-L1 cells in culture. *J. Agric. Food Chem.* **52**, 246–250.
- Reusch R. N. and Sadoff H. L. (1979), 5-*n*-Alkylresorcinols from encysting *Azotobacter vinelandii*: Isolation and characterization. *J. Bacteriol.* **139**, 448–453.
- Reusch R. N. and Sadoff H. L. (1981), Lipid metabolism during encystment of *Azotobacter vinelandii*. *J. Bacteriol.* **145**, 889–895.
- Reusch R. N. and Sadoff H. L. (1983), Novel lipid components of the *Azotobacter vinelandii* cyst membrane. *Nature* **302**, 268–270.
- Rouser G., Siakotos A. N., and Fleisher S. (1966), Quantitative analysis of phospholipids by thin layer chromatography and phosphorus analysis of spots. *Lipids* **1**, 85–86.
- Segura D., Cruz T., and Espin J. (2003), Encystment and alkylresorcinol production by *Azotobacter vinelandii* strains impaired in poly-beta-hydroxybutyrate synthesis. *Arch. Microbiol.* **179**, 437–443.
- Su C.-J., Reusch R. N., and Sadoff H. L. (1981), Isolation and characterization of several unique lipids from *Azotobacter vinelandii* cysts. *J. Bacteriol.* **147**, 80–90.
- Suzuki Y., Kurano M., Esumi Y., Yamaguchi I., and Doi Y. (2003), Biosynthesis of 5alkylresorcinols in rice: incorporation of a putative fatty acid unit in the 5-alkylresorcinol carbon chain. *Bioorg. Chem.* **31**, 437–452.
- Tluscik F., Kozubek A., and Mejbaum-Katzenellenbogen W. (1981), Alkylresorcinols in rye (*Secale cereale* L.) grains. VI. Colorimetric micromethod for the determination of alkylresorcinols with the use of diazonium salt, Fast Blue B. *Acta Soc. Bot. Polon.* **50**, 645–651.
- Tsuge N., Mizokami M., Imai S., Shimazu A., and Seto H. (1992), Adipostatins A and B, new inhibitors of glycerol-3-phosphate dehydrogenase. *J. Antibiot.* **45**, 886–891.
- Yamada H., Shiomi K., Xu Q., Nagai T., Shibata M., Oya I., Takahashi Y., and Omura S. (1995), New glycosidases inhibitors, panosialins D and wD produced by *Streptomyces* sp. OH-5186. *J. Antibiot.* **48**, 205–210.