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ORIGINAL PAPER

Biosynthesis of ω-alicyclic fatty acids induced by cyclic precursors and change of membrane fluidity in thermophilic bacteria *Geobacillus stearothermophilus* and *Meiothermus ruber*

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Abstract Two thermophilic strains belonging to *Geobacillus stearothermophilus* and *Meiothermus ruber*, which naturally do not synthesize ω-alicyclic fatty acids (ω-FAs) were cultivated with cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl carboxylic acids. Gas chromatography–mass spectrometry analysis of fatty acid methyl and picolinyl esters showed that both strains are able to synthesize ω-FAs when cultivated with the appropriate precursor. The incorporation of cyclic acids influenced the whole FA composition as well as membrane fluidity. Membrane fluidity of intact cells was studied by measuring the fluorescence polarisation of the probe 1,6-diphenyl-1,3,5-hexatriene incorporated into membrane lipid bilayers. Cytoplasmic membrane became more fluid with increasing content of ω-FAs. This is caused by considerable changes in lipid packing within the membrane induced by the presence of ω-FAs not found in the natural environment of *Geobacillus* and *Meiothermus* strains.

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Introduction

Members of the genus *Alicyclobacillus* are the only known thermophiles with ω-alicyclic fatty acids (ω-FAs) as a major membrane component (Chang and Kang 2004; Goto et al. 2007; Siristova et al. 2009). The presence of ω-FAs is likely associated with the resistance of *Alicyclobacillus* to high temperatures and acidic conditions (Kannenberg et al. 1984; Chang and Kang 2004; Goto et al. 2007). The reason may be a close packing of the rings of ω-FAs in membrane structure, which stabilize it and thus form a protective coating for the cell membrane (Kannenberg et al. 1984; Chang and Kang 2004). The claim is supported by several experiments in which increasing temperature and decreasing pH strongly increased the amount of cyclohexane lipids (Kannenberg et al. 1984; Moore et al. 1993) or by an experiment with mutants of *Alicyclobacillus acidocaldarius* deficient in ω-cyclohexyl FA biosynthesis, which grew poorly at high temperatures and low pH (Moore et al. 1993).

ω-Cyclohexyl FAs are synthesized via FA biosynthesis from a cyclohexyl carboxylic acid starter unit, such as coenzyme A thioester (Handa and Floss 1997). Cyclohexyl carboxylic moiety is either formed from shikimic acid (a glucose metabolite) or could be supplied from external sources. The biosynthetic pathway from shikimic acid involves a series of dehydrations and double bond reductions interspersed in such a way that an intermediate is never aromatic (De Rosa et al. 1974; Kaneda 1991; Moore et al. 1993; Handa and Floss 1997). The FA synthase of

ω-FA pathway is considered to be catalytically identical to that producing branched-chain FAs (Kaneda 1991).

The majority of glycerophospholipids in the bacterial membrane are bilayer forming, existing under normal physiological conditions in a liquid crystalline state. The liquid crystalline state is considered to be the biologically active state of the membrane (Suutari and Laakso 1994; Denich et al. 2003).

Environmental factors such as temperature, pressure, pH, ionic strength and nutrient composition greatly influence membrane structure and composition (Suutari and Laakso 1994). FA composition of microbial membranes is continually altered in order to retain fluidity during environmental changes by changing fatty acyl chain and head group composition (Suutari and Laakso 1994; Denich et al. 2003). Thermophilic microorganisms adapt to higher temperatures by increasing the proportion of *iso*-branched and longer chain FAs, while decreasing lower-melting *anteiso*-branched and short chain FAs (Suutari and Laakso 1994). Both thermophilic species, i.e. *Geobacillus stearothermophilus* and *Meiothermus rubber*, contain a large proportion of polar lipids in their cytoplasmic membrane including phospholipids and unusual glycolipids. The hydrophobic regions are predominantly *iso*- and *anteiso*-branched FAs, while straight-chain FAs are minor components (Albers and Driessen 2008; Siristova et al. 2009). Neither *G. stearothermophilus* nor *M. ruber* naturally produces ω-FAs (Siristova et al. 2009).

Methyl esters, in general, have been the most analyzed FA derivatives and their chromatographic and mass spectrometry properties provide a wealth of information. However, the mass spectrometry of FAMEs yields only limited information on the structure of FAs, especially non-straight-chain. The mass spectra of ω-cycloalkyl FAMEs have been described several times (Hamilton and Christie 2000; Dobson 1998; Harvey 1984).

Molecular ion intensities are found to decrease on going from cyclohexyl to cyclopropyl derivatives according to the increasing ring tension (De Rosa and Gambacorta 1975). The cyclopropyl derivative does not show a molecular ion. Ions $[M–CH_3O]^+$ and $[M–CH_3OH]^+$ are the only significant ions in the area of molecular ion. The relative intensity of both of these ions increases from cyclohexyl to cyclopropyl FAMEs. Other ions are formed by chain splitting, i.e. α-cleavage which would result in fragments $[M\text{-}41]^+$, $[M\text{-}55]^+$, $[M\text{-}69]^+$, and $[M\text{-}83]^+$, respectively. These ions have a very low abundance (De Rosa and Gambacorta 1975; Oshima and Ariga 1975; Schogt and Begemann 1965) and are sometimes completely missing (Dreher et al. 1976). In all cases, a strong Mc-Lafferty rearrangement ion is observed at *m/z* 74, accompanied by *m/z* 87. Although these data are more than 30 years old, new data (Hu and Floss 2006) confirm these findings.

In our work, we therefore identified ω-cycloalkyl FAs as picolinyl esters by GC–MS. This approach reveals the fine differences between the spectra of the straight- or branched-chain FAs and ω-cycloalkyl picolinyl esters. Picolinyl esters exhibit typical prominent ions at *m/z* 92, 108, 151 and 164. The molecular ion is always abundant and it is always odd-numbered. Ions at *m/z* below 92 can be ignored. In the area of the molecular ion the clear gap of ions $[M\text{-}41]^+$, $[M\text{-}55]^+$, $[M\text{-}69]^+$, and $[M\text{-}83]^+$, respectively, showed loss of the ring. Ions at $[M\text{-}19]^+$ and $[M\text{-}41]^+$ are also present. Thereafter, there is a regular series of ions 14 Da apart caused by cleavage at the successive methylene groups.

We continued with our study of thermophilic bacteria lipids and focused in this work on unique ω-FAs. We chose two thermophilic strains *Geobacillus stearothermophilus* and *Meiothermus ruber* as model microorganisms suitable to batch cultivation experiment with four cyclic acids precursors. The aim was to observe whether ω-FAs are synthesized and how the presence of new structures affects FA composition and membrane fluidity. The changes in membrane fluidity occurring as a response to variation of the FA composition were evaluated.

Materials and methods

Microorganisms and cultivation conditions

Geobacillus stearothermophilus CCM 2062 (Czech Collection of Microorganisms, Brno, Czech Republic) was cultivated in bacillus medium B10: bacterial peptone 5 g L^{-1}, beef extract 3 g L^{-1}, $MnSO_4 \cdot H_2O$ 0.01 g L^{-1}, pH 7.0 (Catalog of Cultures 1999). *Meiothermus ruber* CCM 4212 was cultivated in thermus medium B39: yeast extract 4 g L^{-1}, tryptone 8 g L^{-1}, NaCl 2 g L^{-1}, pH 7.5 (Catalog of Cultures 1999).

Batch cultivation was carried out under aerobic conditions in 500-mL Erlenmeyer flasks filled with 200 mL of media. The flasks were incubated for 24 h at 55°C and 200 rpm. The biomass of both strains was harvested in a late exponential phase, concentrated by centrifugation (11,424×*g*; MEDIFRIGER-BL, Spain) and lyophilized.

Precursor acids

Cyclopropyl (95%), cyclobutyl (98%), cyclopentyl (99%) and cyclohexyl (98%) carboxylic acids (Sigma-Aldrich) were added to the media in amounts of 0.5, 1.5 and 4.5 mmol L^{-1} at the zero time of cultivation. At these

concentrations none of the precursors influenced the growth phase.

Fatty acid extraction and FAME analysis

Extraction procedure was based on the method of Bligh and Dyer (1959). 0.8 volumes of aqueous sample (lyophilized cells suspended in 10 mL water) were added to 3 parts of a chloroform/methanol mixture (1:2, v/v) to give one phase. The mixture was vigorously stirred for 60 min at room temperature, 1 volume of chloroform/water mixture (1:1, v/v) was added and the sample was centrifuged at $6{,}000\times g$ for 15 min. The lower chloroform phase containing the total lipids was separated, dried under nitrogen, and weighed. The total lipids were reacted with 3% conc. HCl in methanol at 80°C for 1 h. Fatty acid esterification occured and FAMEs (fatty acid methyl esters) were extracted with 2 mL of hexane, dried, and analyzed.

Gas chromatography–mass spectrometry of FAMEs was done on a GC–MS system consisting of Varian 450-GC. Varian 240-MS ion trap detector with electron impact ionization, and CombiPal autosampler (CTC, USA). The sample was injected onto a 25 m × 0.25 mm × 0.1 μm Ultra-1 capillary column (Supelco, Czech Republic) under a temperature program: 5 min at 50°C, increasing at 10°C min^{-1} to 280°C and 15 min at 280°C. Helium was the carrier gas at a flow of 0.52 mL min^{-1}. All spectra were scanned within the range *m/z* 50–600. The structures of FAMEs were confirmed by comparison of retention times and fragmentation patterns with those of the standard FAMEs (Supelco, Czech Republic). Calculations of the total amount of FAs were carried out by software (MS Workstation, v. 6.9, Varian) supplied with the device on the basis of total ion current.

Picolinyl esters of FAs

A solution of potassium *tert*-butoxide in tetrahydrofuran (0.5 mL, 1.0 M) was added to nicotinyl alcohol (1 mL). After mixing, the FAMEs (~0.5 mg) in dry dichloromethane (1 mL) were added, and the mixture was held at 40°C for 30 min in a closed vial. After cooling to room temperature, water and hexane were added, and the organic phase was collected, dried over anhydrous sodium sulfate, and evaporated (Rezanka 1990).

A FA picolinyl ester mixture was analyzed on the instrument described above with an Ultra-1 capillary column. Injection temperature (splitless injection) was 100°C. The temperature program was as follows: 100°C for 1 min, subsequently increasing at 20°C min^{-1} to 180°C and at 2°C min^{-1} to 280°C, which was maintained for 1 min. The carrier gas was helium at a linear velocity of 60 cm s^{-1}. All spectra were scanned within the range *m/z* 70–650.

Membrane fluidity

Membrane fluidity of intact cells was measured as described by Laroche et al. (2001), Chu-Ky et al. (2005) and Tymczyszyn et al. (2005). Briefly, DPH (1,6-diphenyl-1,3,5-hexatriene, 0.72 mg) was dissolved in 5 mL of tetrahydrofuran. Cells were resuspended in water and 10 μL DPH (0.62 mM) and shaken (60 rpm) for 20 min in darkness. The suspension was then centrifuged and the pellet was resuspended in 2.5 mL of water.

Measurements were done on a PerkinElmer Luminescence spectrometer Model LS 55 equipped with excitation and emission polarizers at constant temperature in water bath at 55°C (optimum cultivation temperature for the bacteria); excitation and emission wavelengths were 340 and 432 nm, respectively.

Steady-state fluorescence anisotropy (r) was calculated according to the following equation:

$$r = \frac{I_{VV} - G \times I_{VH}}{I_{VV} + 2 \times G \times I_{VH}} \text{ with } G = \frac{I_{VH}}{I_{HH}} \text{ (Shinitzky 1984),}$$

where I_{VV} and I_{VH} represent the fluorescence intensity obtained with the vertical and horizontal orientations, respectively, of the excitation and emission polarizers. $G = I_{HV}/I_{HH}$ is a correction factor accounting for the polarization bias in the detection system (Tymczyszyn et al. 2005).

For each measurement, fluorescence intensities were corrected by subtracting the intensity of light measured both with unlabeled cells in the binary water/glycerol mixture and with DPH in the binary medium.

Results and discussion

The results of our identification of picolinyl esters are illustrated by Supplementary Figs. S1–S4. If some spectra are mutually very similar, as in Supplementary Figs. S1 and S2, the cyclopropyl and cyclobutyl FAs can be distinguished based on retention times (Kaneda 1977). ΔECL (equivalent chain length) has an increasing trend (+0.35 for cyclopropyl, +0.45 for cyclobutyl, +0.53 for cyclopentyl and +0.64 for cyclohexyl, respectively). Analysis of FAMEs and picolinyl esters enabled us to determine both qualitatively and quantitatively not only FAs commonly found in these two bacteria but also unnatural ω-cycloalkyl FAs produced after the addition of an appropriate precursor, i.e. ω-cycloalkyl carboxylic acid.

Tables 1 and 2 summarize the percentage of FAs before and after addition of respective precursors. Both strains incorporated precursors into lipid structures (maximum incorporation of cyclohexyl carboxylic acid 21.9% for *M. ruber* and up to 56.0% for *G. stearothermophilus*).

Table 1 Fatty acid content of *Geobacillus stearothermophilus* CCM 2062 before and after addition of respective precursors (percent of total FA)

Fatty acid	No precursor	Cyclopropyl carboxylic acid			Cyclobutyl carboxylic acid			Cyclopentyl carboxylic acid			Cyclohexyl carboxylic acid		
		0.5 mM	1.5 mM	4.5 mM	0.5 mM	1.5 mM	4.5 mM	0.5 mM	1.5 mM	4.5 mM	0.5 mM	1.5 mM	4.5 mM
Odd *iso*	39.5	40.5	33.2	23.0	36.5	28.6	27.4	31.4	26.6	29.1	31.2	21.1	10.5
Even *iso*	6.9	6.9	7.4	7.2	6.4	7.3	7.0	6.5	6.0	5.3	6.7	6.3	6.0
Odd *anteiso*	23.0	21.4	22.7	20.6	19.5	17.1	14.2	22.2	20.4	16.3	21.5	19.9	19.4
Branched	0.0	0.0	0.0	0.0	1.0	1.9	3.0	0.0	0.0	0.0	0.0	0.0	0.0
Straight	28.4	21.1	17.4	15.4	23.7	20.5	18.1	24.9	14.4	6.4	23.3	16.3	8.1
Unsaturated	2.2	3.6	5.0	5.1	4.0	5.6	5.9	2.0	1.7	1.5	1.9	1.1	0.0
ω-cycPr-15:0	×	1.4	2.1	6.3	×	×	×	×	×	×	×	×	×
ω-cycPr-16:0	×	3.7	7.2	14.8	×	×	×	×	×	×	×	×	×
ω-cycPr-17:0	×	1.4	5.0	7.6	×	×	×	×	×	×	×	×	×
ω-cycBu-15:0	×	×	×	×	5.9	14.5	16.3	×	×	×	×	×	×
ω-cycBu-16:0	×	×	×	×	0.5	0.8	1.3	×	×	×	×	×	×
ω-cycBu-17:0	×	×	×	×	2.5	3.7	6.8	×	×	×	×	×	×
ω-cycPe-16:0	×	×	×	×	×	×	×	8.7	15.2	23.5	×	×	×
ω-cycPe-17:0	×	×	×	×	×	×	×	1.1	2.1	3.3	×	×	×
ω-cycPe-18:0	×	×	×	×	×	×	×	3.2	13.6	14.6	×	×	×
ω-cycHe-17:0	×	×	×	×	×	×	×	×	×	×	6.4	14.8	24.1
ω-cycHe-18:0	×	×	×	×	×	×	×	×	×	×	1.9	4.8	6.2
ω-cycHe-19:0	×	×	×	×	×	×	×	×	×	×	7.1	15.7	25.7
Total ω-cyc	×	6.5	14.3	28.7	8.9	19.0	24.4	13.0	30.9	41.4	15.4	35.3	56.0

iso, branch point is on the penultimate carbon; *anteiso*, branch point is on the ante-penultimate carbon; *branched*, branch point is on different carbon than penultimate or ante-penultimate; *cycPr*, cyclopropyl; *cycBu*, cyclobutyl; *cycPe,* cyclopentyl; *cycHe* cyclohexyl; *ω-cyc*, ω-alicyclic FA; ×, not present; 0.0, less than 0.1%

Table 2 Fatty acid content of *Meiothermus ruber* CCM 4212 before and after addition of respective precursors (percent of total FA)

Fatty acid	No precursor	Cyclopropyl carboxylic acid			Cyclobutyl carboxylic acid			Cyclopentyl carboxylic acid			Cyclohexyl carboxylic acid		
		0.5 mM	1.5 mM	4.5 mM	0.5 mM	1.5 mM	4.5 mM	0.5 mM	1.5 mM	4.5 mM	0.5 mM	1.5 mM	4.5 mM
Odd *iso*	34.0	31.1	27.4	21.1	32.2	28.2	24.2	32.2	27.1	21.9	28.0	21.3	11.4
Even *iso*	4.8	4.3	2.9	1.7	4.5	3.6	2.1	4.4	3.7	2.8	3.2	2.2	1.6
Odd *anteiso*	18.4	20.7	22.7	24.0	19.3	21.7	23.2	19.2	20.9	24.1	22.4	25.9	26.5
Straight	23.5	25.5	28.3	30.3	24.6	27.5	30.3	25.6	29.2	30.6	24.5	25.3	28.4
Unsaturated	7.6	7.5	8.1	8.6	7.5	8.0	8.7	8.5	9.3	9.3	8.2	8.4	10.2
2-Hydroxy	2.5	1.7	0.0	0.0	1.4	0.6	0.0	0.7	0.0	0.0	0.8	0.0	0.0
3-Hydroxy	2.4	0.7	0.0	0.0	0.9	0.0	0.0	0.7	0.0	0.0	0.8	0.0	0.0
ω-cycPr-15:0	×	1.6	2.3	3.6	×	×	×	×	×	×	×	×	×
ω-cycPr-16:0	×	3.4	4.7	6.9	×	×	×	×	×	×	×	×	×
ω-cycPr-17:0	×	1.5	2.9	3.8	×	×	×	×	×	×	×	×	×
ω-cycBu-15:0	×	×	×	×	3.9	5.4	6.5	×	×	×	×	×	×
ω-cycBu-16:0	×	×	×	×	0.6	1.1	2.1	×	×	×	×	×	×
ω-cycBu-17:0	×	×	×	×	2.0	2.6	2.9	×	×	×	×	×	×
ω-cycPe-16:0	×	×	×	×	×	×	×	3.5	5.4	5.9	×	×	×
ω-cycPe-17:0	×	×	×	×	×	×	×	0.4	0.8	1.1	×	×	×
ω-cycPe-18:0	×	×	×	×	×	×	×	2.9	3.6	4.3	×	×	×
ω-cycHe-17:0	×	×	×	×	×	×	×	×	×	×	4.2	8.3	9.7
ω-cycHe-18:0	×	×	×	×	×	×	×	×	×	×	1.9	2.5	3.3
ω-cycHe-19:0	×	×	×	×	×	×	×	×	×	×	3.8	6.1	8.9
Total ω-cyc	×	6.5	9.9	14.3	6.5	9.1	11.5	6.8	9.8	11.3	9.9	16.9	21.9

iso, branch point is on the penultimate carbon; *anteiso*, branch point is on the ante-penultimate carbon; *branched*, branch point is on different carbon than penultimate or ante-penultimate; *cycPr*, cyclopropyl; *cycBu*, cyclobutyl; *cycPe*, cyclopentyl; *cycHe*, cyclohexyl; *ω-cyc*, ω-alicyclic; ×, not present; 0.0, less than 0.1%

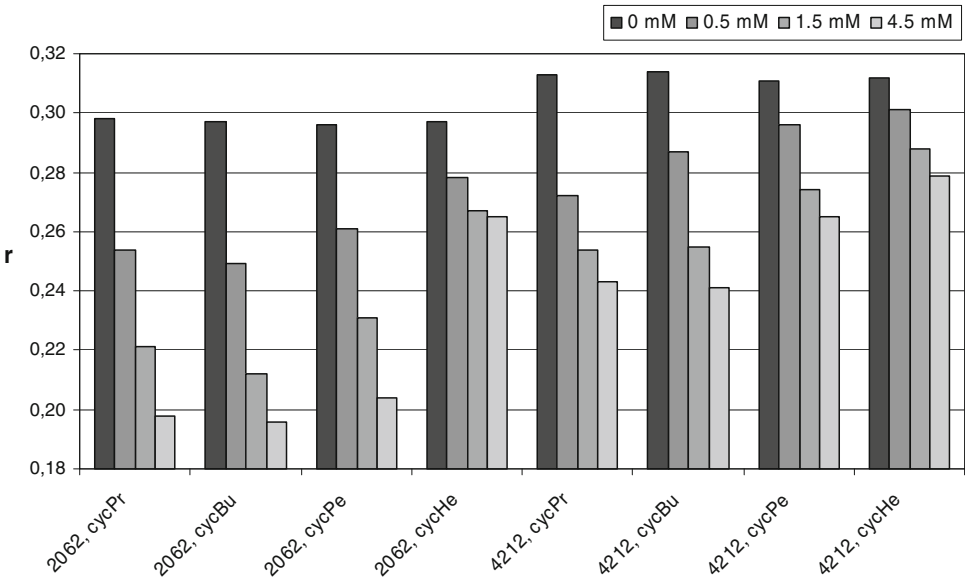

Fig. 1 Fluorescence anisotropy (r) values of whole cells (*G. stearothermophilus* CCM 2062 and *M. ruber* CCM 4212) cultivated with 0, 0.5, 1.5 and 4.5 mmol L^{-1} of cyclic acid precursors (*cycPr* cyclopropyl-, *cycBu* cyclobutyl-, *cycPe* cyclopentyl-, *cycHe* cyclohexyl-carboxylic acids) and measured by DPH

The tables include ω-FAs with total carbon atoms in the range of 15–19.

The addition of the cyclic acids caused the expected occurrence of respective ω-FAs. With increasing concentration of any cyclic acid in the medium the amount of *iso*-odd-numbered FAs in both strains decreased, the most significant decrease being observed after addition of cyclohexyl carboxylic acid (74% decrease for *G. stearothermophilus* and 67% drop for *M. ruber*). The amount of *iso*-even-numbered FAs in *M. ruber* also decreased markedly (the most significant was the 67% drop in cyclohexyl carboxylic acid) while in *G. stearothermophilus iso*-even-numbered FAs decreased moderately but only after the addition of cyclopentyl and cyclohexyl carboxylic acid. The remaining cyclic acids induced hardly any change.

Further features were strain-specific. The strain *G. stearothermophilus* showed a decrease in *anteiso*-FAs (the largest 46% drop with cyclohexyl carboxylic acid) and a decrease in straight-chain FAs (the most significant 78% decrease with cyclopentyl carboxylic acid). Unsaturated FAs increased after addition of cyclopropyl and cyclobutyl carboxylic acids and decreased after addition of cyclopentyl and cyclohexyl carboxylic acids.

By contrast, the content of *anteiso*-, straight-chain and unsaturated FAs in *M. ruber* increased. The largest increase in *anteiso*- and unsaturated FAs (44% and 34% rise, respectively) occurred after the addition of cyclohexyl carboxylic acid. The 27% increase in straight-chain FAs was approximately the same after addition of any cyclic acid.

A specific characteristic of the genus *Meiothermus* is the occurrence of 2-hydroxy FAs. Most strains also have lower but significant amounts of 3-hydroxy FAs (Chung et al. 1997; Ferreira et al. 1999). Both 2-hydroxy and 3-hydroxy FAs were found in our strain of *M. ruber* cultivated without any precursor. However, the amount of hydroxy FAs rapidly decreased after addition of only 0.5 mmol L^{-1} of any cyclic acid and completely disappeared when the concentration of cyclic acids was increased.

In addition to FA composition, membrane fluidity was estimated in whole cells by fluorescence polarization measurements using the membrane probe 1,6-diphenyl-1,3,5-hexatriene (DPH). The DPH fluorescence anisotropy in both strains decreased with increasing concentration of any cyclic acid (Fig. 1), the decrease being more pronounced in *G. stearothermophilus*. The smallest change in anisotropy values in both strains was induced by the addition of cyclohexyl carboxylic acid. A decrease in anisotropy values means that cytoplasmic membrane becomes more fluid (Chu-Ky et al. 2005; Tymczyszyn et al. 2005).

Data reported previously on *Bacillus subtilis* mutant and *A. acidocaldarius* point to variable unnatural levels of ω-FAs found after the addition of appropriate precursors (De Rosa et al. 1974; Dreher et al. 1976; Blume et al. 1978). It was reported that general branched-chain FA synthetase can synthesize a wide variety of unusual FAs provided that it is supplied with appropriate primers (Kaneda 1991). Our results show that, like other bacteria, even the thermophilic strains, which under natural conditions do not synthesize ω-FAs, are capable of producing them in the presence of a relevant precursor.

The large differences in the responses to the presence of precursors in culture media are given by the considerable phylogenetical distance between the two tested strains (Siristova et al. 2009). We deliberately chose one strain phylogenetically closer and another phylogenetically more distant from the genus *Alicyclobacillus*, which naturally

produces ω-FAs. *G. stearothermophilus* and *Alicyclobacillus* sp. were previously classified as belonging to the same genus *Bacillus* and both are Gram-positive and thermophilic bacteria. On the other hand, *M. ruber* falls among Gram-negative bacteria and the only common feature with *Alicyclobacillus* sp. is likely its thermophility (Siristova et al. 2009).

The presence of the cyclic acids had a lower effect on *M. ruber* lipids than *G. stearothermophilus*. The percent incorporation of cyclic acids and the increase in membrane fluidity were lower in *M. ruber*. Except for incorporation of ω-FAs, further changes in FA composition are very similar to those that are induced by increasing temperature. Generally, increase in growth temperature has a fluidizing effect upon the cytoplasmic membrane (Denich et al. 2003). The response of thermophilic bacteria to the fluidizing effect is an increased amount of higher melting FAs (Ray et al. 1971; Nordström and Laakso 1992). In our batch cultivations, of *M. ruber* FAs with higher melting point (*iso*-, hydroxy- and longer-chain length FAs) were replaced by FAs with lower melting point (*anteiso*-, unsaturated and shorter-chain length FAs) which caused a fluidizing effect.

G. stearothermophilus responded to the presence of the cyclic acids by a larger increase in membrane fluidity (except for the case of cyclohexane acid). Reizer et al. (1985) reported that elevation of growth temperature causes in *G. stearothermophilus* a large increase in the content of high-melting-point FAs (palmitic and stearic acids), the content of other FAs decreasing. In our experiments we found a decrease in *iso*- and *anteiso*-FAs, which was, however, accompanied by a concomitant decrease in straight chain FAs also including palmitic and stearic FA. These results show that the adaptation of membrane lipids to the presence of cyclic acids differs from the thermoadaptive compensation. Figure 1 shows a large increase in membrane fluidity when both strains were fed with cyclopropyl, cyclobutyl and cyclopentyl carboxylic acid and a smaller increase with cyclohexyl carboxylic acid. It appears that the cells cannot properly organize the ω-cyclopropyl, ω-cyclobutyl and ω-cyclopentyl FAs in their membrane to suit its stability and fluidity needs. The size of the ring plays also an important role (Fig. 1). Blume et al. (1978) in their study of transition temperature of lipids with incorporated ω-FAs also attributed an importance to the size of the ring.

The data on the degree of cyclic acid incorporation and membrane fluidity suggest that ω-cyclohexyl FAs are the most suitable for both strains to maintain membrane dynamics and order in the requisite state. Our findings on the influence of ω-cyclohexyl FAs differ from those of Kannenberg et al. (1984) who found a lower membrane fluidity and a higher degree of order of di-ω-cyclohexyldodecanoylphosphatidylcholine in liposomes compared with 1,2-dimyristoyl-*sn*-glycero-3-phosphatidylcholine and 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine.

In conclusion, *G. stearothermophilus* and *M. ruber* incorporate four cyclic acids into cellular lipids and thus generate new unnatural structures. The incorporation of new FAs is accompanied by significant changes in the content of other FAs. All cyclic acids in culture media have a fluidizing effect on cytoplasmic membrane. The cyclohexyl acid has only a moderate fluidizing effect compared to other tested cyclic acids with smaller rings.

These features resemble the fluidizing effect of increased temperature. Unnatural precursors cause the biosynthesis of new lipid structures that may not be readily organized within the bilayer and affect thereby its stability and fluidity.

G. stearothermophilus was more affected by the presence of cyclic acids in culture medium than *M. ruber* and appears to be more sensitive to environmental changes affecting the membrane.

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