Modulation of the *Lactobacillus acidophilus* La-5 lipidome by different growth conditions

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Probiotics are bacteria used in the food industry due to their potential health benefits. In this study, the plasma membrane of the probiotic *Lactobacillus acidophilus* La-5 was investigated using state-of-the-art high-resolution shotgun lipidomics. Comparisons of the lipidome of the plasma membrane were done after altering the fatty acid composition by supplementing *L. acidophilus* La-5 with saturated, mono-, di- and tri-unsaturated fatty acids during fermentation. The plasma membrane with the highest degree of saturation resulted in a lipid composition with the highest proportion of cardiolipin (CL) and lowest proportion of monolysocardiolipin (MLCL). No significant changes were found for other lipid classes. The bacteria grown with di- and tri-unsaturated fatty acids were expected to have more unsaturated plasma membranes than bacteria grown with mono-unsaturated fatty acids. This was also the case for MLCL, but the numbers of double bonds for CL were quite similar for these three samples. The results indicate that *L. acidophilus* La-5 possesses a molecular mechanism for remodelling and optimizing the fatty acid composition of CL and MLCL species and the molar ratio of CL and MLCL. This study contributes new knowledge on the previously uninvestigated lipidome of *L. acidophilus* La-5.

INTRODUCTION

Several *Lactobacillus* species are known for their health benefits and are widely used commercially both in starter cultures for fermented foods and as probiotics in functional foods (Naidu et al., 1999). Together with the cell wall, the plasma membrane constitutes the barrier of the cell, protecting the bacterium from the potentially stressful environment in food and the gastrointestinal tract. Modification of the plasma membrane lipid composition is known to change the ability of bacteria to survive freezing, freeze-drying and low pH (Corcoran et al., 2007; Li et al., 2009; Wang et al., 2005b). The understanding of such effects is, however, limited by the fact that mostly the fatty acid composition of the plasma membrane lipids has been investigated previously (Liu et al., 2014; Muller et al., 2011; Murga et al., 2000); the molecular composite of lipid species, i.e. the lipidome, of lactic acid bacteria has, at present, not been thoroughly investigated.

Several studies have dealt with the fatty acid composition of the plasma membrane and how the composition might be altered through fermentation conditions and fatty acid supplementation (Kankaanpää et al., 2004; Li et al., 2009; Muller et al., 2011; Wang et al., 2005b). They found that survival during freezing and drying in production and survival in the gastrointestinal track could be affected by the fatty acid composition of the plasma membrane. Di-unsaturated fatty acids in the plasma membrane had a beneficial effect on freezing survival (Wang et al., 2005b). Saturated and mono-unsaturated fatty acids in the plasma membrane, however, lead to more resistance to the acidic environment in the gastrointestinal tract (Corcoran et al., 2007; Muller et al., 2011). In addition, a high proportion of oleic acid in the plasma membrane of *Lactobacillus acidophilus* La-5 had a positive effect on survival of the bacteria during dry storage (Hansen et al., 2015).

The genome of a strain closely related to *L. acidophilus* La-5, *L. acidophilus* NFCM, has been sequenced, and analysis of the genome showed that the strain lacks the enzymes to synthesize fatty acids *de novo* (Altermann et al., 2005). This means supplementation of exogenous fatty acids is vital for growth, which has also been found for the related *Lactobacillus johnsonii* (Muller et al., 2011) and...
Lactobacillus delbrueckii strains (Partanen et al., 2001). More specifically, oleic acid (C18:1cis9) has been found to be essential and to promote growth of lactobacilli (De Man et al., 1960; Partanen et al., 2001), and oleic acid was therefore added in the media of all fermentations in the present study. Many lactobacilli are able to regulate the membrane fatty acid composition by modifying oleic acid and creating the cyclopropane fatty acid C19:0 (Fernández Murga et al., 2001; Wang et al., 2005a). Production of C19:0 is induced in the stationary growth phase of the cells, but can also be induced according to the environment. Furthermore, it has been hypothesized that the presence of C19:0 in the plasma membrane can improve bacterial resistance to freezing and freeze-drying (García, 2011; Li et al., 2009; Louesdon et al., 2015). How L. acidophilus La-5 or other lactobacilli might be able to regulate the lipidome, dependent on the fatty acids available for incorporation in the plasma membrane, has, however, not been studied before.

Previous studies of Lactobacillus species have not investigated the whole lipidome, but extend to identifying the lipid composition and, in some cases, quantifying each lipid class (Exterkate et al., 1971; Machado et al., 2004; Fernández Murga et al., 2001). A more recent study, using a novel method of matrix-assisted laser desorption-ionization time-of-flight mass spectrometry on whole cell bacteria, identified the lipid species of Lactobacillus sanfranciscensis and Lactobacillus plantarum (Calvano et al., 2011). We have found only one study on Lactobacillus species that gives the overall distribution for major lipid species. The lipids were identified by fast atom bombardment mass spectrometry (Drucker et al., 1995).

The present study was conducted to measure the effect of altering the fatty acid composition on the plasma membrane of the probiotic bacteria L. acidophilus La-5 and to see the response in terms of how the Lactobacillus species distributed the fatty acids between lipid classes. Four versions of L. acidophilus La-5, with varying degree of unsaturation in the plasma membranes, were made by different fatty acid supplementation during fermentation. High-resolution Fourier transform mass spectrometry (FTMS) was applied for a semiquantitative characterization of the L. acidophilus La-5 lipidome and the response to supplementation with different fatty acids.

**METHODS**

**Bacterial strain and fermentation media.** Stock cultures of the strain, Lactobacillus acidophilus La-5, was obtained from Chr. Hansen A/S, Hørsholm, Denmark, and stored at −50°C before utilization. A portion of the stock culture was thawed at room temperature before inoculation. The inoculation material for the fermentations was grown with 1% (v/v) stock culture in de Man, Rogosa and Sharpe medium (MRS broth; Lactobacilli MRS broth, Difco), which includes 1 g Tween 80 l−1 (a C18:1 source), for 17 h at 37°C. The fermentation medium was also MRS broth from Difco (Control sample), or MRS with 0.15 g Tween 20 l−1 (a polysorbate surfactant like Tween 80, but primarily containing C12:0 and C14:0 rather than C18:1; Sigma-Alrich), 10 mg linoleic acid l−1 (C18:2; Sigma-Aldrich) or 10 mg α-linolenic acid l−1 (C18:3; Sigma-Aldrich) additionally added.

**Fatty acids determined by gas chromatography.** The fatty acids were directly converted to fatty acid methyl esters by trans-esterification (Rozès et al., 1993). Fatty acid analysis of MRS medium was performed directly on the liquid solutions. For the direct methylation, a 1 M solution of sodium methoxide (30% sodium methylate in methanol; Merck) in methanol (Sigma-Aldrich) was used. The trans-esterification proceeded during 30 min of overhead stirring (Heto Mastermix, Heto-Holten A/S). The fatty acid methyl esters were extracted with n-heptane (Pro analysis, Merck) and analysed by gas chromatography (HP 6890, Hewlett Packard) equipped with a capillary column (Omegawax 320, 30 m × 0.320 mm × 0.25 μm, Supelco). The oven temperature programme was 50°C for 1 min; from 50°C to 180°C at 15°C min−1; from 180°C to 240°C at 3°C min−1 and held at 240°C for 10 min. Injection volume was 1μl and split ratio was 1:25. Hydrogen was used as carrier gas and the flow was 30 ml min−1. The results were analysed with Chemstation software (Agilent Technologies), and fatty acid methyl esters were identified by comparing retention times with those of known standards. The areas of the individual peaks were used to determine the relative percentage of each fatty acid present.

**Fermentation.** Fermentation of L. acidophilus La-5 for each of the four types of media was conducted anaerobically in 35 l fermenters, at 37°C and pH 6, with a constant agitation of 370 r.p.m.; one fermentation for each of the four types of media. The pH was controlled by the addition of 13.4% ammonium hydroxide (Brenntag Nordic A/S). Bacterial growth throughout the fermentations was followed by measuring the OD600 and the bacterial culture was harvested when it had reached the stationary growth phase, as determined by the ammonium hydroxide consumption rate. Growth was unaffected by the supplementation of exogenous fatty acids and the OD600 and cell counts (c.f.u.) of the bacterial cultures at the end of fermentation were 13.62/5.0 × 10⁸ (grown in MRS), 15.36/6.05 × 10⁸ (grown with Tween 20), 12.66/5.45 × 10⁸ (grown with C18:2) and 13.96/7.60 × 10⁸ (grown with C18:3).

**Harvest, formulation and pellet freezing.** Bacterial cells from each fermentation culture were harvested in a desludging disc centrifuge (CSC.C-06-476, Westfalia) and subsequently washed by mixing one part bacterial culture to nine parts 20% (w/w) sucrose solution. The resuspended cells were then centrifuged in a batch centrifuge (Cryofigue 5500i, Heraeus) to remove medium and wash solution leftovers. For cryo-protection the cells in the pellet were mixed with a 30% (w/w) sucrose solution (0.3 g solution per g bacterial pellet), before they were frozen in liquid nitrogen in pellets of approximately 5 mm in diameter. The frozen pellets were stored at −196°C before freeze-drying later the same day. Freeze-drying was performed at a chamber pressure of 0.3 mbar (0.3 kPa) with temperature increasing from −42°C to 32°C with 1.5°C min−1 (Hetoisic freeze dryer, CD-10-1, Heto Lab equipment, Heto-Holten A/S). The freeze-drying was ended when the weight of the product had been stable for at least 2 hours. The dry product was stored in alu-PE bags at −50°C until further use.

**Lipid extraction.** The lipid extraction was carried out twice for each of the four freeze-dried cultures. Briefly, the freeze-dried bacteria samples were rehydrated and washed twice in MilliQ H₂O and then suspended to obtain a nominally OD600 of 20 per ml 155 mM ammonium acetate in water. The OD600 values were assessed by measuring samples of known dilutions. Hereafter the extraction was carried out at 4°C. The suspension (1000μl) was added to 500μl glass beads (Sigma) and shaken for 12.5 min at 1400 r.p.m. The lysed cells were diluted to 0.4 OD600 with 155 mM ammonium acetate in a total volume of 200μl. To each sample, 990μl chloroform/methanol
Lipid profiling by mass spectrometry. Lipid extracts were analysed in negative ion mode by high-resolution FTMS and ion trap fragmentation (ITMS²) using an LTQ Orbitrap XL instrument (Thermo Fisher Scientific) equipped with a TriVersa NanoMate nanoelectrospray ion source (Advion Biosciences) (Ejsing et al., 2009; Schwudke et al., 2006). FTMS data were recorded using a max injection time of 250 ms, automated gain control at 1e6, two microscans and a target resolution of 100 000 (FWHM at m/z 400). All ITMS² data were recorded using collision-induced dissociation, normalized collision energy at 35 %, isolation width at 2 amu, activation Q parameter at 0.2, one microscan and data-dependent acquisition of the ten most abundant precursor ions per cycle with dynamic exclusion, such that each precursor ion was fragmented only once. Lipid species were identified and quantified by ALEX software, SAS and Tableau, as previously described (Almeida et al., 2015; Husen et al., 2013). Lipid species detected by FTMS analysis were identified using an m/z tolerance setting of ± 0.004 amu and annotated using sum composition (e.g. CL 72:3). Based on a priori knowledge we used the search constraints for identifying cardiolipin (CL) species with C index=52 to 80 and double bond (DB) index=0 to 12, monolysocardiolipin (MLCL) species with C index=40 to 68 and DB index=0 to 12 and phosphatidic acid (PA), phosphatidylglycerol (PG), triglyceroacylglycerol (TGDG) or quadrglyceroacylglycerol (QGDG) species with C index=26 to 42 and DB index=0 to 9. We note that with these search constraints there were no conflicting identifications of isobaric CL, MLCL, PA, PG, TGDG or QGDG species. Lipid species abundance was estimated by intensity profiling using the FTMS data, whereby the intensity of individual lipid species were normalized to the sum of intensities of all monitored lipid species. Total lipid class abundance was calculated as the sum of intensities of all lipid species belonging to a given lipid class. The fatty species abundance was estimated by intensity profiling using an m/z tolerance setting of ± 0.25 amu. The relative intensities of detected acyl anion fragments were normalized to the sum of intensities of all monitored lipid species. Statistical analysis. Univariate analyses were carried out by Student’s t-test (2-tail paired with significance measured at a probability level of P≤0.05, three technical replicates) using Excel (Office 2010, Microsoft).

RESULTS

Lipidome analysis of L. acidophilus La-5 and changes in lipid classes induced by fatty acid supplementation

To investigate how the composition of lipid classes and fatty acid composition within each lipid class respond to alteration of the fatty acids available, four types of fermentation media were used. L. acidophilus La-5 was fermented in MRS (hereafter denoted Control), or in MRS with Tween 20 (containing C12:0 and C14:0, hereafter denoted Tween 20), C18:2 (hereafter denoted C18:2) or C18:3 (hereafter denoted C18:3). The fatty acid composition of the MRS medium with Tween 80, which was the basis of all four fermentations, was also determined (Table 1). It can be observed from the table that the MRS medium used for the fermentations contained a large amount of C18:1cis9 and some C18:0 and C16:0.

The total lipid extracts were analysed by high resolution FTMS and the profile of lipid classes were compared (Fig. 1). The lipid classes identified included CL, MLCL, PA, PG, TGDG and QGDG. The relative intensities only reflect lipid composition in a semiquantitative way, as each lipid class shows its own slightly different linear relationship between quantity and MS intensity.

The supplementation of fatty acids did not affect which lipid classes were present in the four bacterial cultures. In general, the four samples showed a low content of most lipid classes such as PA, PG, TGDG, QGDG; the intensities of these lipid classes show very modest response to fatty acid supplementation, as content of these lipid classes were almost the same for all samples. Most notably was the supplementation of Tween 20, which altered the abundance of CL and MLCL by comparison to the lipid class profiles of the three other samples (Fig. 1). The relative intensity of CL was greater for the Tween 20 sample (80 %) compared with the other three samples (60 %). The relative intensity of MLCL was, on the other hand, smaller (10 %) for the Tween 20 sample compared with the other three samples (30 %). Bacteria grown with Tween 20 were supplemented with medium- and long-chain saturated fatty acids, such as C12:0 and C14:0, and the differences in CL and MLCL intensities indicate that L. acidophilus La-5, among other things, responds by changing the ratio between the lipid classes, CL and MLCL.

Due to low abundance of other lipid species, the high concentration of CL and MLCL and changes in ratios of these components and the importance of CL in relation to

<table>
<thead>
<tr>
<th>Fatty acid designation and name</th>
<th>Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C12:0 (Lauric)</td>
<td>0.1</td>
</tr>
<tr>
<td>C14:0 (Myristic)</td>
<td>1.1</td>
</tr>
<tr>
<td>C16:0 (Palmitic)</td>
<td>11.3</td>
</tr>
<tr>
<td>C16:1 (Palmitoleic)</td>
<td>0.2</td>
</tr>
<tr>
<td>C18:0 (Stearic)</td>
<td>14.0</td>
</tr>
<tr>
<td>C18:1cis9 (Oleic)</td>
<td>68.9</td>
</tr>
<tr>
<td>C18:1cis11 (Vaccenic)</td>
<td>3.1</td>
</tr>
<tr>
<td>C18:2cis9,12 (Linoleic)</td>
<td>0.0</td>
</tr>
<tr>
<td>C18:3cis9,12,15 (Linolenic)</td>
<td>ND</td>
</tr>
<tr>
<td>C19:0,10 (Dihydrosterculic)</td>
<td>0.1</td>
</tr>
</tbody>
</table>

ND, Not detected.

1992
Changes in molecular composition of CL and MLCL species

The molecular composition (described as lipid class and sum of fatty acid moieties’ chain length and double bonds) of CL and MLCL lipid species was different for the four bacterial samples. The Tween 20 sample was clearly distinctive with many lipid species enriched with shorter and more saturated fatty acid moieties (Figs 2 and 3). The three most abundant CL species were CL 68 : 2, CL 66 : 2 and CL 64 : 2, each comprising to about 10 % of the total pool of CL. The fatty acid composition of these lipid species could also be described, with the most abundant molecular species being: CL (68 : 2) 16 : 0-16 : 0-18 : 1-18 : 1, CL (66 : 2) 14 : 0-16 : 0-18 : 1-18 : 1 and CL (64 : 2) 12 : 0-16 : 0-18 : 1-18 : 1 or 14 : 0-14 : 0-18 : 1-18 : 1. The three most abundant MLCL species were MLCL 50 : 1, MLCL 48 : 1 and MLCL 46 : 1, each comprising to about 15 % of the total pool of MLCL. These species primarily had the following fatty acid composition: MLCL (51 : 1) 16 : 0-16 : 0-18 : 1-18 : 1, MLCL (48 : 1) 14 : 0-16 : 0-18 : 1-18 : 1 or 14 : 0-14 : 0-18 : 1-18 : 1.

For the Control, C18 : 2 and C18 : 3 samples, on the other hand, the four most abundant CL species were CL 74 : 4, CL 73 : 4, CL 72 : 3 and CL 71 : 3 each comprising between 6 and 12 % of the total CL (Fig. 2). Of these species, the most abundant molecular species were CL (74 : 4) 18 : 1-18 : 1-19 : 1-19 : 1, CL (73 : 4) 18 : 1-18 : 1-19 : 1-19 : 1, CL (72 : 3) 16 : 0-18 : 1-19 : 1-19 : 1 and CL (71 : 3) 16 : 0-18 : 1-19 : 1-19 : 1. The two most abundant MLCL species were MLCL 55 : 3 (about 15–25 %) and MLCL 53 : 2 (about 14–20 %) (Fig. 3). Of these species, the most abundant molecular species were MLCL (55 : 3) 18 : 1-18 : 1-19 : 1 and MLCL (53 : 2) 16 : 0-18 : 1-19 : 1.

Among the less abundant CL and MLCL species, differences could be detected between the three samples, Control, C18 : 2 and C18 : 3. There were several CL and MLCL species with a high number of double bonds, which could be detected for the C18 : 2 and C18 : 3 samples, but were undetectable in the Control sample. These include CL 74 : 6, CL 73 : 6 and CL 72 : 6, as well as, MLCL 55 : 5, MLCL 54 : 6 and MLCL 54 : 5. It is interesting that there are no CL species with a higher number of double bonds, seeing as how this lipid is composed of four fatty acids. Furthermore, the maximum number of double bonds in both the CL and MLCL species is six.

Responses in CL and MLCL chain length and unsaturation

To evaluate the general composition of CL and MLCL we investigated the total chain length (the chain length in CL and MLCL fatty acid moieties), as well as the double bond index (the number of double bonds in fatty acid moieties) for each of the four fermentation samples. Differences could be detected in chain length and unsaturation of the fatty acid moieties of CL and MLCL upon supplementation with Tween 20, di- or tri-unsaturated fatty acids to the fermentation medium (Fig. 4). The fatty acid moieties of CL and MLCL species of the Tween 20 sample were clearly shorter and more saturated than the other samples. This can be observed from both the chain length and double bond distributions (Fig. 4) and the mean chain length, unsaturation and average fatty acid composition (Table 2).

The chain lengths of both CL and MLCL for the C18 : 2 and C18 : 3 samples were similar to the Control sample. However, for CL, the Control sample had a larger part of lipid species with a total chain length of 74 (about 19 %) compared with the C18 : 2 and C18 : 3 samples (9 and 11 %), and a smaller part of lipid species with a total chain length of 70 (12 %) compared with the C18 : 2 and C18 : 3 samples (17 and 16 %). The same pattern could be seen for the chain lengths of MLCL (Fig. 4). These variations led to a small but significant difference in the mean chain length of CL for the C18 : 2 sample compared with the Control sample (Table 2).

The number of double bonds in CL and MLCL species was influenced by supplementation of C18 : 2 and C18 : 3 in a complex way. Overall, the mean number of double bonds for CL was quite similar for the Control, C18 : 2 and C18 : 3 samples, although the C18 : 3 sample did show a small but significant increase of double bonds compared with the Control sample. For MLCL, conversely, both the C18 : 2 and C18 : 3 sample showed a clear and significant increase in the number of double bonds (Table 2).

Table 2 also enables comparison of the average fatty acid composition of CL and MLCL, as the average fatty acid

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Comparative lipidomics of L. acidophilus La-5

Fig. 1. Lipid class profile of L. acidophilus La-5 (Control, Tween 20, C18:2 and C18:3). Error bars display SD (based on three technical replicates).
of the classes is provided. There is a general tendency that the average fatty acid length is shorter for MLCL, and for the Control and C18:3 samples the effect is statistically significant. For the Tween 20 sample the fatty acids are, on average, significantly less saturated in MLCL than CL, whereas the opposite is seen for the C18:3 sample. This indicates that fatty acids are not randomly distributed between lipid classes and L. acidophilus La-5 possesses some ability to direct fatty acids to lipid classes.

**DISCUSSION**

In this study, the lipidome of L. acidophilus La-5 was investigated. The use of high resolution FTMS analysis enabled us to not only identify lipid species of CL and MLCL, but also investigate the composition of the distinct species within each lipid class. No previous studies on Lactobacillus species have used high resolution FTMS analysis, nor has the effect on the composition of lipid classes and species when supplying bacteria with fatty acid supplements during fermentation been investigated.

We observed that the lipid classes identified were the same upon supplementation with either Tween 20 (C12:0/C14:0), di- or tri-unsaturated fatty acids. The lipid classes included the lipids identified in another study of L. acidophilus (Fernández Murga et al., 2001), and additionally identified MLCL, PA and QGDG, but not diglycosyldiacylglycerol (DGDG). DGDG could be an intermediate for TGDG and QGDG, as the glycosyltransferase responsible for transferring glyco-residues is able to transfer up to four residues to the precursor, diacylglycerol (Hölzl & Dörmann, 2007). Furthermore, MLCL species...
have not been identified in other lipid studies on Lactobacillus species (Calvano et al., 2011; Drucker et al., 1995; Exterkate et al., 1971; Fernández Murga et al., 2001; Machado et al., 2004; Murga et al., 2000; Tymczyszyn et al., 2005). One reason for this difference could be that the composition of the lipid classes is dependent on the bacterial species and strain and only one research group has investigated the lipid classes of the L. acidophilus species within the last 15 years (Fernández Murga et al., 2001; Murga et al., 2000), and this was not the same strain as L. acidophilus La-5. A second reason could be that the previous studies have not analysed the lipid composition to the same depth and detail as the present study. The methods used for L. acidophilus were thin layer chromatography and low-resolution fast atom bombardment mass spectrometry (Fernández Murga et al., 2001). The present study is one of only a few studies using mass spectrometry to identify the lipid species, and previously none of them have used state-of-the-art high-resolution FTMS-based lipidomics (Calvano et al., 2011; Drucker et al., 1995; Fernández Murga et al., 2001). Further studies using high-resolution MS techniques will show if the present findings can be generalized beyond L. acidophilus La-5.

Supplementation of Tween 20 gave rise to two kinds of response: increase of the proportion of CL and changes of fatty acid composition within the CL and MLCL classes. The fatty acid chains of both CL and MLCL were, as could be expected, shorter and more saturated for the Tween 20 sample than for the three other samples. The addition of C18:2 or C18:3 to the fermentation medium was expected to increase the number of double bonds. Interestingly, the number of double bonds for CL was quite similar for the Control, C18:2 and C18:3 samples. A comparison

![Figure 3](image-url)
of the number of double bonds in the average fatty acid of CL and MLCL showed that it was the same for the Control sample, whereas the number of double bonds was higher in the average fatty acid of MLCL for the C18:2 and C18:3 samples, significantly for C18:3 (Table 2).

A hypothesis is that MLCL in *L. acidophilus* La-5 is not an intermediate component, but an end product in the regulation of membrane fluidity and bilayer stability. From model studies of membranes consisting of CL, it was found that CL with four C14:0 moieties favoured the liquid crystalline phase (L*) lamellar phase). CL with four C18:1 moieties, on the other hand, favoured the inverted hexagonal phase (HII) (Sankaram *et al.*, 1989). This can be explained by the packaging parameter and CL’s relatively small headgroup compared to the volume of the four fatty acids. Similar to CL, with only saturated fatty acids is MLCL with three C18:1 moieties, which also favours the L* phase (Powell & Marsh, 1985). This knowledge, together with the results in the present study, could suggest that *L. acidophilus* La-5 regulates the abundance and unsaturation of CL and

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**Fig. 4.** Differences in total chain length and unsaturation of the lipid classes CL and MLCL of *L. acidophilus* La-5 (*Control*, *Tween 20*, C18:2 and C18:3). CL chain length, as number of carbon atoms in the sum of fatty acid moieties (a); MLCL chain length (b); unsaturation of CL, as number of double bonds in the sum of fatty acid moieties, a cyclopropane ring is equivalent to a double bond (c); unsaturation of MLCL (d). Error bars display SD (based on three technical replicates).
MLCL to maintain a suitable number of lipids, which prefer either the Lφ phase or HII phase. This notion is still on a theoretical level, but it agrees with the observations of lower intensity of MLCL in the sample supplemented with Tween 20 and the larger amount of double bonds in MLCL in the C18 : 2 and C18 : 3 samples. In a previous study of L. acidophilus La-5 with a similar fermentation set-up, the membrane integrity following freeze-drying and survival during dry storage was investigated (Hansen et al., 2015). Supplementation of fatty acids with different levels of unsaturation had no or only very little effect on the membrane integrity, which may be connected to compensation by adjustment of the level of CL and MLCL. The modified compositions of the fatty acids had, however, a greater effect on the survival of the bacteria during dry storage (Hansen et al., 2015) beyond the level where a simple adjustment of the CL and MLCL levels could compensate.

This study is, to our knowledge, the first to use state-of-the-art high-resolution lipidomics to investigate the plasma membrane lipid composition of a Lactobacillus species. In summary, we found that the composition of lipid classes was affected by the addition of fatty acid supplements, and supplementation of Tween 20 altered the lipid composition, as well as the fatty acid chain length and unsaturation level of CL and MLCL. The supplementation of di- and tri-unsaturated fatty acid affected the unsaturation of CL and, to a higher degree, MLCL. The results indicate that L. acidophilus possesses a molecular mechanism for remodelling and optimizing the fatty acid composition of CL and MLCL species, and the molar ratio of CL and MLCL. This observation contributes with new knowledge of the lipidome and lipid metabolic capacity of L. acidophilus La-5. Clearly, further studies using complementary biophysical and biochemical techniques and approaches are needed to clarify if Lactobacillus bacteria can remodel CL and how the fatty acid composition of CL and MLCL affects the plasma membrane dynamics.

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