Impact of pH on Changing the Fatty Acid Composition and Growth of Lactobacillus plantarum and Lactobacillus casei

Ibourahema COULIBALY1,4, Elisée KPOROU KOUASSI2, Souleymane TRAORE3, Daouda KONE4

1,4 Training Unit and Research in Agroforestry, Laboratory of Microbiology and Food Inginiering (LMFI), University Jean Lorougnon Guède, BP : 150 Daloa, Ivory Coast.
2 Laboratory of Biochemistry and Microbiology, Bioactives Natural Substances (BIONAS), University Jean Lorougnon Guède, BP : 150 Daloa, Ivory Coast.
3 Training Unit and Research in Science and Food’s Technology, Laboratory of Nutrition and Food Security, ( LNFS ), University Nangui Abrogoua Abidjan, BPV : 151 Abidjan, Ivory Coast.
4 Plant Physiology Laboratory, Faculty of Biosciences, University of Cocody, FHB Abidjan 22 BP 582 Abidjan 22, Ivory Coast.

Abstract: Fatty acid composition and growth of Lactobacillus plantarum and Lactobacillus casei, were studied at different pHs of the culture media in a fermenter with according interest in lactobacillic acid production of the cultures. In this study, we notice, the increasing of total fatty acid content of the bacterial cells with increasing culture age. The production of lactobacillic acid was affected in lactobacillus by culture age and pH of the media, but in a very different manner. In Lb. casei cultures, the relative proportion of lactobacillic acid was highest when the pH was lowest (pH 4.5), whereas in Lb. plantarum cultures, the proportion of lactobacillic acid was highest at pH 7.0. The pH of the medium affected not only the relative proportion of lactobacillic acid, but also biomass production and total fatty acid accumulation of the cultures. Thus, by controlling the pH of the cultures, the volumetric yield of lactobacillic acid could be improved considerably compared to cultures without pH control.

Keywords: pH, fatty acid, lactobacillic acid, Lactobacillus plantarum.

1. Introduction

Cyclopropane fatty acid (CFA) formation is a postsynthetic modification of the lipid bilayer that occurs as cultures of Escherichia coli and many other bacteria enter stationary phase. We report the first distinct phenotype for this membrane modification; early stationary phase cultures of strains lacking CFA[1].

The main cyclopropane fatty acids of lactobacilli, lactobacillic acid (11,12-methyleneoctadecanoic acid; cy9:0[llc]) and dihydrosterculic acid (9,10-methyleneoctadecanoic acid; cy9:0[9c]), are formed by methylation of cis-vaccenic (18:1[9c]) and oleic acid (18:1[9c]), respectively. However, dihydrosterculic acid has generally been found only if oleic acid is added into the medium [2]. The reaction is catalyzed by cyclopropane fatty acid (CFA) synthase, a soluble enzyme found in the cell cytoplasm, and it is known to require S-adenosyl-L-methionine (SAM) as the alkylating agent. A free monounsaturated fatty acid cannot act as lipid substrate, but it must be in an acylated form, bound to membrane lipids, which means that the enzymatic reaction takes place in a hydrophobie environment [2, 3].

In spite of many investigations, the physiological significance of the synthesis of CFAs as well as the factors controlling the onset of their accumulation, still remain obscure [4]. The regulatory and physiological aspects of CFA formation have been most thoroughly studied in Escherichia coli[5]. In addition, the effects of cultural conditions on cyclopropane fatty acid formation have been studied to some extent, e.g., in cultures of other Enterobacteriaceae as well as in Lactobacilliaceae, and Pseudomonales [4,6-13]. Unfortunately, the regulatory mechanisms controlling the CFA production seem to differ from species to species, and no general conclusions can be made.

Early studies of CFA-producing bacteria found that these modified fatty acids first appear in the late exponential or early stationary phase of growth. In Azotobacter vinlandii, CFAs are made only during encystment [14]. The basis of the timed appearance of CFAs is reported to be the induction of CFA synthase in several bacteria, including Pseudomonas spp. [15,16], Proteus vulgaris[17], and Lb. plantarum[18], but growth-phase-specific induction of the enzyme was not obvious in early studies of E. coli. It has more recently been shown that E. coli produces a sharp peak of CFA synthase activity, which is easily missed, in the transition from exponential growth to stasis [19]. The purpose of the work presented here was to study the effect of pH on growth and fatty acid composition of two different Lactobacillus strains, Lactobacillus biacnieri TKK B-1059 and Lactobacillus plantarum G100. These strains were previously shown to produce appreciable amounts of CFAs, especially lactobacillic acid if a medium free of oleic acid was used [20]. Our final goal was to achieve a high volumetric production of lactobacillic acid, which we consider a commercially interesting compound because of its biological activity: Lactobacillic acid among other cyclopropane fatty acids is claimed to affect the properties of cell membranes.

Volume 5 Issue 6, June 2016
www.ijsr.net
Licensed Under Creative Commons Attribution CC BY
It is unanimously agreed that the extent of the cyclopropanation of the monounsaturated fatty acids represents one of the major adaptive responses of the bacterial cells in order to stabilize the membrane fluidity known as “homeoviscous adaptation” [21]. However, the role of CFAs in membrane fluidity adjustments remains unclear. According to the hypothesis of Härtig et al. [22], the cyclopropanation of the monounsaturated fatty acids represents one of the major adaptive responses of the bacterial cells in order to stabilize the membrane fluidity. The bacterial cells are able to pack into the acyl chain array of the phospholipid because of their higher lipid melting points and their poorer ability to pack into the acyl chain array of the phospholipid bilayer in comparison those of with unsaturated fatty acids [23]. But contrary effects were obtained for measurements of membrane physical changes due to cyclopropane formation.

2. Materials and Methods

2.1 Strain and growth media

*Lb. plantarum* G100 was used for these studies. The bacteria were maintained in MRS agar medium [24] at 4°C and subcultured every 4 week. The composition of MRS medium is given in Table 1.

| Raegent* | Amount g/L |
|----------|------------|
| Glucose  | 20         |
| Peptone casein | 10     |
| Beef extract | 10     |
| Yeast extract | 5.0    |
| K₂HPO₄, 3H₂O | 2.6     |
| Sodium acetate | 5.0     |
| Diammonium citrate | 1.7    |
| MgSO₄,7H₂O | 0.2      |
| MnSO₄,4H₂O | 0.05     |
| Tween 80  | 1.0       |

*All reagents used were given by wv and were pro-analysis grade*

For preparation of inocula for fermenter experiments, a modified MRS medium according to …… (MRS50-T) was used (MRS medium containing 50 g glucose/L, but no Tween 80). The media employed in fermenter cultivations contained (per 1 L of tap water): 50 g of glucose, 20 g of yeast extract, 20 g of tryptone, 1 g of diammonium citrate, 0.05 g of MnSO₄*4H₂O, 0.1 g of MgSO₄*7H₂O, and either 1 g (medium A) or 10 g (medium B) of CH₃COONa*3H₂O.

2.2 Fermentation and cultivations

To study the effect of pH on growth and fatty acid composition of *Lb. plantarum* G100, fermenter experiments were carried out in a 2-L Braun Biostat MD fermenter (B. Braun Melsungen, Germany) with a working volume of 1 L. The inoculum was cultivated in two stages: First 0.2 mL of bacterial culture grown at 37°C in MRS50-T medium for 6 h was transferred into 5 mL of MR50-T medium and allowed to grow to the exponential phase (150-200 Klett units). This culture (0.5 mL) was used to inoculate an Erlenmeyer flask containing 50 mL of MR50-T medium. The flask was shaken at 60 rpm in a Certomat orbital shaker/incubator (type R/HK) at 37°C until the culture reached exponential phase (150-200 Klett units) after which it was used to inoculate the fermenter containing either 1L of medium (*Lb. plantarum* G100). During the preparation of inocula, growth was monitored with a Klett-Summerson colorimeter (filter no. 66).

The température in ail fermenter cultivations was 37°C and stirring speed 100 rpm. The aération rate was 0.16 L/min. The pH of the cultures (4.5-7.0) was controlled automatically by adding 10% NH₄OH. During the cultivations, samples (2 x 5 mL) were withdrawn for the analyses of growth, fatty acid composition, and glucose consumption of the bacteria until the stationary phase of growth was reached.

2.3 Data analyses

The samples (5 mL) taken during the fermenter cultivations were centrifuged for 15 min (6000g). The glucose content of the growth medium (supernatant) was analyzed using the DNS-method of Fischer and Stein [25]. The cells were washed with tap water, freeze-dried, and weighed to estimate the growth of the cultures as dry weight. The dried cells were stored in -20°C for 1-5 d before fatty acid analysis.

To analyze the fatty acid composition, the freeze-dried cells were suspended in excess of saponification reagent and analyzed as described by Suutari et al. [26]. GC analysis of fatty acid methyl esters was carried out by Hewlett-Packard model 5890A gas chromatograph equipped with a flame ionization detector, a capillary liquid System, and a model 7673A automatic liquid sampler. The GC conditions were HP-FFAP WCOT (25 m x 0.2 mm x 0.3 /un) column; carrier gas He at 1 mL/min; split ratio 1:20; inj. vol. 1 /mL; column inlet pressure 150 kPa; inj. temp. 250°C; det. temp. 250°C; temp. program from 70 to 200°C at 25°C/min. Data analysis was performed with HP 3365 ChemStation software. The compounds were identified by GC peak retention times relative to fatty acid methyl ester standards (Sigma, St. Louis, MO) and verified with a mass-selective detector (Hewlett-Packard model 5971A) as described by Johnsson et al. [20]. The absolute amounts of fatty acids were calculated by using heptadecanoic acid methyl ester (Sigma) as an international standard. Results of all the analyses are mean values of two parallel samples analyzed separately.

3. Results and Discussion

3.1 Impact of pH on growth of *Lb. plantarum* G100

During the cultivation of lactic acid bacteria, acid production causes a dramatic decrease in pH of the medium, and finally the growth ceases at a pH characteristic of the bacterial strain. Consequently, the biomass yields of *lactobacillus* are relatively low in cultures with uncontrolled pH. If lactic acid is neutralized by base addition during cultivation, the growth can continue longer and thus the biomass yields can be improved [27]. In this work, we studied the growth and fatty acid composition of *Lb. plantarum* in media of varying pHs. The pH values for the cultures were chosen according to preliminary experiments. We could not perform cultivations where the pH value of the cultures was significantly outside the chosen limits, since the bacteria could not grow in these conditions.
Table 2. Effect of pH growth and fatty acid composition of Lactobacillus casei

| pH  | Culture time | Dry wt g/L | Glucose used g/L | C14:0 | C16:0 | C16:1 | C18:0 | C18:1(11c) | Cy9:0 | FAC mg/g dry wt | Vac/Cy | Vcy mg/L |
|-----|--------------|------------|-----------------|-------|-------|-------|-------|------------|-------|---------------|--------|----------|
| 4.5 | 6 h          | 0.41       | 9.0             | 0.70/1 | 30.5/4.2 | 5.0/0.8 | 5.7/0.8 | 51.5/7.0 | 4.5/0.6 | 13.6          | 11.4   | 0.3      |
|     | 12 h         | 1.44       | 18.5            | 0.60/1 | 36.2/5.6 | 4.7/0.7 | 5.9/0.9 | 36.0/5.6 | 15.8/2.4 | 15.4          | 2.3    | 3.5      |
|     | 18 h         | 3.64       | 42.8            | 0.40/1 | 37.6/6.8 | 3.0/0.5 | 9.5/1.7 | 8.6/1.6 | 40.7/7.4 | 18.1          | 0.2    | 26.8     |
|     | 24 h         | 3.98       | 50.0            | 0.30/1 | 37.2/6.9 | 2.7/0.5 | 11.2/2.1 | 5.6/1.1 | 42.7/7.9 | 18.6          | 0.1    | 31.6     |
|     | 30 h         | 3.96       | 50.0            | 0.40/1 | 37.5/6.9 | 2.8/0.5 | 11.2/2.1 | 5.0/1.0 | 42.9/8.1 | 18.7          | 0.1    | 32.1     |

FAC = fatty acid content of the cells (mg/g dry wt) Vac/Cy = content of C18:1(11c) per content of C19:0(11c) Vcy = volumetric concentration of cy9(11c) (mg/L medium)

Table 3: Effect of pH growth and fatty acid composition of Lactobacillus plantarum G1000

| pH  | Culture time | Dry wt g/L | Glucose used g/L | C14:0 | C16:0 | C16:1 | C18:0 | C18:1(11c) | Cy9:0 | FAC mg/g dry wt | Vac/Cy | Vcy mg/L |
|-----|--------------|------------|-----------------|-------|-------|-------|-------|------------|-------|---------------|--------|----------|
| 5.0 | 6 h          | 0.83       | 6.2             | 1.8/0.2 | 41.8/5.1 | 7.7/0.9 | 2.7/0.3 | 24.3/3.0 | 20.6/2.5 | 12.2          | 1.2    | 2.1      |
|     | 9 h          | 1.58       | 10.9            | 1.6/0.3 | 42.0/7.0 | 7.1/1.2 | 2.8/0.5 | 24.4/4.4 | 21.8/3.6 | 16.7          | 1.1    | 5.8      |
|     | 12 h         | 3.21       | 22.5            | 1.4/0.2 | 40.8/6.8 | 5.5/0.9 | 2.5/0.4 | 20.7/3.4 | 28.8/4.8 | 16.5          | 0.7    | 15.3     |
|     | 18 h         | 4.42       | 38.0            | 1.4/0.2 | 40.1/6.9 | 5.0/0.9 | 2.3/0.4 | 19.7/3.4 | 31.5/4.2 | 17.6          | 0.6    | 23.9     |
|     | 24 h         | 4.01       | 50.0            | 1.2/0.4 | 29.3/9.4 | 5.3/1.7 | 1.9/0.6 | 38.8/12.4 | 23.4/7.5 | 32.0          | 1.7    | 30.1     |

FAC = fatty acid content of the cells (mg/g dry wt) Vac/Cy = content of C18:1(11c) per content of C19:0(11c) Vcy = volumetric concentration of cy9(11c) (mg/L medium)

The results of all the analyses performed during the fermenter cultivations are collected in Table 2 and Figure 1 further represent the growth pattern of Lb. plantarum at different pH values. Lb. casei gave a slightly better biomass yield when the pH of the medium was kept at 4.5 than when cultivated at pH 5.5. Instead, in Lb. plantarum cultures, the final dry weight was bigger at pH 6.0 than at pH 5.0. If the pH of the medium was adjusted to 7.0 by base addition, the

Figure 1: Impact of medium’s pH on Lactobacillus casei growth, ◆ = pH 4.5; ■ = pH 5.5 and □ = pH 7.0

Volume 5 Issue 6, June 2016

www.ijsr.net
Licensed Under Creative Commons Attribution CC BY

Paper ID: ART20168
http://dx.doi.org/10.21275/v5i6.ART20168
2548
According to the fatty acid analyses, myristic (C14:0), palmitic (C16:0), hexadecenoic (C16:1), stearic (C18:0), cis-vaccenic (18:1ω7c), and lactobacillic (c19:0ω7c) acid accounted for more than 95% of the total amount of cellular fatty acids in both bacterial strains studied (Tables 2 and 3). Furthermore, oleic acid (18:1ω9c) and dihydrosterculic acid (c19:0ω9c) could be detected in traces.

The pH of the medium affected the fatty acid composition of both Lactobacillus strains studied. For comparison, the fatty acid compositions of Lb. Casei cells in stationary phase and Lb. plantarum cells at the end of exponential phase when grown at different pHs are illustrated in figures 3 and 4, respectively.

In Lb. casei cultures, the effect of pH on the relative amounts of fatty acids was quite clear: The proportion of lactobacillic acid increased from 3.4 to 42.9% when lowering the pH of the medium (Table 2). Moreover, the relative proportion of cis-vaccenic acid was much higher at pH 7.0 than at 4.5 (56.1 and 5.0%, respectively). The relative proportions of the saturated fatty acids, palmitic and stearic acid were in contrast lower at pH 7.0 than at pH 4.5. In Lb. plantarum cultures, lactobacillic acid biosynthesis was proposed by Smith and Norton [4] to be controlled by CFA synthase activity as well as by SAM and fatty acid substrate (cis-vaccenic acid) levels.

Furthermore, S-adenosylhomocysteine (SAH) hydrolase activity of the cells might play an important rôle in the régulation of lactobacillic acid formation, since high activities of SAH hydrolase prevent production inhibition of CFA synthase by SAH [29]. In Lb. plantarum cultures, it has previously been shown that lowering the pH of the medium caused an in-crease in the amount of lactobacillic acid in the bacterial cells and that this was mainly owing to an induction in CFA synthase activity [30]. Accord- ing to our results (Fig. 3), Lb. casei cultures responded to changes in pH of the medium in a similar manner. However, it has to be pointed out that in the studies with Lb. Plantarum [30] and also with E. coli [31], only the relative proportions of cyclopropane fatty acids at different pHs were compared. At least in Lb. casei cultures, the absolute amount of lacto-bacillic acid was bigger at pH 5.5 than at 4.5 in the stationary growth phase, although the relative proportion was smaller (Table 2). This might be owing to higher fatty acid substrate (cis-vaccenic acid) levels at pH 5.5, since the pH of the medium seemed to affect also the total fatty acid accumulation, the fatty acid content of the cells being much lower at pH 4.5 than at pH 5.5 (Fig. 3). In conclusion, the volumétric production of lacto-bacillic acid, which we here wanted to maximize, was in Lb. casei cultures best at pH 5.5 (36.4 mg/L, Fig. 3). This was over 2.5 times more than in shake flasks at uncontrolled pH (13.8 mg/L, unpublished results).

In Lb. plantarum cultures, the effect of pH was more obscure. In con- trast to Lb. casei cultures, both the absolute and relative amount of lactobacillic acid was highest and the proportion of cis-vaccenic acid lowest at pH 7.0 at the end of exponential growth phase (Table 3). Both at pH 5.0 and 6.0, the amount of cis-vaccenic acid increased considerably at the beginning at the stationary growth phase (Table 3), and a quick cell lysis occurred soon after the cessation of growth (Fig. 2). Instead, at pH 7.0, the growth was slow (Fig. 2); no cell lysis occurred and the cells were able to use cis-vaccenic acid for lactobacillic acid synthesis until the stationary phase of growth (Table 3). As illustrated in Fig. 4, the pH of the culture medium affected again not only the fatty acid pattern, but also the total fatty acid accumulation of the cultures, with cellular fatty acid content being highest at pH 6. However, in Lb. plantarum cultures, the CFA synthase activity was not likely to be increased at low pH with culture aging, although the total fatty acid synthesis was enhanced, and therefore, cis-vaccenic acid content was not diminished at pH 5.0 and 6.0 during growth as in Lb. casei cultures (Tables 2 and 3). This indicates that the regulatory mechanisms controlling lactobacillic acid biosynthesis in Lb. plantarum were different from those in Lb. casei and Lb. plantarum. However, since the maximal dry weight was reached at pH 6.0, the best volumétric production of lactobacillic acid (37 mg/L) was achieved at pH 6.0 (Fig. 4) in spite of having a higher relative proportion of lactobacillic acid at pH 5.0 and 7.0. As a result, the production was at pH 6.0 over five times higher than in shake flasks at uncontrolled pH.

Impact of culture age on fatty acid content of the cells at different pH

The total fatty acid content (mg/g cells) of both Lactobacillus strains increased with increasing culture age. The changes in the fatty acid patterns during cultivations can be seen from Tables 2 and 3. The major change in Lb. casei cultures at pH 4.5 and 5.5 was the increase in both absolute and relative amounts of lactobacillic acid and a concomitant decrease in cis-vaccenic acid with increasing culture age. This naturally led to a dramatic decrease in the cis-vaccenic acid/lactobacillic acid ratio during cultivation (Table 2). Cyclopropane fatty acid accumulation has previously been reported to occur with increasing culture age in some other lactobacilli as well. However, this phenomenon has been related to the naturally occurring acidification in cultures with uncontrolled pH [10]. Here, we could detect substantial

Figure 2: Impact of medium’s pH on Lactobacillus plantarum growth. ▲= pH 5.0; ■= pH 6.0 and □= pH 7.0.
accumulation of lactobacillic acid with culture aging, although the pH was kept constant throughout the cultivation, thus indicating that the decrease in pH of the culture is not alone responsible for the enhancement of CFA production, but other factors also have to be involved in controlling the lactobacillic acid accumulation with culture aging.

When cultivated at pH 7.0, the ability of Lb. casei to produce lactobacillic acid from cis-vaccenic acid was clearly restricted, and thus, it was merely the accumulation of cis-vaccenic acid along with an increase in the absolute amount of palmitic acid that caused the increase in the total fatty acid content of the cells. Still, a decrease in the cis-vaccenic acid/lactobacillic acid ratio occurred with increasing culture age also at pH 7.0 (Table 2). Thus, the interchange of cis-vaccenic and lactobacillic acid occurred in Lb. casei cells with increasing culture age to some extent at all the pH values studied.

In Lb. plantarum cultures, the effect of culture age on fatty acid composition was not similar to that in Lb. casei cultures. The amount of lactobacillic acid did increase during the cultivations, but at the end of exponential growth phase, severe cell lysis occurred at pH 5.0 and 6.0, thus causing dramatic changes in the fatty acid pattern of the cells (Table 3). Instead, at pH 7.0, the absolute amount of cis-vaccenic acid and consequently the ratio of cis-vaccenic acid to lactobacillic acid decreased clearly with increasing culture age.

### References

[1] Chang, Y.Y., Cronan J.E Jr. “Membrane cyclopropane fatty acid content is a major factor in acid resistance of Escherichia coli”, Mol Microbiol.,33 pp 249-59, 1999.

[2] Chang, Y. Y., Eichel, J. & Cronan, J. E., Jr “Metabolic instability of Escherichia coli cyclopropane fatty acid synthase is due to RpoH-dependent proteolysis”. J.Bacteriol, 182, pp 4288-4294, 2000.

[3] George, K. M., Y. Yuan, D. R. Sherman, and C. E. Barry III. “The biosynthesis of cyclopropanated mycolic acids in Mycobacterium tuberculosis. Identification and functional analysis of CMAS-2”. J. Biol. Chem. 270 pp27292-27298,1995.

[4] Gitter, B., R. Diefenbach, H. Keweloh, and D. Riesenberg. “Influence of stringent and relaxed response on excretion of recombinant proteins and fatty acid composition in Escherichia coli”. Appl. Microbiol. Biotechnol.,43 pp89-92,1995.

[5] Gottesman, S. “Proteases and their targets in Escherichia coli”. Annu. Rev. Genet.30 pp465-506, 1996.

[6] Grogan, D. W., and J. E. Cronan, Jr. “Use of lambda phasmids for deletion mapping of non-selectable markers cloned in plasmids”. Gene 22 pp75-83, 1983.

[7] Grogan, D. W., and J. E. Cronan, Jr. “Cloning and manipulation of the Escherichia coli cyclopropane fatty acid synthase gene: physiological aspects of enzyme overproduction”. J. Bacteriol.,158 pp286-295, 1984.

[8] Crowfoot, P. D., and A. L. Hunt. “The effect of oxygen tension on methylene hexadecanoic acid formation in Pseudomonas fluorescens and Escherichia coli”. Biochim. Biophys. Acta.,202 pp550-552, 1970.

[9] Jacques, N. A., and A. L. Hunt. “Studies on cyclopropane fatty acid synthesis: effect of carbon
source and oxygen tension on cyclopropane fatty acid synthetase activity in *Pseudomonas denitrificans*. “Biochim. Biophys. Acta.” 337 pp 453–470, 1980.

[10] Jacques, N. A. “Relationship between cyclopropane synthetase and the formation of membrane lipids in *Mycobacterium tuberculosis*. Proc. Natl. Acad. Sci. USA 92 pp6630-6634, 1995.

[11] Su, C. J., R. Reusch, and H. L. Sadoff. “Fatty acids in phospholipids of cells, cysts, and germinating cysts of *Azotobacter vinelandii*.” *J. Bacteriol.*, 137 pp 1434-1436, 1979.

[12] Taylor, F. R., and J. E. Cronan, Jr. “Cyclopropane fatty acid synthase of *Escherichia coli*: stabilization, purification, and interaction with phospholipid”. *Biochemistry.*, 15 pp 3292–3300, 1979.

[13] Wang, A.-Y., and J. E. Cronan, Jr. “The growth phase-dependent synthesis of cyclopropane fatty acids in *Escherichia coli* is the result of an RpoS (KatF)-dependent promoter plus enzyme instability”. *Mol. Microbiol.*, 11 pp 1009-1017, 1994.

[14] Sinensky M. “Homeoviscous adaptation: a homeostatic process that regulates the viscosity of membrane lipids in *Escherichia coli*”. *Proc. Natl. Acad. Sci. U. S. A.*, 71 pp 522-525, 1974.

[15] Grogan D. W., Cronan J. E., Jr “Cyclopropane ring formation in membrane lipids of bacteria”. *Microbiol. Mol. Biol. Rev.* 61 pp 429-441, 1997.

[16] Guillot A., Obis D., Mistou M. Y. 2000. “Fatty acid membrane composition and activation of glycine-betaine transport in *Lactococcus lactis* subjected to osmotic stress”. Int. J. Food Microbiol. 55:47–51

[17] Härtig C., Lothfagen N., Harms H. “Formation of trans fatty acids is not involved in growth-linked membrane adaptation of *Pseudomonas putida*”. *Appl. Environ. Microbiol.*, 71 pp 1915-1922, 2005.

[18] Bang, I. S., Kim, B. H., Foster, J. W. & Park, Y. K. “OmpR regulates the stationary-phase acid tolerance response of *Salmonella entericserovar Typhimurium*. *J Bacteriol.*, 182, pp 2245–2252 2000.

[19] Crowfoot, P. D., and A. L. Hunt. “The effect of oxygen tension on membrane hexadecanoic acid formation in *Pseudomonas fluorescens* and *Escherichia coli*”. *Biochim. Biophys. Acta.* 202 pp 550–552, 1970.

[20] Weber, F., J. S. Isken, and J. A. de Bont. “Cis/trans isomerization of fatty acids as a defence mechanism of *Pseudomonas putida* strains to toxic concentrations of toluene”. *Microbiology*, 140 pp 2013–2017, 1994.

[21] Yuan, Y., and C. E. Barry, III. “A common mechanism for the biosynthesis of methoxy and cyclopropyl mycocids in *Mycobacterium tuberculosis*. Proc. Natl. Acad. Sci., USA 93 pp 12828-12833, 1996.

[22] Yuan, Y., D. C. Crane, J. M. Musser, S. Sreevatsan, and C. E. Barry III. “MMAS-1, the branch point between cis- and trans-cyclopropane containing oxygenated mycolates in *Mycobacterium tuberculosis*”. *J. Biol. Chem.*, 272:10041-10049, 1997.

[23] Yuan, Y., R. E. Lee, G. S. Besra, J. T. Belisle, and C. E. Barry III. “Identification of a gene involved in the biosynthesis of cyclopropanated mycocids in *Mycobacterium tuberculosis*. Proc. Natl. Acad. Sci. USA 92 pp6630-6634, 1995.

[24] Zalkin, H., J. H. Law, and H. Goldfine. “Enzymatic synthesis of cyclopropane fatty acids catalyzed by bacterial extracts”. *J. Biol. Chem.*, 238 pp 1242-1248, 1963.

[25] Zambrano, M., D. Siegle, M. Almiron, A. Tomo, and R. Kolter. “Microbial competition: *Escherichia coli* mutants that take over stationary phase cultures”. *Science.*, 259 pp 1757-1760, 1993.

[26] Matagaras M, Metaxopoulos J, Galiotou M, Drosinos DH. “Influence of PH and temperature on growth and bacteriocin production by *Leuconostoc mesenteroides* L124 and *Lactobacillus curvatus* L442”. *Meat Sci.*, 64 pp 265-271, 2003.

[27] Ogawa J, Kishino S, Ando A, Sugimoto S, Mihara K, Shimizu S. “Production of conjugated fatty acids by lactic acid bacteria”. *J. Biosci. Bioeng.*, 100 pp 355-364, 2005.

[28] Oliveira R, Flores A; Silva R; Perego P; Converti A; Gioielli L; Oliveira M. “Effect of different prebiotics on the fermentation kinetics, probiotic survival and fatty acid profiles in nonfat symbiotic fermented milk”. *Int. J Food. Microbiol.*, 128 pp 467-472 (2009).

[29] Rodrigues D, Rocha-Santos T, Gomes A, Goodfellow B, Freitas A. “Lypolysis in probiotic and symbiotic cheese: The influence of probiotic bacteria, prebiotic compounds and ripening time on free fatty acid profile”. *Food Chem.*, 131 pp 1414-1421, 2012.

[30] Wrolstad R. “Nutritional roles of carbohydrates. In: Food Carbohydrate Chemistry. R. Wrolstad Editor. Wiley-Blackwell, UK. p147-164, 2012.