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Cyclopropane fatty acids in *Lactococcus Lactis*
Dealing with industrial stress

"We CAN deal with it! Thanks to cyclopropane"

Abstract

The cyclopropanation of the membrane fatty acids is an adaptation inherent to a large number of organisms allowing them to deal with numerous types of stress. The organisms capable of producing cyclopropane fatty acids are spread through two branches of the phylogenetic tree, the prokaryotes and the eukaryotes, leading to the conclusion that this feature must have been developed by the ancestral organism.

In all these organisms the genetic regulation induces the CFA synthesis at the onset of the stationary phase, which is characterized by nutrient limitation. At this point unsaturated acids of the cis-conformation are modified into CFAs.

In this paper it will also show that the CFA synthesis is not limited to the stress induced by starvation. Several other types of stress, which can be encountered by organisms will also be discussed, with emphasis put on the stress types encountered by *Lactococcus Lactis* during industrial usage of named bacterium. An answer will be sought to the question: To what extent do CFAs help the bacterium *Lactococcus Lactis* deal with the stress conditions it encounters during industrial production?

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Introduction

For many people the term biotechnology is a 21st century invention directly related to the, for some, controversial topic of genetic engineering, however in reality biotechnology is a much broader topic predating our recorded history. The process of cheese making relies largely on bacteria like *Lactococcus Lactis* and is thought to have its origin between 8000BC and 3000BC. In recent years biotechnology has, with an ever increasing rate, become a tool for humanity to help solve its many problems. Also in modern bio-industry many microorganisms are being used as production organisms and as part of the industrial processes they are often subjected to extreme conditions. There are many adaptations microorganisms have developed to help them deal with difficult conditions. For example, halophile bacteria collect osmoprotectants inside their membrane to counter the extreme osmotic pressure. Another such adaptation, which is present in numerous organisms are the Cycopropane Fatty Acids.

A cyclopropane fatty acid is any fatty acid which contains a propane ring (figure 1). In this paper the term is mainly used for a fatty acid in a biological membrane. CFAs are produced from unsaturated fatty acids during the late exponential or early stationary growth phase.

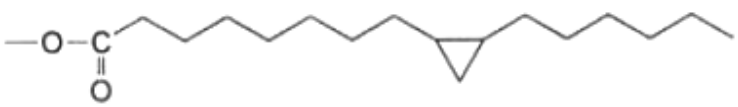

Figure 1: A schematic representation of fatty acid containing a cyclopropane ring.

The discovery of the CFAs was first announced in 1951 by Hofmann, Lucas and Sax. During their research, which was targeted at unraveling the fatty acid composition of *Lactobacillus arabinosus,* they showed that the lipids of *L. arabinosus* essentially contained four different fatty acids. Three of these were previously known, namely palmitic acid, stearic acid, cis-11,12-octadecenoic acid (cis-vaccenic acid). The fourth fatty acid they found was a previously unrecorded, unsaturated fatty. By using 'both chemical and physical methods' the chemical formula was determined to be $C_{19}H_{35}O_2$ and it was established that this fatty acid contained a cyclopropane ring. Giving homage to the organism from which the fatty acid was first isolated it was named lactobacillic acid.[1] Research based on the discovery of Hofmann, Lucas and Sax later showed that lactobacillic acid was not the only cyclopropane fatty acid and that the occurrence of CFAs is not exclusive to the prokaryotes.

In this paper the author will attempt to instill a deeper understanding of the Cyclopropane Fatty Acids and this information will be used to answer the question: To what extent do CFAs help the bacterium *Lactococcus Lactis* deal with the stress conditions it encounters during industrial production?

Distribution of CFAs

The cyclopropane fatty acids had first been discovered in *L. arabinosus,* a bacterium. Since then the development of the liquid-gas chromatography technology in 1950 further research on complex lipids was made considerably simpler. As a result the presence of lactobacillic acid, and other cyclopropane fatty acids (CFAs), has been shown in the phospholipids of numerous organisms.[2]

Prokaryotes

In the domain of the bacteria CFAs seem to be the most abundant. Apart from the already mentioned *Lactobacillus* genus (Gram negative), many other genera were found to contain CFAs. These genera are well distributed throughout the prokaryotic part of the phylogenetic tree and include Gram negative, as well as Gram positive bacteria. Among these are strict anaerobes, selective aerobes, as well as obligate aerobes. It has been shown that the enzymes used in the production of the CFAs are highly conserved and thus, with the widespread sequencing of whole genomes, it has been made considerably easier to find organisms that are capable of producing these enzymes. Organisms incapable of producing these enzymes are certain not to have CFAs in their membranes, however it cannot be said with certainty that all organisms equipped with the enzymes do produce CFAs. Ultimately, when having found an organism, with the genes to produce the enzymes necessary for making CFAs, only a lipid analysis can prove that it really does produce CFAs. In these prokaryotes where the production mechanism is present, but synthesis does not take place, research has shown that certain precursors are missing.[2]

Table 1: Distribution of CFAs among bacterial genera

genera producing CFAs		**Genera incapable of producing CFAs**	
Gram positive	**Gram negative**	**Gram Positive**	**Gram negative**
Arthrobacter	Alcaligenes	Corznebacterium	Leptospira
Bifidibacterium	Azotobacter	Streptomyces	Moraxella
Clostridium	Bordetella		Neisseria
Lactobacillus	Campylobacter		Rhodobacter
Lactococcus	Caulobacter		Spirochaeta
Pediococcus	Chlorobium		Treponema
Streptococcus	Citrobacter		
	Enterobacter		
	Helicobacter		
	Klebsiella		
	Nitrobacter		
	Proteus		
	Pseudomonas		
	Rhizobium		
	Salomonella		
	Serratia		
	Thiobacillus		
	Vibrio		
	Yersinia		

The data displayed in Table 1 was taken from [2] and Lactococcus was added. Of the listed genera at least one species has been determined to produce CFAs for the genus to be placed in the 'genera producing CFAs' column.

At this point it should be noted that in bacteria which can, and do produce CFAs, the lipid analysis can produce negative results for CFA presence. In literature two prominent reasons have been given for this enigma. The first reason has been found to be the reagents used in the lipid analysis, which have an adverse effect on the outcome. The cyclopropane ring is chemically rather reactive (chapter 3.3 biochemical properties) and when reacting with the reagents the CFA can be transformed into a branched fatty acid.[2] The second reason for a false negative has been related to the production of the CFAs, which is dependent on the prevailing physiological conditions. It is very possible that an organism, which has been tested to a negative result, does produce CFAs under different growth conditions.[3]

Eukaryotes

Even though they seem to be most abundant in bacteria cyclopropane fatty acids are not a trait exclusive to prokaryotes. In eukaryotes, the occurrence and distribution of cyclopropane fatty acids has been investigated in seeds, leaves and other tissues of several eukaryotic species, to the conclusion that several genera of protozoa (*Crithidia*, *Herpetomonas*, *Leptomonas*, *Leishmania, Tetrahymena*)[2,5] synthesize CFAs, as does the slime mold *Physaram polycephalu*.[2] Additionally, though they are not widely distributed in higher plants, they do occur in the seed oils of the plant order Malvales.[4] Also, CFAs have been discovered in the tropical millipedes *Graphidostreptus umuliporus*[2] and *Graphidostreptus tumuliporus*[6]. In these species the CFAs can be found with a considerable concentration in the females and an even higher concentration in the eggs, however no traces of the CFAs can be found in the males. It has been suggested that the females produce the CFAs for their eggs, where they play an important role in the lipid structure. The exact function of the CFAs in the eggs has not yet been determined.

It would seem that the CFAs are much less common in the eukaryotes when compared to the prokaryotes, but they seem to be equally well distributed.

Archaea

It should be mentioned that since the cyclopropane fatty acids exist in the bacterial domain, as well as in the eukaryotic domain, they are likely to have their origins in the ancestral organism, which bacteria and eukaryotes have in common. Thus it would be logical for archea, having split off from the eukaryotes, and thus sharing the same ancestral organism, to have cyclopropane fatty acids as well. However, to this point no CFAs have been found in any bacteria. The reason for this has been proposed to be the same as for the non-CFA producing prokaryotes, the absence of the needed precursors.[2]

Biosynthesis

The term biosynthesis of cyclopropane fatty acids incorrectly describes the process in which CFAs are produced. Based on the fact that the fatty acids, which get turned into cyclopropane fatty acids, are already assembled into phospholipids and already form part of a bi-layer membrane, this process should be viewed as a postsynthetic modification.
As already mentioned in the chapter 'Distribution of CFAs' there are certain precursors needed for the synthesis of CFAs. These precursors are unsaturated fatty acids (UFAs) of the cis conformation. There are two types of UFAs, the cis form and the trans form. In the cis conformation the two hydrogen atoms are on the same side thus the phospholipid has a stronger kink. (Figure 2) These UFAs are produced from acetyl CoA via the well-established polyketide pathway Research has shown that only cis UFAs get transformed into CFAs, and only if the double bond is sufficiently far from the end of the acetyl chain.

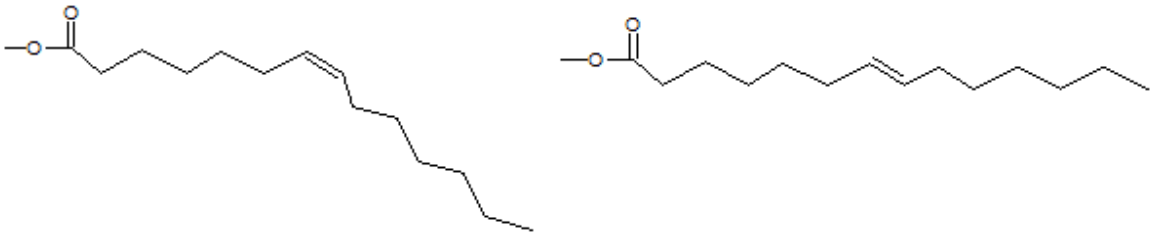

Figure 2: schematic representation of a cis (left) and a trans (right) unsaturated fatty acid.

Pathway

The synthesis of a CFA from a cis-UFA requires the methylation of the two double bonded (cis) carbons. The source of this methyl group (C1) has been determined to be the S-methyl group of a S-adenosylmethionine (AdoMet). (Figure 3)

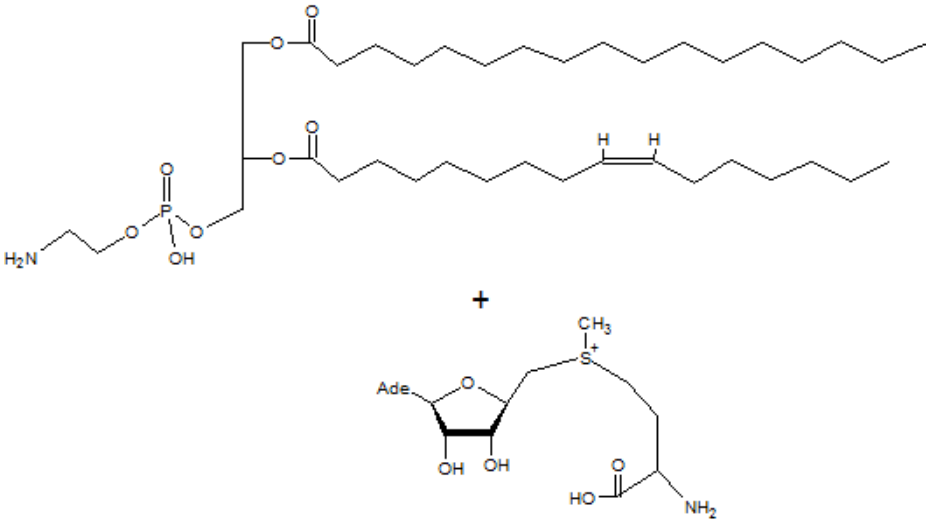

Figure 3: Schematic representation of a cis-UFA, note the two hydrogen atoms on the same side of the fatty acid chain, and the AdoMet, note the methtl group attached to the sulphonium cation.

The proposed method for the attachment of the AdoMet to one of the cis carbons is a S_N1 reaction. In this particular reaction the sulphonium cation draws the electrons from the double bond, thereby attaching the methyl group to one of the former cis carbons and making a carbocation of the other cis carbon. (Figure 4)

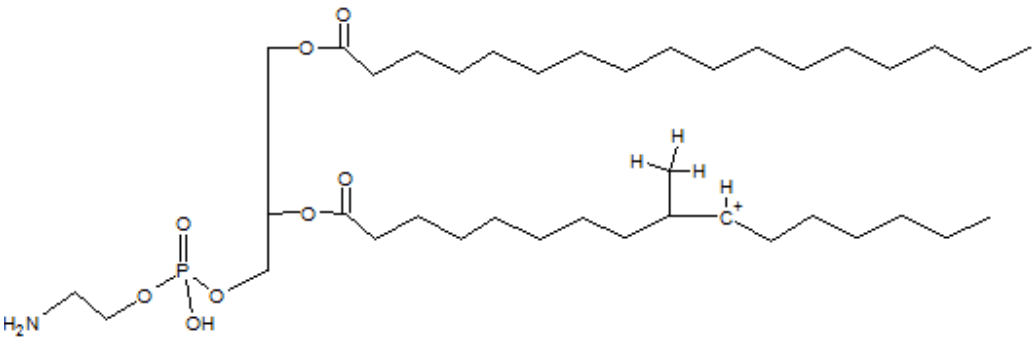

Figure 4: Schematic representaion of the carbocation intermediate.

At this point a number of different pathways are available to quench the cation, one of which is the formation of the cyclopropane ring. In this pathway one of the methyl protons is drawn by

the cation, whose respective proton is passed on to the sulphonium, there by producing the cyclopropane ring. (Figure 5)

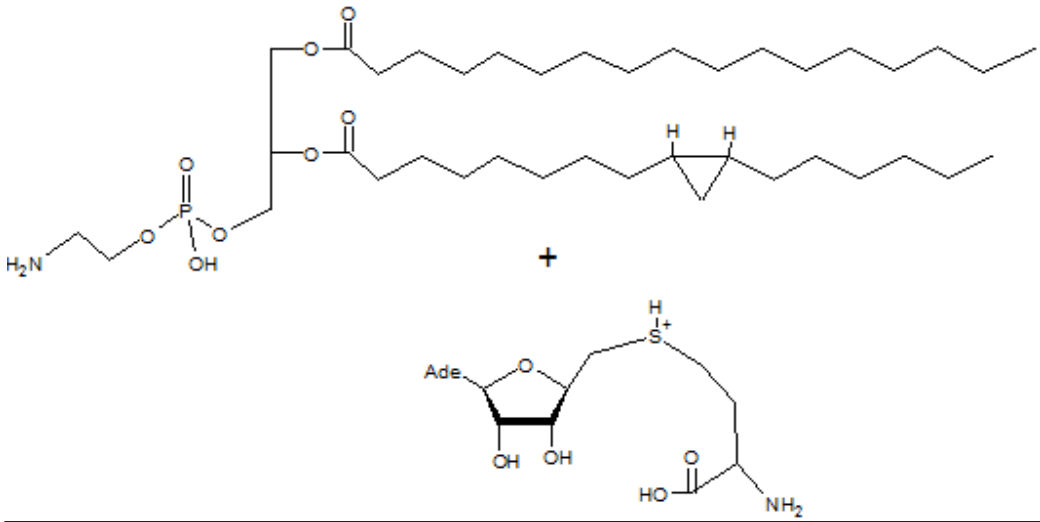

Figure 5: Schematic representation of the cyclopropane fatty acid. Note the Proton replacing the methyl group on the sulphonium cation.

The reaction is catalyzed by the so called CFA synthase which speeds up the process and assures that CFAs are produced, and none of the other possibilities.[2]

Biochemical properties

The three carbon atoms forming the cyclopropane ring have bonding angel 60 degrees, which is, chemically speaking, an unfavorable arrangement. There is a lot of angle strain (27.5kcal/mol) on the structure, which should, chemically speaking, considerably weakening the bonds. For this reason, and because the synthesis of cyclopropane in phospholipids has a high energetic cost, it was for some time proposed that cyclopropane fatty acids were a type of C_1 storage, easily accessible for methylation reactions when needed. This notion was disproven on the basis that in the phase where the CFAs are produced the hypothetically stored methyl groups would already be needed.[2]

It was discovered that in the membranes the cyclopropane rings, once they are formed, are very stable and are considered to be permanent membrane components. An experiment where the cells were brought back to exponential growth, after they had entered stationary growth, showed that the propane rings were generally not dismantled. However, an exception to this rule was found, in an experiment were the cells were exposed to toluene. Here the cyclopropane rings reverted to cis-UFAs and these cis-UFAs were converted into a trans-UFAs. It was determined that trans-UFAs offer better protection against toluene than either cyclopropane fatty acids, or cis-UFAs.

Membrane fluidity

Another theory on the function of the CFAs was proposed, which has gained considerable support since its proposition, and it now generally accepted. It was determined that because of the high cost and the phase in which they were produced, the CFAs were preparing the cells for the conditions of the stationary phase, where resources are no longer abundant. Up to this day the mechanism of protection has not conclusively been determined.

The fluidity of a bilayer membrane is influenced by numerous factors, such as the length of the fatty acids and the degree of saturation, as well as the temperature. In general it can be said that with decreasing fatty acid chain length , as well as decreasing saturation, the fluidity of the membrane increases. The same holds true for an increase in temperature. This is contrasted by the change induced by the cyclopropanation of a cis-UFA, which decreases the membrane fluidity. The cyclopropane rings restrict the overall mobility of the fatty acid and reduce the effect of the temperature on the membrane fluidity.

Regulation of the CFA synthesis

The regulation of cyclopropane fatty acid synthesis has been investigated most thoroughly in *Escherichia coli*. Based on the theory that the biosynthesis of CFAs has originated from the ancestral organism it can be assumed that the regulation is the same in all organisms. The assumption is supported by research done on several other CFA producing organisms. In this research it was shown that the genes responsible for the genetic part of the CFA synthesis regulation have considerable homology. Thus the model organism *E. coli* can be used to explain the regulation of the cyclopropane fatty acid synthesis as a substitute for the less well studied *L. lactis*.

PpGpp

For bacteria, such as *E. coli*, and for certain plants it is known that when they undergo amino acid starvation, as they do in the stationary phase, they bring about the stringent response. This mechanism is characterized by the accumulation of the Guanosine pentaphosphate (ppGpp). The gene coding for ppGpp is called rel^+. It has been shown that in rel^+ knockouts (*relax*, or Δ*relA*) the stringent response is not induced and also that there is no production of CFAs. The accumulation of large concentrations of ppGpp has also been documented for other types of starvation, such as carbon and general nutrient deficiencies. It is generally accepted that for most types of stress an organism can undergo there is an increase in ppGpp. These alterations in the intercellular ppGpp level have an effect on the regulation of up to 1/3 of all genes, causing the cell to conserve its resources, stopping cellular growth and cell division. One mechanism induced by ppGpp, which is well studied during amino acid starvation, is the binding of ppGpp to the σ subunit of the RNA polymerase thereby inhibiting protein synthesis and conserving the amino acids. At the same time ppGpp increases the metabolism of amino acids.[7]

One of the many other effects ppGpp has on the *E. coli* genome is the up-regulation of the *rpoS* gene.

Rpo genes

Experiments in *E. coli* have shown that the activity of the CFA synthase increases in the late exponential phase, spikes in the early stationary phase and then diminishes rapidly. Although many environmental conditions (chapter 'Industrial Stress') have been found capable of influencing the CFA biosynthesis, the genetic regulation is believed to be the most crucial factor in the regulation of CFA synthase activity.

Two functional promoters of the *cfa* gene, the gene coding for the CFA synthase protein, have been identified. One of these promoters is recognized by the σ^{70} subunit of the RNA polymerase. σ^{70}, encoded by the *ropD* gene, is the so called housekeeping sigma factor, which transcribes most genes in the exponential phase, and also the transcription of the CFA synthase throughout all growth phases of the cell. The other promoter is recognized by the σ^{38} subunit of the RNA polymerase. σ^{38}, encoded by the *rpoS* gene, it the sigma factor generally correlated with the stationary phase and starvation. Thus, the activation of σ^{38} is logical at the indicated phase transition, since the phase transition is caused by the no longer abundant resources. This suggest that the σ^{38} promoter is crucial to the activation of the *cfa* gene and thus rapid accumulation of cyclopropane fatty acids in the stationary phase. [2]

The *cfa* gene

The isolation of the *cfa* gene revealed that the gene is less than 1.5 kbp in length, making it a rather small gene. It was also discovered that the gene is rather unique, since there are no known homologues other than the *cfa* genes in other organisms. In the sequence of the gene there are three motifs, which have been shown to be homologous to proteins known to bind with AdoMet. After folding of the protein these three regions are likely to form an AdoMet binding site. The *cfa* gene transcribes a single protein, the CFA synthase, which consist of 382 amino acids and has a size of approximately 40 kDa. Based on size exclusion chromatography experiments the CFA synthase had been predicted to have a size of approximately 90 kDa. This suggests that CFA synthases form dimmers when they are active producing CFAs.[2]

Degradation of CFA synthase

The stress response discussed in the subchapters above makes clear how the high concentrations of CFA synthase come to be. However, there is another factor which is involved in the regulation of the CFA synthesis, which is the rapid disappearance of the CFA synthase following its induction in stationary phase of the growth curve. Given that the stationary phase is characterized by a almost complete inactivation of protein synthesis it is highly unlikely that the CFA synthase is degraded by a specially made enzyme. It was experimentally discovered, by using a biotin labeled CFA synthase, that the CFA synthase has a very short in vivo half-life of 5 min. Further results from tests with different strains of *E. coli* supported the hypothesis that the diminished CFA synthase activity is mainly attributable to the instability of the enzyme itself. However it has also been proposed that CFA synthase might be the target of a so far unidentified energy-independent heat shock regulon protease.[8]

Industrial stress

As stated in the chapter '*Rpo* genes' many environmental conditions can have an influence on the biosynthesis of CFA in bacteria. In this chapter several types of stress, which the bacterium *Lactococcus Lactis* encounters during its industrial use, will be discussed.

Temperature

The optimal growth temperature of *L. lactis* is 30°C. When a cell culture is subjected to a temperature of 42°C cellular growth will stop. Survival rates after an exposure of 30 minutes can be considered, with the cells having returned to a preshock growth rate after 1 hour. *L. lactis* is capable of resisting moderately increased temperatures with little adverse effects. After a heat shock of 50°C for 30 minutes, from an initial growth temperature of 30°C, the survival is significantly decreased (1/1000). It is important to note that if the 50°C heat shock is preceded by a pretreatment of 42°C for 10 minutes the survival rate is increased by 10%. [10]

The adaptation of *L. lactis* to low temperature contrasts its resistance to high temperature. Where a temperature significantly higher than the optimum the cellular growth stops at lower temperature there is a mere decrease in growth rate. It should be mentioned that it the culture freezes there obviously is no further growth. However, the bacteria have a high survival rate and once unfrozen they will start growing again, again a contrast with the cell death at too high temperatures. [10] This feature is used by many scientists, also industrialist scientists, in the process of Freeze-Drying, a method often used for the preservation and long-term storage of bacterial cells. However, it has been found that this process can have a negative effects on the viability of many cell types, particularly in the exponential growth phase. It has also been determined that the increased bacterial survival rate of stationary phase cells is directly related to the amount of cyclopropane fatty acids. [11]

Acid

The lactic acid bacteria, which includes *L. lactis*, ferment sugars thereby producing lactic acid. From this it is logical to conclude that they are frequently subjected to acid stress. *L. lactis* is capable of withstanding a pH of 4.5 in stationary phase with no significant increase in cell death, however the survival of the *Lactococcus* cells decreases rapidly at a pH lower than 4.5. The *L. lactis* subspecies *cremoris* is known to be capable of resisting a pH of 3.0. [10] If the cells are in the exponential phase their resistance to the acidity generally decreases. It should be noted that lactic acid is a weak organic acid which is deprotonated at low pH. In its deprotonated state it can easily be exported. A strong correlation has been found between the CFA concentration in the bacterial membrane and the resistance of those bacteria to lethal pH. The mechanism of protection has not yet been unraveled, but it has been suggested that an increase in CFA levels in the membranes decreases proton permeability across the membrane.[9]

Osmotic pressure

The membrane of *L. lactis*, as all other cell membranes, is permeable to water and hence will allow water to defuse in and out of a cell, according to the concentrations of salt on the inside, and outside of the membrane. If the concentration of salts inside the cell is higher than outside water will enter the cell, and if the concentration of salts outside it higher water will leave the cell. This puts stress on the cell, given that having too much water in the cell would cause the cell to burst, and too little water inside the cell would cause it to dry up. Bacteria have developed mechanisms to deal with this problem, for example the osmoprotectants, which help raise the osmotic values on the cells interior, for high external salt concentrations on the outside. [10] Research dedicated to cyclopropane fatty acids has discovered that increases in osmotic stress are mirrored in an increase of the membrane CFAs. It would appear that the CFAs assist in the handling of osmotic stress, though no mechanism for this assistance has been proposed yet.

Conclusion/Discussion

In this essay an overview of the cyclopropane fatty acids was given. It was established that the cyclopropane fatty acids are a protection mechanism and are present in the prokaryotes and eukaryotes. In eukaryotes the occurrence of CFAs is considerably less than in prokaryotes. This is possibly due to the fact that most eukaryotes do not encounter such extreme conditions. When CFAs are found in eukaryotes it is because they need special protection, for example *Malvales* seeds need to endure the conditions inside the intestines of avians. Generally speaking the archaea also have the necessary genetic equipment to form CFAs, but in this domain, as well is in numerous generea of the bacteria, the CFAs are not produced due to a lack of precursors. The removal of a trait or ability in nature is mostly due to selection, so it can be imagined that in organisms not subjected to large amounts the energetic cost lead to the disablement of this trait. This explanation possibly holds for some bacteria and the eukaryotes, but of archea it is known that they survive under extreme condition. The loss of the ability to form CFAs in archea might be explained by the different membrane type which these organisms have. Archea has, for a large part, phospholipid monolayer membranes, which are even more rigid than the CFA membranes, and thus the ability to make CFAs is superfluous.

The precursors from which the CFAs are produced are cis unsaturated fatty acids. The generally accepted pathway for CFA synthesis is an S_N1 reaction in which the methyl group from an S-AdoMet is attached first to one of the cis carbon and then the other. Though the pathway is generally accepted it is not clear how the modification of the fatty acids within the membrane actually works. It was thought that this was a method for storing methyl groups, but the timing of the CFA synthesis disproved this. Later it was found that cyclopropane rings in the membrane make the membrane more rigid and thus it was concluded that CFAs are a protection mechanism. Cyclopropane is known for its chemically unfavorable arrangement, due to the considerable angle stress, and its reactivity. In biological membranes however it was shown that the CFAs are very stable, staying in the membrane even if the cell finds itself back in more favorable conditions. This first led to the conclusion that the CFAs cannot be removed from the membrane. This proved to be incorrect, since an experiment on the resistance against toluene showed that the cyclopropane can be dismantled. It can be concluded that, if the membranes need to adapt to different conditions, they can dismantle the CFAs, but without a sufficiently strong stimulus they will not.

The chapter ‘regulation of CFA synthesis’ elaborates the regulation of CFA synthesis making use of an example, the amino acid starvation. This starvation, as an example for any kind of environment related stress, causes the production of ppGpp, a universal stress signal molecule. During amino acid starvation ppGpp causes the transcription of the *rpoS* gene, whose product, the sigma factor 38, in turn activates the *cfa* gene. This causes the production of large numbers of CFA synthase and as a result lots of CFAs are produced in a short time. Because of their short half-life the CFA synthases are quickly degraded and as a result the amino acids which make the synthases can be used for other purposes, which is a handy arrangement when amino acids are what is needed.

Under exponential growth conditions and under amino acid starvation conditions two different sigma factors are active and for both of these factors a promoter has been found for the

cfa gene. Given the knowledge that there are numerous other *rpo* genes it might be concluded that under different stress conditions these other sigma factors become activated by ppGpp and up-regulate the CFA production, for example during heat shock the sigma factor 32.

Excessive heat is one of the many stress types encountered by *L. lactis* during industrial processes. It has been shown that a heat shock of 50^{o}C kills the bacteria, however it was also discovered that if the bacteria are first acclimatized to heat before the heat shock, their survival chance is considerably increased. A reason for this might be that during the pretreatment the stress indicator ppGpp is released and signals the need for a higher concentration of CFAs. It has been proven that increased amounts of CFAs increase the survivability of Lactococcus lactis when under heat stress. It is known that the mobility of fatty acids in the membranes increases with increasing temperature. At too high temperatures this mobility possibly destroys the membranes integrity. It is possible that by reducing the mobility of the fatty acids, the CFAs increase the temperature at which the membrane functions are lost.

Another temperature related stress encountered by bacteria is freezing, in this paper specifically the process of freeze drying. It has been found that in the viability count, after thawing, of cells taken from the stationary phase is considerably higher than that of cells taken from the exponential phase. This increased survival has also been related to the presence of more CFAs. It would appear that cyclopropane fatty acids stabilize membrane lipids against turnover and degradation.

The bacterium *L. lactis* is known for producing lactic acid, thereby acidifying its cytoplasm and, after export of the acid, its environment. Hence it is logical that *L. lactis* is adapted to acidic conditions. The method, which has been proposed for this suggests that the acid is exported and acidic molecules are kept outside. The role of the CFAs in this has not yet been determined, even though it was proven that they do play a role. However, it can be taken as a certainty that their role is related to the permeability of the membrane, either making transport of the deprotonated lactic acid to the exterior easier or reducing the influx of charged molecules, possibly both.

The last stress type discussed in this essay was the osmotic stress. This type of stress comes into being when large differences exist between the concentrations of salts on the inside of a cell as compared to the outside. It was shown that with more CFAs the cells can handle more osmotic stress. A mechanism for the CFAs action has as of yet not been proposed, but multiple reasonable hypothesis can be formulated. In aqueous solutions salts disintegrate into the ions from which they are formed, hence the same mechanism as proposed for acid stress would function for them. Another mechanism might be that the cell membrane, which is more rigid due to the many CFAs acts as an additional cell wall, thereby allowing the cell to withstand more osmotic pressure.

In this paper the cyclopropane fatty acids were discussed with respect to the question, ‘To what extent do CFAs help the bacterium *Lactococcus Lactis* deal with the stress conditions it encounters during industrial production?’. It was established that in at least three industrial stress types encountered by *L. lactis* there was a direct relationship between the amounts of CFAs in the membrane and the resistance to the stress inducing factor. It was further shown that there is a high likeliness that CFAs are produced for any stress encountered by the organism and as a result it was suggested the CFAs help deal with these stress types too. From this it can be concluded

that cyclopropane fatty acids do help the bacterium *L. lactis* in dealing with the stress conditions it encounters during industrial production. However, a conclusive statement with reference to the extent of this assistance is considerably more difficult, due to multiple factors. In first instance numerous stress types, which the organism encounters during industrial production, have not yet been examined with respect to the CFAs. Though it has been suggested that CFAs assist with all stress types, this has to be made fact, before one can make conjectures based on it. For the stress types which have been researched there is insufficient numeric data. The current scientific knowledge states trends, which is highly insufficient to resolve the question at hand. Finally, searching for the extent of help due to the CFAs, one needs to know the help given not only by the CFAs, but also by other factors. It was stated in the introduction that the CFAs are only one adaptation to difficult conditions of many. Seeing as these factors are not discussed in this paper, nor are they all known, a conclusive statement to the extent of the help given by the cyclopropane fatty acids cannot be made.

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