



**e-ISSN: 2278-5191**

# **STUDY OF MEMBRANE FATTY ACIDS OF GRAM NEGATIVE BACTERIA AND ITS INFLUENCE TOWARDS THE TERRESTRIAL ECOSYSTEM**

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### **ABSTRACT:**

*Membrane fatty acids being the fundamental components of gram negative bacterial lipids contribute structural integrity, protection against the chemical and mechanical damage. Nature provides an enormous diversity in the composition of fatty acids to the different species of bacteria according to its habitat.Each bacterial species lives in different environment consist different grades and different types of fatty acid. With the help of some new and efficient analytical techniques the chemical complexity of fatty acid has been determined and it is now revealed that some polysaturated fatty acid, mono saturated fatty acids are found to be common amongst some groups of gram negative bacteria. This review indicates the recent year research done on many aspects of gram negative bacterial fatty acids and their influence towards the ecosystem. Some fatty acids discussed here may potentially be used as an active agent for recognizing certain important bacterium species in ecosystems.*

## **KEYWORDS:**

*Gram negative bacteria, Fatty acids, Ecosystem.*

### **1. INTRODUCTION**

The fatty acid is an important carboxylic acid that exists either saturated or unsaturated in all the cell membrane of gram negative bacteria [1].Membrane fatty acid as part of lipids in gram negative bacteria plays important role in maintaining structural integrity and physiological functions of cell membrane [2]. Studies have mainly focused on maintaining different growth media for bacteria and methods for isolation of the fatty acids. Accumulation of knowledge regarding the chemistry of fatty acids has been lagging far behind the progress made in the studies of carbohydrates and proteins.

In recent year, membrane fatty acid has attracted attention of many researchers in both gram negative and gram positive bacteria [3, 4, 5, 6, 7, 8, and 9]. The biochemistry of the bacterium of fatty acids has been elaborated [10] till; there is a lacuna in the understanding the influence of membrane fatty acidspresent in the cell membrane of gram negative bacteria on terrestrial ecosystem.Therefore, the present study is to do

comparative on fatty acid profile of gram negative bacteria as well as to understand the influence of towards terrestrial ecosystem.

#### **2. MEMBRANE FATTY ACIDS**

Biological membranes are not homogenous lipid mixtures, but rather, they are composed of dynamic lipid and proteins cluster referred to as micro domains that differ in their composition from their flanking regions [11]. Total fatty acid content in the most species of bacteria ranges between 1 to 10% of dry cell weight [12, 13]. Lipids are a large and diverse group of naturally occurring organic compound that share common physical properties, such as their solubility in non-polar organic solvent and in general insolubility in water. In terms of membrane composition, lipid can be classified into different groups: glycerolipids, sphingolipids and terpene- derived lipids. Fatty acid may contribute complex lipids, although they can be found as free entities in the membrane [14].

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**Fig 1**.Cartoon illustration of the lipid complex membrane formation [100].

Lipid micro domainsform within the cell membrane has its unique composition that establishes environments that favor the activity of specific fatty acids and proteins [14].There isa compelling reason to believe that lipid domains exist, and they have been clearly demonstrated in model lipid monolayer and bilayer, their precise

nature, compositions, size and dynamic in biological membranes still remain controversial [10].The number of fatty acids occurs in bacteria is vast.

According to the chemical nature and structural formula, fatty acids are categorized into many groups as shown in following figure:



**Fig 2**. Types of membrane fatty acid present in every cell of bacteria

### *a. Saturated straight chain Fatty acids*

Fatty acids having a chain of less than 12 carbon atoms have been detected in small amounts in virtually all species of gram negative bacteria. These fatty acids commonly found as follows acids: formic  $(C_1)$ , acetic  $(C_2)$ , propionic  $(C_3)$ , butyric  $(C_4)$ , caproic  $(C_6)$ , Caprylic  $(C_8)$ , and Capric  $(C_{10})$ . The higher fatty acids constitute a muchlargerproportion of the total fatty acid content of the cell. The acids included in this group are lauric (C), myristc (C<sub>12</sub>), palmitic acid (C<sub>14</sub>), steric (C<sub>16</sub>), arachidic  $(C_{18})$ , behenic  $(C_{22})$ , lignoceric  $(C_{24})$ , and octaconsonoic  $(C_{28})$ . In bacteria palmitic acid occurs more frequently and usually in larger amount than any other saturated fatty acid found in the bacteria [15].There is less fatty acid reported to be having an odd number of carbon atom like evidence of  $C_{15}$  and  $C_{17}$  straight chain saturated acids in species *Pseudomonas aeruginosa*[16]. These fatty acids have yet to be identified with certainty [17].

#### *b. Branched chain Fatty acids*

Branched fatty acid is also a common constitute of bacterial lipids. This type of complex fatty acid has been isolated fromthe corynebacteria[14, 18, and 19]. These include chronic and diptheric acids, both of which have the same empirical formula,  $C_{35}H_{68}O_2$  although they appear different in their structural details. Several derivatives of this fatty acid have been found in the same bacterial organisms.

Hausmann and Craigh[24] have shown that isootanoic, or methylheptanoic, acid  $(C_8H_{12}O_2)$  is produced by *Bacillus polymyxa*as a component of polymyxin B.

Akashi and Saito [20] have detected a branched chain  $C_{15}$ and  $C_{17}$  acid in the lipids of strains of some gram positive bacterial strain like *sarcina, Bacillus subtilis and Bacillus natto.* This compound was tentatively namedsarcinic acid. In later studies in the *Bacillus*  species, Saito  $[26, 27]$  showed that the C<sub>15</sub> was 15-Methylhexadecanoic acid.

Asselineau[23] has reported another two branched fatty acid, whichin *Bacillus subtilis,* and a C15 compound and the other a  $C_{16}$  substance. Further characterization of this is not being reported yet. They have also reported the presence of  $C_{19}$  branched chain fatty acid in the gram negative bacterial strain *Pasteurelapestis*. The detailed study of this has not been made yet.

#### *c. Hydroxy fatty acids*

These are the fatty acid, which has carboxyl group and a hydroxyl group at different positions in the fatty acid chain [28]. Fatty acids like β- Hydroxybutyric, β-Hydroxydecanoic, β-δ-β Methyl valeric acid and Dihydroxysteric are some examples for hydroxyl fatty acid [29, 30, 31, 10, 32, 33, and 34]. Crowder and Anderson [34] have discovered the presence of Dihydroxystearic acid.Asselineau and Lederer[18] discussed detail about the fatty acid called Corynomycolic acid  $(C_{32}H_{64}O_3)$ , and caorynolic acid, a dihydroxy compound also known as corynine  $(C_{52}H_{104}O_4)$ . These substances, have many similarities to the mycolic acid found in mycobacteria [10].β-Hydroxybutyric fatty acid has been found in several bacteria including species of *Azabacter*[29,108], *Chromobacterium*[29], *Bacillus* [30, 31, and97], *Pseudomonas* [29], and *micrococcus* [98]. Mevalonic acid has been isolated from culture of lactobacilli and other microorganisms [137, 138]. β-Hydroxydecanoic fatty acid occur in *E.Coli*[32], *Serratia species* [33], and various species of *Pseudomanaspyocyanea*[99].

### *d. Cyclopropane fatty acid*

Cyclopropane fatty acid is unique in its chemical structure by having a cyclopropane in fatty acid chain [25]. Earlier it was believed that only  $C_{19}$  cyclopropane occurred in bacteria. This belief was based on the fact that no other such acid had been encountered in all bacteria that had been studied [10]. However, after frequent use of more sensitive techniques and apparatus has shown that any cyclopropane does occur in bacteria. Most probably  $C_{17}$  fatty acid found in the lipids of  $E$ . *coli*in 1959 [38] might be the first cyclopropane to be isolated and studied successfully. However, Hofmann and Lucas [45] first reported the presence of cyclopropane fatty acid as cis-11, 12- methylene – octadecanoic acid [100, 101, 102, and 103]. Thereafter, Dauchy and Asselineau [44] showed that such acid was indeed present in *E. coli*bacteria. The location of the ring the fatty acid chain is still under study. Most of this fatty acid is found and studied in gram negative bacteria. It is studied in very few in gram negative bacteria i.e. *E. coli, P. pestis* and in *A. tumefaciens* [32, 38, 36 and 15] as compared with gram positive bacteria. It is not yet determined whether this acid occurs naturally as D or L form of isomer. Lactobacillus acid has been identified in *Latobacillusarabinosus*[36, 43], *Lactobacillus casei*[36, 105], *lactobacillus delbrueckii*[36], and *Agrobacterium tumefeciens* [36, 37]. The possible occurrence of shorter cyclopropane fatty acid having a carbon number C and C has also been reported in *C. butyricum*[39,104].

#### *e. Unsaturated fatty acid*

Unsaturated fatty acid constitutes the major portion of the bacteria in all cell bacteria. Most of this fatty acid is under C18. Very few literatures are successfully studied and published on this unsaturated fatty acid. Unsaturated fatty acid like octadecenoic acid commonly known as mono-saturated C<sup>18</sup> fatty acid "oleic acid" was isolated in the year of 1952 from *L. arabinosus*[43].It was reported in same paper that this octadecenoic acid is of two different types. These are cis-11-octadecenoic and cis-9-octadecenoic. Octadecenoic acid of *L. arbinosus*was actually in cis- vacccenic acid (i.e, cis-11 octadecenoic). Oleic acid is cis-9-octadecenoic acid [10]. The unsaturated fatty acid called cis-vaccinesaresole octadecenoic acids that present in *L. casei*[105] and in *A. tumefeciens* [37]. According to the study of Hofmann and Tausig [41], major octadecenoic acid present in the

bacteria is cis- vessenic accompanied by small amount of oleic acid. Octadecenoic acid present in *E. coli* as cisvecenic acid was reported by Law [32]. Unsaturated fatty acid having 16 carbons and less has also been reported by Asano and Takahasi [22] in *C. diphtheria*  and  $C_{10}$  and  $C_{12}$  unsaturated fatty acid was reported to be present in *Brucellasuis* by Gubarev et al. [106]. Hexadecenoic acid was reported to be found in *streptococci species* [41]. Alimova [21] reported the presence of unsaturated fatty acid  $C_{18}$  and  $C_{20}$  in the corynebacteria. Later they have reported the presence of unsaturated fatty acid  $C_{21-28}$  [21, 22].

### **3. Fatty acids spectra of some gram negative bacteria**

Fatty acid spectra usually are collection of all fatty acids information present in the particular microorganism [35]. At the present time there are not enough fatty spectra has been made. Here, I have referred some thorough fully characterized in this respect are *Thiobacillusthiooxidans*[10], *Agrobacterium tumifacian*[40, 37], *Escherichia coli*[38], *Clostridium butyricum*[15], *Streptococcus species*[36, 41], *Lactobacillus debruekii*[15], *Lactobacillus casei*[36, 71], and *Lactobacillus arabinosus*[15, 43]. Compare to information availability lipids have been somewhat less intensively studied in other gram negative bacteria as comparative to gram positive bacteria. These gram negative bacteria are easily found in different growth conditions in nature i.e. in terrestrial ecosystems.

The fatty acid composition of these organisms is shown in **Table 2**. But, before this table I would like to show the Fatty acidspectra of all common Grams negative is shown in following table [10].

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**Table 1**.*Fatty acid of common gram negative bacteria*



### **Table 1***.–***(***Continued***)**

## **4. Comparative fatty acid spectra of few representative gram negative bacteria**

As I mentioned earlier, I have been referring only few gram negative bacteria, although the identification and isolation of fatty acids has been done successfully on many interesting bacteria. Reason behind this is because, very few bacterial spectra has full information about the fatty acids. In case of gram negative bacterial spectra it is even lesser than gram positive's fatty acids spectral. For example, the gram negative bacterial which have been most thoroughly characterized in this respect are *Escherichia coli* [44, 32, 38], *Thiobacillus*species [46],

*Agrobacterium tumefacience, Streptococcus species* [62, 72], *Camonas species* [17], *Shewanellaputrefaciens* [48], *Cyclobacteriummarinus* [49], and *Shigellaflexneri* [50]. The fatty acid composition of these gram negative bacteria is shown in **Table 2**. There are many bacteria whose fatty acid study has been less intensively studied and according to me they have not given considerable information about the fatty acids. Here in **Table 2**, I have taken the quantitative measure as the center point regardless of their qualitative nature. The qualitative nature of growth media of bacteria often regulates the

physiology of bacteria and may also cause the quantity of its components like fatty acid itself [45]. **Table 2***. Fatty acid of some gram negative bacteria*



 $+<sup>d</sup>$  = Present but amount is either less than 1% or not determined.

 $c = Cyclopropane acid$ .

**International Journal of Pharmaceutical, Biological and Chemical Sciences (IJPBCS) | APR-JUN 2015 |VOLUME 4 | ISSUE 2 | 01-19 | www.ijpbcs.net**

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### **4. Influence of bacteria to terrestrial ecosystem**

### a. *Escherichia coli*

*Escherichia coli*, commonly abbreviated as *E. coli* is a gram negative, facultative anaerobic, rod – shaped bacteria [51]. In terrestrial ecosystems, it is usually found in tropical soil having normal pH 5.8 -7 [52], temperature of 23  $^0C - 25$   $^0C$ . It is reported [52] that

when simple nutrients (Glucose and salts) were added to natural soil *E .coli* was shown to immediately increase by about three logs in its growth within 24 hours. The influence of the *E .coli* to habitat in tropical soil has not been fully studied. However, other related studies have been reported in many papers [38,53].



**Fig 3**.Scanning electron microscopy image and cartoon illustration of *E. coli* bacteria.

### b. *Agrobacterium tumefaciens*

*Agrobacterium tumefaciens*is a gram negative rod shaped, flagellated, soil bacteria [54] that causes crown gal tumorsat wound sites of infected dicotyledonous plants which is characterized by a growth of tumor or gall on the infected plant, often the junction between the root and the shoot. [55].*Agrobacterium tumefaciens* is

reported to be found in normal soil of neutral pH 7 [56].The influence of bacteria *Agrobacterium tumefaciens*to soil has not been fully understood. However, its important influence towards the agriculture biotechnology and its nature of pathogenicity is well described in many papers and references [54, 57]

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**Fig 4**. Scanning electron microscopic image and cartoon illustrated image of *Agrobacterium tumefaciens*[*International society of microbial cology*]

### c. *Camonas*species

The *camonas*species is a strickly aerobic straight Gram – negative rod shapedpolarly flagellated bacteria [58, 59, and 60]. There are many types of *Camonas* species like *Camonaskoreensis, Camonastestosternai and Camonasacidovorans* but all of them share similar physical structure. These bacteria have attracted many researchers to study its influence to the terrestrial ecosystem and to the human health. Some paper has reported *Camonas*species to be opportunistic bacteriafor both plant and human [59, 61]. The genus *Camonas*

includes many species [28, 34, 36, {411}] are reported to be successfully degraded the aromatic compounds and are considered to be an important species in biodegradation of toxic waste. The typical habitats of these species are in fresh water and marine habitat with low nutrient levels [58] but, some species like *Comanastestosternai* have been reported to be non fermentative,chemoorgano–trophic bacteria that rarely attack sugars and well grow on organic acids and amino acids [62].

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Fig 5. Scanning electron microscopic (SEM) image of *Casmanas* species (A) Colony of *Camonas* species [111] (B) Single polar flagelled*Camonas*species [222]

### d. *Shewanellaputrefaciens*

*Shewanellaputrefaciens*are facultative anaerobic, gram negative, flagellated chemolithotrophicbacteria [63]. The natural habitat is usually in ground waste water, but, it is also found in ground soil,and in river [64]. The properties of being able to reduce metal by

*Shewanella*species is reported [63, 64, 65, 66, 67 and 68]. *Shewanellaputrefaciens*are typical electro activechemolithotrophic bacteria [70], which can adsorb and transforms a range of metal ions [71, 72- 73] trough interaction of metal ions with outer membrane lipids [74, 75].



**Fig 6**.Scanning electron microscopic image of *Shewanellaputrefaciens*. (A) Colony of *Shewanellaputrefaciens*[112] (B) Single flagellated*Shewanellaputrefaciens* bacterium[www.x-divers.ru/aricles/technologies/36/full.html]

The *Shewanella*species have a very interesting ability for human welfare i. e known as bio electrochemical system [76, 77]. The bio electrochemical system is able of microorganism to create an electrochemical potential by transferring electron to extracellular electron acceptor [78-79]. *Shewanella*species in terrestrial ecosystem have a great deal of interest in their application for recalcitrant wastewater remediation using microbial fuel cells and microbial electrolysis cell [80] by reducing metal like gold, cobalt and ferrous to its corresponding anions. The process it follows to complete bio electrochemical is by using electrode as electron donors or electron donors for

respiration, electron transfer of microbial cells, coupled with the reduction of pollutant leading to their reduced mobility and sequestration [63]. Furthermore, this species can live at extreme pH up to 2 and interestingly inorganic contaminants act as electron acceptors while in its bio electrochemical system [71, 81].*Shewanella* species are reported to be pathogenic both for fish and human health [80] other than bio electrochemical system.

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### *e.Cyclobcteriummarinus*

*Cyclobacteriummarinas*are gram negative, Oxidative chemoorganotrophic bacteria, mostly found in soil, marine water and in oil [83, 84]. The *Cyclobacterium*species have a very unique ring like and mostly non - flagellated [811]. Studies have reported *Cyclobacterium*species to be growth on red algae and other marine life [86]. *C. marinus*required NaCl for

growth and variety of organic compound in seawater for its growth [85]. The influence of these bacteria like pathogeneticity, the property of being able to reduce metal and other influences on terrestrial system is not clearly understood yet. The lipid composition of the *Cyclobacterium*has been studied thoroughly and reported to be having mostly polar lipidsi.e. glycolipid type.



**Fig 7.***Cyclobacterium* species SEM, scanning electron microgaraph image (A) Scanning electron micrograph image of *Cyclobacteriummarinus*[109] (B) Transmission electron microscopy image of*cyclobacteriummarinus*[110]

### e. *Shigellaflexneri*

*Shigellaflexneri*is a rod shaped,lactose fermenting, nonspore forming, non-motile, facultative anaerobic Gram negative bacteria [88]. Naturally *Shigellaflexneri*are found in a low pH environment,  $37 \text{ }^{\circ}\text{C}$  of temperature [87]. The required conditions not easy to find in terrestrial ecosystems hence, the *ShigellaFlexneri*israrely

found in soil. *Shigellaflexneri* are found in the human gastrointestinal tract, which seems to be fulfilling the require environment for its growth [87]. The influence of *Shigellaflexneri* to terrestrial ecosystem is not clearly studied, but, its influence of the human health has been studied far enough.



Fig 8.Transmission electron microscopic image of *Shigellaflexneri* [<sup>©</sup>Dennis Kunkel Microscopy, Inc]

The *Shigella* species have been reported to be pathogenic for human health like *Shigelladysenterriae*, one of four *Shigella species*, that causes classic bacillary dysentery, which is most severe form of shigellosis ( an enterobacterium disease) [89]. The same paper reported that *Shigellaflexneri*and *Shigellaboydii*can cause mild or

severe infections. It is further need to study on *Shigella* species and its interaction with the terrestrial ecosystem.

### f. *Thiobacillus*species

Most of the *Thiobacillus*species share same basic physical property. *Thiobacillus species* have been reported to be very important gram negative bacteria that present mostly in extreme environments like in low pH environments [90].*Thiobacillus species* have high economic importance in bioleaching which helps in the recovery of the lost minerals like ferrous, suphate and suphur. These bacteria usually use in leaching out of acid drainage situation. These bacteria use in the extraction of metals from ores [90]. The extraction of minerals from the ores by means of bioleaching is more environmental friendly [91]. There are different bioleaching techniques to extract ores from different source like hydrometallurgical for extraction of metal from solid

ores. *Thiobacillus species* usually attach to the surface of ore during the bioleaching[92]. During mineral extraction by the use of *Acidithiobacillusferroxidans* formation of crystal boundaries around ore restrict the attachment of bacteria often [93]. Later the formation of the biofilm takes place. The Layer is formed as continuous extracellular polymeric substance in a few weeks after the initial attachment of cells and the extraction of minerals is done by bioleaching. These extracellular polymeric substances are the LPS which consist of fatty acids **[**94, 95, and 107**]**.



Fig 9. (A) Scanning electron microscopic image of *Thiobacillusferrooxidans*[<sup>(c)</sup> [Dennis Kunkel Microscopy, Inc\]](http://www.denniskunkel.com/) (B) Highly magnified microscopic image of *Thiobacillusferrooxidans* [109].

### **4. CONCLUDING REMARKS**

The study of membrane fatty acids has become one of the important fields in research of membrane lipids. Habitate of gram negative bacteria and its ability to show various characteristic like chemolithotrophic, chemoorganotrophic, pathogenicity, autotrophic and symbiotic has influence some researcher to do study details about the mechanism of the bacteria to performed these property and bioproduct that often involved in the mechanism. Most of the time membrane lipids have been reported to be involved in the reaction, protection and interaction with the specific substance or host of the gram negative bacteria. The fatty acids study of the gram negative bacteria is present in varying concentration and in varying types according to its corresponding bacteria. Different bacteria having little similarity in its fatty acid profile show almost similar characteristic and show similar influence to the terrestrial ecosystem for example:

*Shewanellaputrefaciens, shigellaflexneri, camonas* species *and thiobacllus species* share similarity in having unsaturated fatty acid and cyclopropane fatty acids all of them can be grown in low pH environment.*Shewanellaputerfaciens* and *Thiobacillus* species also share similarities in saturated fatty acids, thus, possibly both of them share similar properties of reducing and oxidizing the metals.

Among the gram bacterial fatty acids profile *Thiobacillus*species have more types of fatty acids and it is followed by Shigella*putrefacien*and Escherichia coli and *Agrobacterium tumefaciens*. *E.coli*, *Agrobacterium tumefacies*and *Camonas* species share similar fatty acid profiles of saturated and unsaturated fatty acids. *Camonas*species havea minimum fatty acid type, it shows highest tolerant to the salt water. Some of these bacteria have been reported been reported to be pathogenic to human, marine life and some have been reported to be pathogenic to plant. Hence, there is a lacuna in understanding the specific fatty acid functions in contributions of the bacterial physiological action on its environment. The study of various fatty acids distribution in the gram negative bacteria has revealed the presence of varying types of fatty acids in different gram negative bacteria but, the major accomplishment in the study of fatty acid and its influence to the variousecosystems are still to be reported.

### **Acknowledgement**

We would like to acknowledge the funding and support for the project "Differential membrane lipid profile and fluidity of *Acidithiobacillus ferroxidans* during the process of adhesion to minerals" under SERB Scheme, DST- with the reference of DO No. SR/S3/ME/0025/2010. The author wants to thank Dr. Preston Devasia for his support in manuscript discussion and improvement

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