



<https://bjm.ui.ac.ir/?lang=en>

Journal of Microbial Biology  
E-ISSN: 3060-7647  
14rd Year, Vol. 14, No. 56, 2025 pp. 1-14  
Received: 26/07/2025 Accepted: 04/10/2025

(Research Paper)

## Fatty Acids metabolism in Stress Condition by Halotolerant *Bacillus*: Extraction and Application

**Zahra Fathi Hassanabadi**

Department of Microbiology, Faculty of Biological Sciences and Technology, Shahid Ashrafi Esfahani University, Isfahan, Iran

[zahra235fathi@gmail.com](mailto:zahra235fathi@gmail.com)

**Maryam Jalili Tabaii**

Department of Microbiology, Faculty of Biological Sciences and Technology, Shahid Ashrafi Esfahani University, Isfahan, Iran

[maryjtabaii@gmail.com](mailto:maryjtabaii@gmail.com)

**Shekoofeh Sadat Etemadzadeh<sup>1</sup>** 

Department of Biology, Faculty of Science, University of Jiroft, Jiroft, Iran

[sh.etemadzadeh@ujiroft.ac.ir](mailto:sh.etemadzadeh@ujiroft.ac.ir)

**Giti Emtiazi**

Department of Cellular and Molecular Biology and Microbiology, Faculty of Biological Science and Technology, University of Isfahan, Isfahan, Iran

[emtiazigiti@gmail.com](mailto:emtiazigiti@gmail.com)

### Abstract

Halotolerant *Bacillus* species, like other bacteria, adapt to stress conditions by modifying their fatty acid profiles to maintain membrane integrity. In this study, the fatty acid profiles of *Bacillus halotolerans* were investigated under stress conditions induced by lead (0.1% Pb), glycerol (0.1%), sodium chloride (0.1% NaCl), silver (0.05 ppm), and iron (0.1 ppm) nanoparticles. Fourier Transform Infrared and Gas Chromatography/Mass Spectrometry were employed to characterize the metabolic responses and identify the produced fatty acids. Among the applied stress conditions, the lead-containing environment exhibited the highest level of fatty acid production (71.5 mg/L) and diversity. Also, different kinds of fatty acids with different applications were detected like omega-3 ( $\alpha$ -Linolenic acid and Eicosapentaenoic acid), omega-6 ( $\gamma$ -Linolenic acid and Linoleic acid), myristoleic acid, palmitic acid, stearic acid, cis-hexadecenoic, cis-octadecenoic acid, and so on. These compounds are applicable in the pharmaceutical, medical, cosmetic, and food industries, whereas other fatty acids produced, such as biodiesel and its derivatives, also serve a wide range of purposes.

**Keywords:** *Bacillus halotolerans*, Fatty acid, FTIR, GC/MS analysis, Stress condition, Lead contamination.

---

<sup>1</sup> Corresponding Author

[3060-7647/](https://doi.org/10.22108/BJM.2025.146090.1643) © 2025 The Authors



## 1. Introduction

Microbial acclimatization to environmental stressors, such as salinity, has been the subject of intensive research because of its biotechnological and industrial significance (1). Halotolerant microbes such as, *Bacillus* spp., are defined by their metabolic flexibility, which improves survival under osmotic stress through dynamic adjustments to their lipid profiles. Fatty acids, which are structural components of bacterial membranes, are key regulators of membrane fluidity, permeability, and stability during changes in osmotic conditions (2). In halotolerant *Bacillus* sp., changes in fatty acid metabolism, including the synthesis of unsaturated and branched-chain fatty acids, are one of the most crucial adaptation mechanisms to avoid the harmful effects of high salt concentrations (2, 3).

Biotechnological applications of bacterial fatty acids extend beyond microbial physiology to include biosurfactant synthesis, biodegradable plastics, biofuels, and the pharmaceutical industry. For example, lipopeptides and glycolipids of *Bacillus* spp. have excellent emulsifying and antimicrobial activities and find applications in bioremediation and food packaging. Microbial fatty acids are renewable alternatives to petroleum-derived chemicals and satisfy the increasing demand for green chemistry products (4-6).

Fossil fuels, such as petroleum, coal, and natural gas, are currently the dominant sources of global energy consumption, although their ability to meet future demand is increasingly uncertain. Excessive use of these resources is also associated with environmental pollution, increased greenhouse gas emissions, and global warming. Therefore, policymakers and scientists are seeking alternative and renewable energy sources, such as biodiesel (4, 7, 8). Biodiesels are methyl esters of fatty acids from renewable resources, mainly produced from vegetable and plant oils, such as soybean, rapeseed, sunflower, *Jatropha*, and palm oils, along with animal fats and waste cooking oils. However, the limited quantities of these conventional feedstocks can only deliver a small fraction of the existing demand (7, 9). Therefore, alternative sources should have the following characteristics: less environmental pollution, economic efficiency,

meeting energy needs and non-interference with food production (7).

Microorganisms are a good option, and microbial oils have many advantages over plant oils and animal fats, such as shorter cycles, less labor required, less space required, no dependence on season and climate, ease of scale-up, and ease of genetic engineering (7, 10, 11). Microbial lipids produced by oleaginous microorganisms including microalgae, *Bacillus* bacteria, fungi and yeasts (7, 9, 12). Oleaginous microorganisms can produce between 20% and up to 80% lipid per dry cell weight in the stationary growth phase, depending on species, culture conditions, etc. (9, 10, 13). The oil content may be influenced by medium composition, nitrogen source, carbon source, C/N ratio, temperature, agitation, pH, NaCl concentration and other additives to the culture medium (7).

Currently, yeasts and algae have been studied more due to their high cellular lipid contents, while only a small number of bacteria can accumulate significant amounts of lipids. Bacteria usually produce lipids in the plasma membrane and as a result have a lipid content of less than 20% of dry weight (12). Some bacterial species, such as *Mycobacterium*, *Streptomyces*, *Rhodococcus*, *Nocardia*, *Pseudomonas*, and *Bacillus*, are prone to produce fatty acids (7, 12). *Bacillus* species, which are widely distributed in the environment, are the largest and most diverse genus of endospore-producing aerobic bacteria and play an important role in industrial processes (14).

The major component of oleaginous microorganisms is triacylglycerol (TAG), composed of C16 and C18 (4); however, some of them may contain polyunsaturated fatty acids (PUFAs), specifically omega-3 fatty acids, which are linked to human health (15, 16). The three main omega-3 fatty acids are alpha-linolenic acid (ALA) 18:3 (n-3), docosahexaenoic acid (DHA) 22:6 (n-3), and eicosapentaenoic acid (EPA) 20:5 (n-3), which are mainly obtained from fish and shellfish (16, 17), but bacterial production of these acids is a promising alternative approach for large-scale production (17).

Despite these developments, the exact regulatory mechanisms of fatty acid metabolism in halotolerant *Bacillus* under stress conditions remain partly unresolved. In addition, optimized extraction procedures are required to enhance the yield and purity for large-scale industrial applications. The objectives of this research were as follows: (1) to investigate the production of fatty acids by *Bacillus halotolerans*, a halotolerant and heavy metal-resistant bacterium, in different culture media and select the most suitable medium (2) to establish an effective protocol for extraction, and (3) to study the type of fatty acids produced in each medium using Fourier Transform Infrared and Gas Chromatography/Mass Spectrometry analysis to assess their industrial and biotechnological potential.

## 2. Materials and Methods

### 2.1. Bacterial Strain

The halotolerant and heavy metal-resistant bacterium *Bacillus halotolerans* strain SCM 034 (GenBank accession number: MT810037), which was previously isolated from a soil sample from Kerman, Iran, was studied for lipid production in this experiment. The strain was grown on nutrient agar (N.A; Ibersco, Iran) plates at 30 °C for 24 h. It can also grow at more than 12% NaCl and in the presence of some metals (7 mM Pb, 7 mM Ca, 5 mM Cr, 3 mM Ni, Mn, and Cu) (3).

### 2.2. Culture Conditions for Lipid Production and Extraction

For lipid production by *B. halotolerans*, 200 mL of nutrient broth (N.B; Ibersco, Iran) medium in Erlenmeyer flasks (500 mL) was prepared and autoclaved (121 °C for 15 min). A 10% v/v inoculum (20 mL of freshly cultured overnight bacterial suspension into 200 mL of culture medium), standardized to 0.5 McFarland (approximately  $1.5 \times 10^8$  CFU/mL;  $OD_{620} \approx 0.08-0.1$ ), was added and incubated on an orbital shaker (170 rpm) at 30 °C for 24 h (3).

In order to extract the lipid produced, the bacterial cells were first disrupted with 6N hydrochloric acid (The pH was adjusted to 2.0) (9). After storing the

sample overnight at 4 °C, centrifugation (3000 rpm, 20 min) was performed and the precipitates were collected. In the next step, chloroform/methanol (2:1, v/v) was added for lipid extraction, and after 20 min shaking and 10 min centrifuging (3000 rpm), the chloroform phase (the lower phase) was collected and dried overnight at room temperature (18, 19).

### 2.3. Selection the Appropriate Medium Supplementation for Maximum Bacterial Growth and lipid Production

In this study, the effects of some compounds in the culture medium, such as lead heavy metal (20), glycerol (21), NaCl (22), silver, and iron nanoparticles (23), were examined on bacterial growth and lipid production. So, N.B media (200 mL) were prepared and lead (0.1%), glycerol (0.1%), NaCl (0.1 %), silver nanoparticles (Silver (Ag) Nano powder, Pishgaman Nano Material Iranian Company (Ag, 99.99%, 20 nm, metal basis)) (0.05 ppm), and iron nanoparticles (Iron Oxide Fe<sub>3</sub>O<sub>4</sub> Nano powder, Pishgaman Nano Material Iranian Company (Fe<sub>3</sub>O<sub>4</sub>, 98+%, 20-30 nm)) (0.1 ppm) were added to each of them (A N.B medium was considered as a control). Then, 10% of the overnight bacterial culture (0.5 McFarland) was inoculated, and the media were incubated for 36 h (170 rpm at 30 °C). During incubation, growth curves were drawn (by recording the absorbance at 620 nm using a spectrophotometer) to investigate the amount of bacterial growth in different media. In another experiment, the lipids produced (after 24 h) were extracted and weighed for each medium.

### 2.4. Fourier Transform Infrared (FTIR) and Gas Chromatography/Mass Spectrometry (GC/MS) analysis of Lipid Produced

N.B containing 0.1% lead, N.B containing 0.1% glycerol, and N.B medium were selected because of the highest bacterial growth and fat production. Therefore, they were analyzed for the structure and type of fat.

First, the dry weight of the cell biomass ( $DW_B$ ) and dry weight of the lipid ( $DW_L$ ) were measured for

200 mL of each medium to calculate the lipid content ( $C_L$ ) (12).

$$C_L (\%) = [DW_L (\text{mg}) / DW_B (\text{mg})] \times 100$$

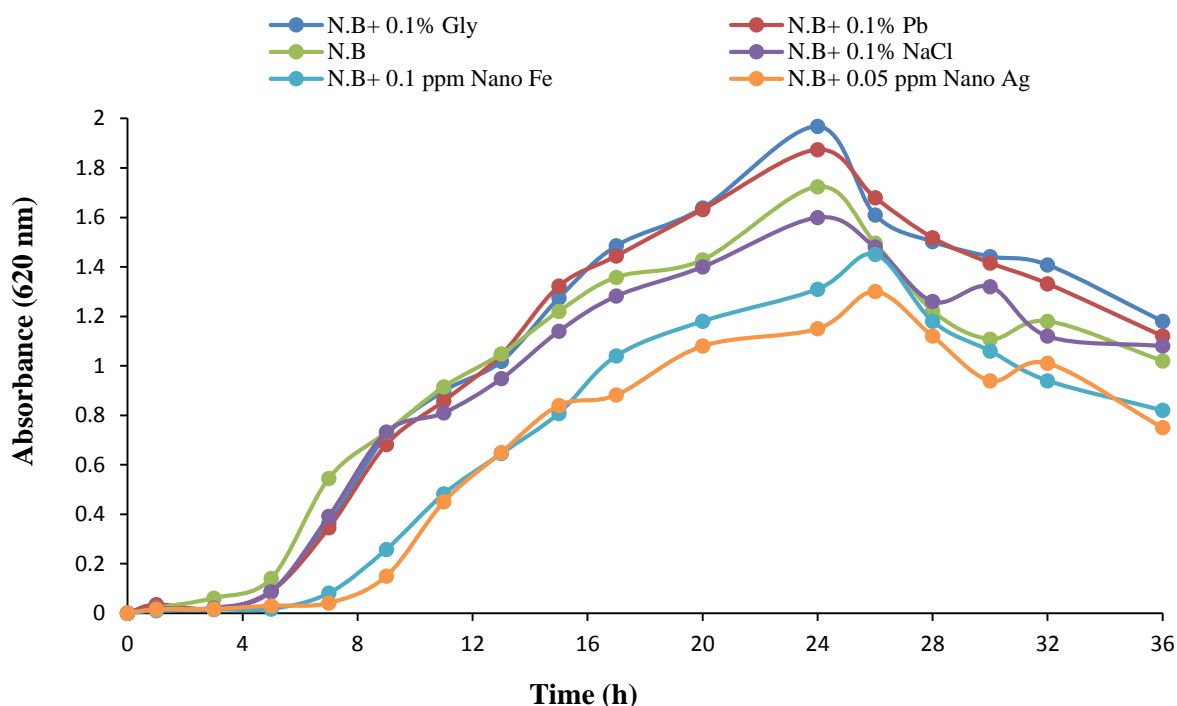
The chemical properties of the extracted lipids were evaluated using FTIR spectroscopy (JASCO, FT/IR-6300, Japan). For FTIR measurements, approximately 2–5 mg of dried lipid powder was used per sample. The powder was directly applied to the crystal surface of the FTIR spectrometer (ZnSe crystal) without any chemical pretreatment or mixing with KBr, as the sample was sufficiently pure and homogeneous.

In addition, GC/MS analysis was performed to identify the lipid components. 1  $\mu\text{L}$  of lipids dissolved in n-hexane was injected into the GC/MS analyzer (Agilent ISIRI 13126-2 Gas Chromatograph, capillary column HP 88, initial temperature of 100 °C and detector temperature of 300 °C).

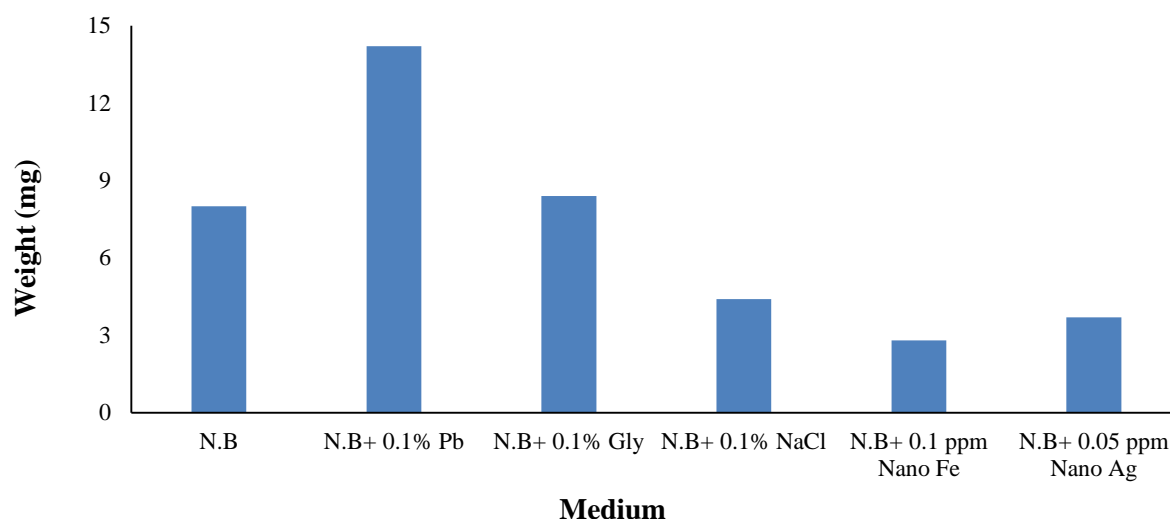
### 3. Results

Lipid extraction from the bacterial culture was performed using chloroform/methanol (a protocol for lipid extraction), and after drying the chloroform phase, its weight was measured.

In this study, the effects of various factors on bacterial growth and lipid production were investigated. As shown in Figure 1, the maximum bacterial growth was observed in N.B containing 0.1% glycerol, N.B containing 0.1% lead, N.B, N.B containing 0.1% NaCl, and finally N.B containing nanoparticles. Nevertheless, the maximum lipid production was in N.B containing lead, N.B containing glycerol, N.B, N.B containing NaCl, and N.B containing nanoparticles, respectively (Figure 2).



**Figure 1.** Growth curves of *B. halotolerans* in different media during 36 h. The maximum growth was in N.B containing 0.1% glycerol.



**Figure 2.** Dry weight of extracted lipid from 200 mL of different media after 24 h incubation. The maximum lipid production was in N.B containing 0.1% lead.

**Table 1.** The amount of dry weight of cell biomass and extracted lipid from 200 ml of different media after 24 h incubation.

Media composition (200 ml)	Dry weight of cell biomass (mg)	Dry weight of lipid (mg)
N.B	186	8.1
N.B+ 0.1% Gly	221	8.4
N.B+ 0.1% Pb	198	14.3

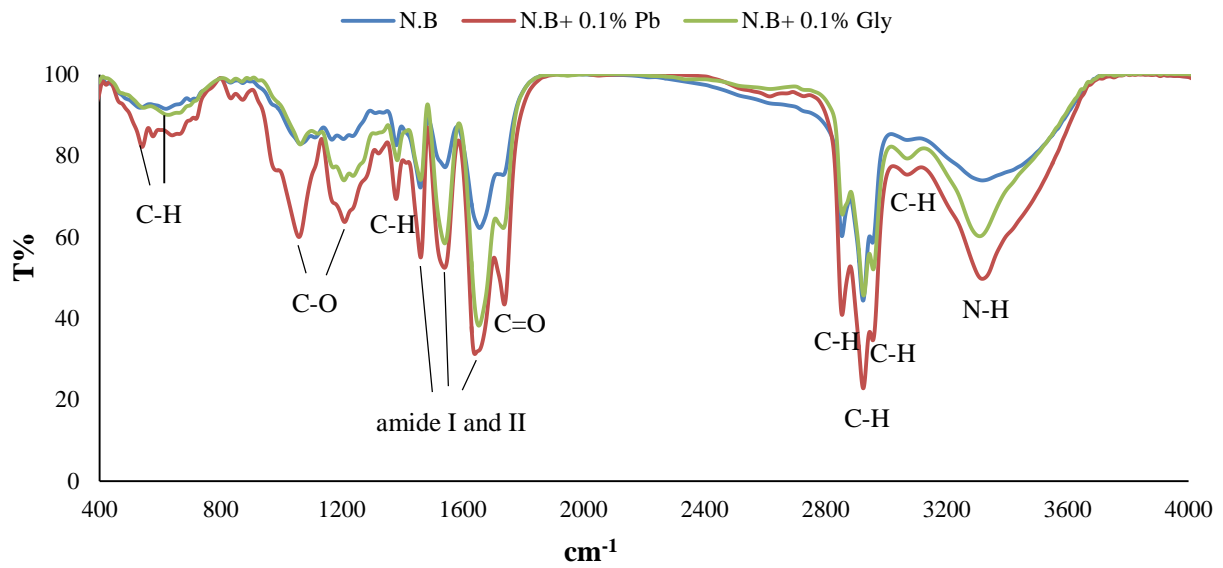
The dry weight of the cell biomass and the dry weight of the lipid were measured for N.B containing lead, N.B containing glycerol, and N.B (Table 1). According to the results, the maximum cell growth was observed in N.B containing glycerol, while the maximum lipid production was observed in N.B containing lead, which corresponds to the results of the previous step. In addition, the lipid contents of N.B containing lead, N.B containing glycerol, and N.B were 7.22%, 3.8%, and 4.35%, respectively, so, the maximum  $C_L$  produced was 7.22%, which was obtained from lead containing medium. Notably, the presence of lead resulted in a ~86% increase in lipid accumulation compared to the control (without additives), indicating a strong stimulatory effect of lead on lipid biosynthesis under the tested conditions.

The results of FTIR analysis showed relatively similar functional groups in the three extracted lipids, and only a few new peaks were created in the spectrum of lipids extracted from lead and glycerol media (Figure 3). The majority of peaks

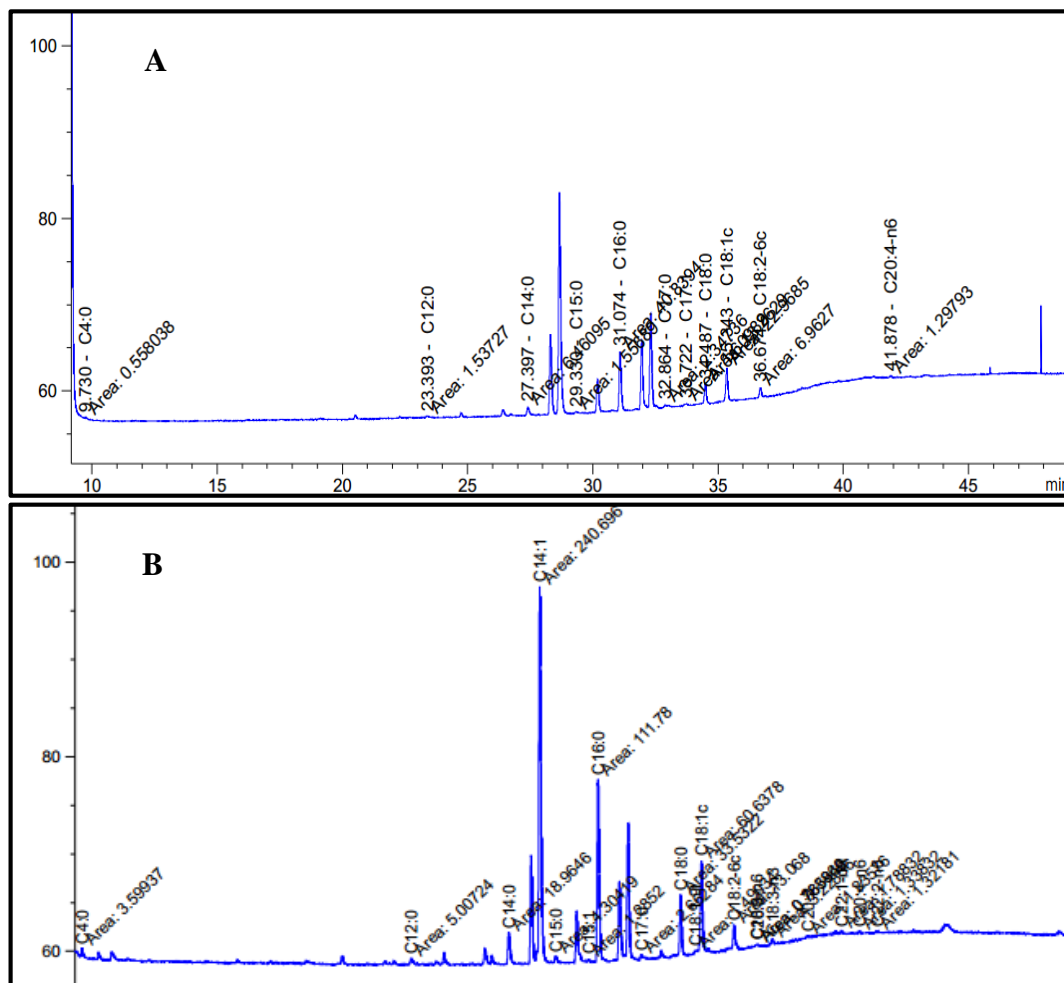
confirmed the presence of lipids in the structure, including C-H groups, which was inferred by the peaks recorded at approximately  $619.038\text{ cm}^{-1}$  (24, 25),  $1380.78\text{ cm}^{-1}$  (26),  $2855.1\text{ cm}^{-1}$  (27, 28),  $2924.52\text{ cm}^{-1}$  (29),  $2955.38\text{ cm}^{-1}$  (30), and  $3069.16\text{ cm}^{-1}$  (31). The C-O group was derived from the peak at  $1063.55\text{ cm}^{-1}$  (32, 33), while the peaks at approximately  $1460.81\text{ cm}^{-1}$  (34),  $1540.85\text{ cm}^{-1}$  (35), and  $1653.66\text{ cm}^{-1}$  (36) indicated amide I and amide II groups. Peaks at approximately  $1735.62\text{ cm}^{-1}$  (37, 38) and  $3318.89\text{ cm}^{-1}$  (39, 40) were assigned to the C=O (carbonyl) and N-H groups, respectively, in proteins. In addition, the peak at approximately  $1207.22\text{ cm}^{-1}$  (41) (C-O group) in the spectra of lead and glycerol and the peaks at approximately  $540\text{ cm}^{-1}$  (42) and  $868.77\text{ cm}^{-1}$  (43) (C-H group) in the lead spectrum indicated the presence of lipids in the extract structure.

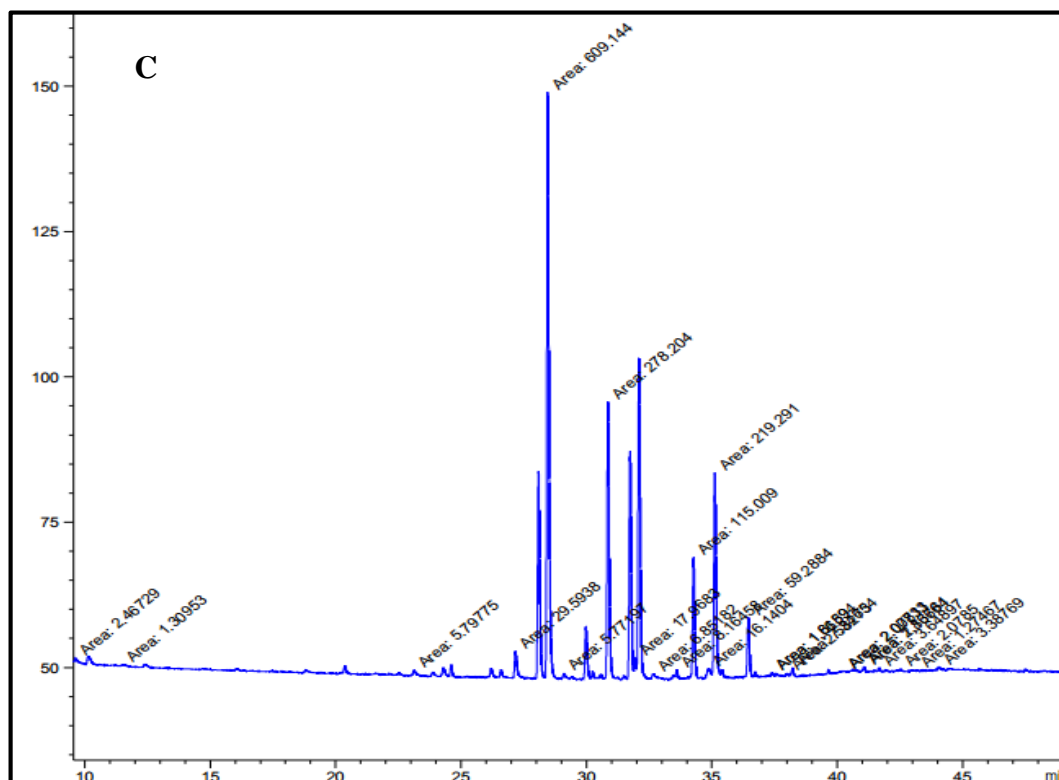
A few minor peaks appeared in the spectra of the Pb- and glycerol-treated samples, but they were weak and lacked sufficient intensity to be

considered characteristic or functionally significant.



**Figure 3.** Fourier transform infrared (FTIR) spectrum of the extracted lipids from N.B containing lead, N.B containing glycerol and N.B media.





**Figure 4.** GC/MS analysis of the extracted lipids, A) from N.B medium, B) from medium containing glycerol, C) from medium containing lead.

The GC/MS results for recognizing the lipid components showed some differences in the presence and quantity of compounds (Figure 4). The lowest diversity of fatty acids (C4-C20), especially unsaturated and branched fatty acids, was observed in N.B medium (Table 2A). C16 and C18 were the major compounds found in this extracted lipid.

In the medium containing glycerol, the production of fatty acids (C4- C22) as well as unsaturated fatty acids was greater (Table 2B),

and the major compounds were C14 and C16, respectively.

Regarding to, the maximum variety of fatty acids was related to lead-containing medium, and C4 to C24 fatty acids were produced. Also, the production of unsaturated and branched fatty acids was significantly higher, and omega-3 ( $\alpha$ -Linolenic acid and Eicosapentaenoic acid) and omega-6 ( $\gamma$ -Linolenic acid and Linoleic acid) essential fatty acids were also detected (Table 2C). The main lipid components extracted from this medium were C14, C16 and, C18, respectively.

**Table 2.** The component of the extracted lipids by GC/MS analysis, A) N.B medium, B) medium containing glycerol, C) medium containing lead.

**A**

Fatty acid	Formula
Butyric acid	C4:0
Lauric acid	C12:0
Myristic acid	C14:0
Pentadecanoic acid	C15:0
Palmitic acid	C16:0
Heptadecanoic acid	C17:0
cis-10-heptadecenoic acid	C17:1 n-7
Stearic acid	C18:0
cis-9-oleic acid	C18:1 n-9
Linoleic acid	C18:2 n-6
cis-5,8,11,14-Eicosatetraenoic acid(Arachidic acid)	C20:4 n-6

**B**

Fatty acid	Formula
Butyric acid	C4:0
Lauric acid	C12:0
Myristic acid	C14:0
Myristoleic acid ester	C14:1
Pentadecanoic acid	C15:0
Cis-10-Pentadecanoic acid	C15:1
Palmitic acid	C16:0
Heptadecanoic acid	C17:0
Stearic acid	C18:0
Trans -9-Elaidic acid	C18:1 n-9
cis-9-oleic acid	C18:1 n-9
Linoleic acid	C18:2 n-6
Arachidic acid	C20:0
$\alpha$ -Linolenic acid	C18:3
cis-11,14-Eicosadienoic acid	C20:2 n-6
Erucic acid(uresic acid)	C22:1 n-9
cis-5,8,11,14,-Eicosatetraenoic acid(Arachidic acid)	C20:4 n-6
cis-13,16-Docosadienoic acid	C22:2 n-6

**C**

Fatty acid	Formula
Butyric acid	C4:0
Hexanoic acid	C6:0
Lauric acid	C12:0
Myristic acid	C14:0
Myristoleic acid ester	C14:1
Pentadecanoic acid	C15:0
Palmitic acid	C16:0
Palmitoleic acid	C16:1

Heptadecanoic acid	C17:0
cis-10-heptadecenoic acid	C17:1 n-1
Stearic acid	C:18
Trans -9-Elaidic acid	C18:1 n-9
cis-9-oleic acid	C18:1 n-9
Linoleic acid	C18:2 n-6
Arachidic acid	C20:0
$\gamma$ -linolenic acid	C18:3 n-6
$\alpha$ -Linolenic acid	C18:3
Linolenic(gondoic acid)	C20:1 n-9
Behenic acid	C22:0
8,11,14-Eicosatrienoic acid(Dihomo- $\gamma$ -linolenic acid)	C20:3
11,14,17-Eicosatrienoic acid	C20:3 n-3
Tricosanoic acid	C23:0
cis-5,8,11,14-Eicosatetraenoic acid(Arachidic acid)	C20:4 n-6
Lignoceric acid	C24:0
cis-5,8,11,14,17-Eicosapentaenoic acid	C20:5 n-3
Nervonic acid	C24:1 n-9

#### 4. Discussion

Fatty acids are organic compounds that are composed of a carboxylic acid group and long aliphatic chains. These molecules typically contain 14 to 24 carbon atoms and can be straight or branched, saturated, monounsaturated (MUFAs), or polyunsaturated (PUFAs) (44, 45). Fatty acids, mainly essential fatty acids (such as  $\omega$ -3 PUFAs and  $\omega$ -6 PUFAs), are important not only in nutritional systems but also for promoting public health (46, 47). MUFAs and PUFAs have been widely used as pharmaceutical, cosmetic, and food materials. Among them, linoleic acid has been reported as the most potent antibacterial agent for Gram-positive bacteria (48). Additionally, Linoleic, oleic, palmitoleic, palmitic, and stearic fatty acids are commonly incorporated into dermatological formulations (49). In a recent study, according to the results of GC-MS analysis, fatty acid compounds (palmitic acid, stearic acid, and others), omega-3 ( $\alpha$ -linolenic acid and eicosapentaenoic acid) and omega-6 (linoleic acid and  $\gamma$  -linolenic acid) were detected, particularly in the medium containing lead, suggesting that these changes help maintain membrane fluidity and integrity, which are crucial for cell survival under stress conditions. Although glycerol, a known carbon precursor,

supported optimal cell growth of *B. halotolerans*, it did not result in the highest lipid yield or diversity. In contrast, exposure to lead, acting as a stressor, appeared to stimulate lipid biosynthesis pathways. This functional shift suggests that under stress conditions, cells prioritize the accumulation of storage compounds including lipids over active proliferation. Similar regulatory mechanisms have been observed in previous studies, highlighting the role of environmental stress in modulating microbial lipid production.

Besides unsaturated essential fatty acids, other saturated and unsaturated fatty acids and their derivatives have wide applications. For example, some of them have inhibitory effects against oral bacteria. Myristoleic acid inhibits *Selenomonas artemidis*, cis-hexadecenoic and cis-octadecenoic acids inhibit *Porphyromonas gingivalis* and *Streptococcus sobrinus*, and lauric acid and myristic acid act synergistically with thymol for growth inhibition (50). Prasath et al. showed that myristic acid inhibit biofilm formation and hyphal development in *Candida albicans* (51). Also, they proved the antifungal, antibiofilm and antivirulence activities of palmitic acid against *Candida*

*tropicalis* (52). While all of these compounds were also produced by our *Bacillus* in this study.

Bhattarai et al. (2007) isolated two antifouling compounds (2-hydroxymyristic acid and cis-9-oleic acid) from a chloroform extract of the marine bacterium, *Shewanella oneidensis* SCH0402. In another study,  $\beta$ -sitosterols and palmitic acid were obtained from the chloroform extract of *Nitraria retusa* leaves. They exhibited antitumor effect by preventing the expansion of transplantable tumor, protecting the lung parenchyma, increasing the proliferation of splenocytes, enhancing the lysosomal activity of host macrophages and cellular antioxidant activity (53). Similarly, in our experiments, cis-9-oleic acid and palmitic acid were obtained from the chloroform extract of all media.

Also, biodiesel is obtained from microorganisms, which are one of the sources of renewable and environmentally friendly energy. Microalgae are an attractive choice for biodiesel production, producing compounds such as palmitic acid (54). This compound was also produced by *B. halotolerans* in our study.

In general, although lead is classified as a heavy and toxic metal, the results of this study revealed its remarkable impact on lipid biosynthesis in *B. halotolerans*. The presence of lead in the culture medium led to approximately an 86% increase in lipid accumulation compared to the control, indicating a strong stimulatory effect under stress conditions. Moreover, lead exposure not only enhanced the total lipid content but also altered the fatty acid profile, indicating that the bacterium may activate adaptive metabolic pathways in response to heavy metal stress.

These findings point to the intriguing possibility of using controlled lead stress to modulate microbial

lipid production, which could have biotechnological applications. However, further studies are needed to explore the underlying mechanisms and assess the safety and feasibility of such approaches. Also, there is a need for optimization methods to increase production and reduce costs by this strain, and then methods to separate different compounds.

#### 4. Conclusions

In the current study, fatty acid synthesis by *Bacillus halotolerans* was investigated in various media. The synthesized fatty acids were identified using FTIR and Gas Chromatography/ Mass Spectrometry, GC-MS. Our analysis revealed a diverse profile of fatty acids with significant industrial and biological relevance, including Omega-3 fatty acids ( $\alpha$ -linolenic acid and eicosapentaenoic acid), Omega-6 fatty acids ( $\gamma$ -linolenic acid and linoleic acid), saturated and monounsaturated fatty acids (myristoleic acid, palmitic acid, stearic acid, cis-hexadecenoic acid, and cis-octadecenoic acid, among others). The effect of metals and nanoparticles on promoting fatty acid synthesis was also investigated in this experiment. The findings revealed that the lead-supplemented medium induced the highest fatty acid yield and diversity, suggesting a potential stimulatory role of this metal on lipid metabolism.

#### Conflict of interests

The authors declare that they have no conflict of interest.

#### Acknowledgment

The authors would like to thank the Shahid Ashrafi Esfahani University for the financial support of this study.

#### References

- (1) Tan Y-S, Zhang R-K, Liu Z-H, Li B-Z, Yuan Y-J. Microbial adaptation to enhance stress tolerance. *Frontiers in Microbiology*. 2022;13:888746. <https://doi.org/10.3389/fmicb.2022.888746>
- (2) Wang J, Liu Y, Ma Y, Wang X, Zhang B, Zhang G, et al. Research progress regarding the role of halophilic and halotolerant microorganisms in the eco-environmental sustainability and conservation. *Journal of*

- Cleaner Production*. 2023;418:138054.  
<https://doi.org/10.1016/j.jclepro.2023.138054>
- (3) Etemadzadeh SS, Emtiazi G. In vitro identification of antimicrobial hemolytic lipopeptide from halotolerant *Bacillus* by Zymogram, FTIR, and GC mass analysis. *Iranian Journal of Basic Medical Sciences*. 2021;24(5):666.  
<https://doi.org/10.22038/IJBMS.2021.53419.12022>
- (4) Madani M, Enshaeieh M, Abdoli A. Single cell oil and its application for biodiesel production. *Process Safety and Environmental Protection*. 2017;111:747-56.  
<https://doi.org/10.1016/j.psep.2017.08.027>
- (5) P Desbois A. Potential applications of antimicrobial fatty acids in medicine, agriculture and other industries. *Recent patents on anti-infective drug discovery*. 2012;7(2):111-22. <https://doi.org/10.2174/157489112801619728>
- (6) Yoshida K, Hashimoto M, Hori R, Adachi T, Okuyama H, Orikasa Y, et al. Bacterial long-chain polyunsaturated fatty acids: their biosynthetic genes, functions, and practical use. *Marine drugs*. 2016;14(5):94.  
<https://doi.org/10.3390/md14050094>
- (7) Subramaniam R, Dufreche S, Zappi M, Bajpai R. Microbial lipids from renewable resources: production and characterization. *Journal of Industrial Microbiology and Biotechnology*. 2010; 37(12): 1271-87.  
<https://doi.org/10.1007/s10295-010-0884-5>
- (8) Chu W-L. Strategies to enhance production of microalgal biomass and lipids for biofuel feedstock. *European Journal of Phycology*. 2017;52(4):419-37.  
<https://doi.org/10.1080/09670262.2017.1379100>
- (9) Yu X, Dong T, Zheng Y, Miao C, Chen S. Investigation of cell disruption methods for lipid extraction from oleaginous microorganisms. *European Journal of Lipid Science and Technology*. 2014;117:730-7.  
<https://doi.org/10.1002/ejlt.201400195>
- (10) Ledesma-Amaro R, Dulermo R, Niehus X, Nicaud J-M. Combining metabolic engineering and process optimization to improve production and secretion of fatty acids. *Metabolic engineering*. 2016;38:38-46.  
<https://doi.org/10.1016/j.ymben.2016.06.004>
- (11) Ledesma-Amaro R, Nicaud J-M. *Yarrowia lipolytica* as a biotechnological chassis to produce usual and unusual fatty acids. *Progress in lipid research*. 2016;61:40-50.  
<https://doi.org/10.1016/j.plipres.2015.12.001>
- (12) Zhang Q, Li Y, Xia L. An oleaginous endophyte *Bacillus subtilis* HB1310 isolated from thin-shelled walnut and its utilization of cotton stalk hydrolysate for lipid production. *Biotechnology for biofuels*. 2014;7(1):1-13.  
<https://doi.org/10.1186/s13068-014-0152-4>
- (13) Ochsenreither K, Glück C, Stressler T, Fischer L, Syltatk C. Production strategies and applications of microbial single cell oils. *Frontiers in microbiology*. 2016;7:1539.  
<https://doi.org/10.3389/fmicb.2016.01539>
- (14) Diomande SE, Guinebretière M-H, Broussolle V, Brillard J. Role of fatty acids in *Bacillus* environmental adaptation. *Frontiers in microbiology*. 2015;6:813.  
<https://doi.org/10.3389/fmicb.2015.00813>
- (15) Armenta RE, Valentine MC. Single-cell oils as a source of omega-3 fatty acids: an overview of recent advances. *Journal of the American Oil Chemists' Society*. 2013; 90(2):167-82.  
<https://doi.org/10.1007/s11746-012-2154-3>
- (16) Gupta A, Singh D, Byreddy AR, Thyagarajan T, Sonkar SP, Mathur AS, et al. Exploring omega-3 fatty acids, enzymes and biodiesel producing thraustochytrids from Australian and Indian marine biodiversity. *Biotechnology journal*. 2016;11(3):345-55.  
<https://doi.org/10.1002/biot.201500279>

- (17) Bianchi AC, Olazábal L, Torre A, Loperena L. Antarctic microorganisms as source of the omega-3 polyunsaturated fatty acids. *World Journal of Microbiology and Biotechnology*. 2014;30(6):1869-78. <https://doi.org/10.1007/s11274-014-1607-2>
- (18) Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*. 1957;226(1):497-509. [https://doi.org/10.1016/S0021-9258\(18\)64849-5](https://doi.org/10.1016/S0021-9258(18)64849-5)
- (19) Etemadzadeh SS, Emtiazi G. Generation of non-toxic, chemical functional bio-polymer for desalination, metal removal and antibacterial activities from animal meat by-product. *Journal of Food Science and Technology*. 2021;58(1):159-65. <https://doi.org/10.1007/s10123-023-00334-4>
- (20) Murata T, Kanao-Koshikawa M, Takamatsu T. Effects of Pb, Cu, Sb, In and Ag contamination on the proliferation of soil bacterial colonies, soil dehydrogenase activity, and phospholipid fatty acid profiles of soil microbial communities. *Water, Air, and Soil Pollution*. 2005;164(1):103-18. <https://doi.org/10.1007/s11270-005-2254-x>
- (21) Rasouli A, Aghaei SS, Zargar M. Bio-production of Single-cell Oil by *Rhodococcus Erythropolis* PTCC 1767 Bacterial using Low-cost Carbon Sources. *Biological Journal of Microorganism*. 2020;9(35):71-85. <https://doi.org/10.22108/bjm.2020.121878.1281> [In Persian]
- (22) El-Sheekh M, Abomohra AE-F, Hanelt D. Optimization of biomass and fatty acid productivity of *Scenedesmus obliquus* as a promising microalga for biodiesel production. *World Journal of Microbiology and Biotechnology*. 2013;29(5):915-22. <https://doi.org/10.1007/s11274-012-1248-2>
- (23) Fathabad SG, Tabatabai B, Walker Dm, Chen H, Lu J, Aslan K, et al. Impact of zero-valent iron nanoparticles on *Fremyella diplosiphon* transesterified lipids and fatty acid methyl esters. *ACS omega*. 2020;5(21):12166-73. <https://dx.doi.org/10.1021/acsomega.0c00566>
- (24) Ranjani S, Faridha Begum I, Santhoshini J, Senthil Kumar N, Ruckmani K, Hemalatha S. *Mimosa pudica* floral nanoparticles: a potent antibiotic resistance breaker. *Inorganic and Nano-Metal Chemistry*. 2020;51(12):1751-58. <https://doi.org/10.1080/24701556.2020.1852429>
- (25) Baskar K, Chinnasamy R, Pandey K, Venkatesan M, Sebastian PJ, Subban M, et al. Larvicidal and histopathology effect of endophytic fungal extracts of *Aspergillus tamaris* against *Aedes aegypti* and *Culex quinquefasciatus*. *Heliyon*. 2020;6(10):e05331. <https://doi.org/10.1016/j.heliyon.2020.e05331>
- (26) Faisal M, Elhussieny A, Ali K, Samy I, Everitt N, editors. Extraction of degradable bio polymer materials from shrimp shell wastes by two different methods. *IOP Conference Series: Materials Science and Engineering*; 2018: IOP Publishing. <https://doi.org/10.1088/1757-899X/464/1/012004>
- (27) Huq M. Green synthesis of silver nanoparticles using *Pseudoduganella eburnea* MAHUQ-39 and their antimicrobial mechanisms investigation against drug resistant human pathogens. *International journal of molecular sciences*. 2020; 21(4): 1510. <https://doi.org/10.3390/ijms21041510>
- (28) Karthik G, Sundaravadivelu M. Studies on the inhibition of mild steel corrosion in hydrochloric acid solution by atenolol drug. *Egyptian Journal of Petroleum*. 2016;25(2): 183-91. <https://doi.org/10.1016/j.ejpe.2015.04.003>
- (29) Naiya TK, Singha B, Das SK. FTIR study for the Cr (VI) removal from aqueous solution using rice waste. *International Proceedings of Chemical, Biological and*

- Environmental Engineering*. 2011;10:114-9.  
<https://B2n.ir/nb6551>
- (30) Ameenudeen S, Unnikrishnan S, Ramalingam K. Statistical optimization for the efficacious degradation of reactive azo dyes using *Acinetobacter baumannii* JC359. *Journal of Environmental Management*. 2021;279:111512.  
<https://doi.org/10.1016/j.jenvman.2020.111512>
- (31) Sandhyarani G, Sowmya L, Venumadhev K. Formulation and evaluation of orodispersible tablets of risperidone. 2017.  
<https://doi.org/10.20959/wjpr20177-8754>
- (32) Mani M, Pavithra S, Mohanraj K, Kumaresan S, Alotaibi SS, Eraqi MM, et al. Studies on the spectrometric analysis of metallic silver nanoparticles (Ag NPs) using *Basella alba* leaf for the antibacterial activities. *Environmental Research*. 2021;199:111274.  
<https://doi.org/10.1016/j.envres.2021.111274>
- (33) Bağda E, Tuzen M, Sari A. Equilibrium, thermodynamic and kinetic investigations for biosorption of uranium with green algae (*Cladophora hutchinsiae*). *Journal of environmental radioactivity*. 2017;175:7-14.  
<https://doi.org/10.1016/j.jenvrad.2017.04.004>
- (34) Mohamed JMM, Alqahtani A, Al Fatease A, Alqahtani T, Khan BA, Ashmitha B, et al. Human Hair Keratin Composite Scaffold: Characterisation and Biocompatibility Study on NIH 3T3 Fibroblast Cells. *Pharmaceuticals*. 2021;14(8):781.  
<https://doi.org/10.3390/ph14080781>
- (35) Ghosh S, Gandhi M, van Hullebusch ED, Das AP. Proteomic insights into *Lysinibacillus* sp.-mediated biosolubilization of manganese. *Environmental Science and Pollution Research*. 2021;28(30):40249-63.  
<https://doi.org/10.1007/s11356-020-10863-4>
- (36) Periyasamy S, Muthuchamy M. Electrochemical oxidation of paracetamol in water by graphite anode: effect of pH, electrolyte concentration and current density. *Journal of environmental chemical engineering*. 2018;6(6):7358-67.  
<https://doi.org/10.1016/j.jece.2018.08.036>
- (37) Lingegowda DC, Kumar JK, Prasad AD, Zarei M, Gopal S. FTIR spectroscopic studies on *Cleome gynandra*—comparative analysis of functional group before and after extraction. *Romanian Journal of Biophysics*. 2012;22(3-4):137-43.  
<https://doi.org/10.5555/20133275620>
- (38) Liu H, Wang L, Wang M, Hou C-J, Ye B-b, Wang Z, et al. Engineered multifunctional fluorinated film based on semicontinuous emulsion polymerization using polymerizable quaternary ammonium emulsifiers. *International Journal of Polymer Science*. 2018;2018.  
<https://doi.org/10.1155/2018/5659137>
- (39) Batool S, Gilani S, Zainab S, Tahir M, Harrison W, Syed Q, et al. Synthesis and Structural Characterization of a Monomeric Mixed Ligand Copper (II) Complex Involving N, N, N', N'-Tetramethylethylenediamine and Mefenamate. *Journal of Structural Chemistry*. 2019;60(7):1156-65.  
<https://doi.org/10.1134/S0022476619070187>
- (40) Karthik G, Sundaravadivelu M. Experimental and theoretical studies of anti-ulcer drugs with benzimidazole rings as corrosion inhibitor for copper in 1 M nitric acid medium. *Journal of adhesion science and Technology*. 2017;31(5):530-51.  
<https://doi.org/10.1080/01694243.2016.1222047>
- (41) Yin Z-g, Wei S, Hu Y-h, Guan Q-j, Zhang C-h, Gao Y-s, et al. Depressing behaviors and mechanism of disodium bis (carboxymethyl) trithiocarbonate on separation of chalcopyrite and molybdenite. *Transactions of Nonferrous Metals Society of China*. 2017;27(4):883-90.  
[https://doi.org/10.1016/S1003-6326\(17\)60100-6](https://doi.org/10.1016/S1003-6326(17)60100-6)
- (42) Eid AM, Fouda A, Niedbala G, Hassan SE-D, Salem SS, Abdo AM, et al. Endophytic

- Streptomyces laurentii* mediated green synthesis of Ag-NPs with antibacterial and anticancer properties for developing functional textile fabric properties. *Antibiotics*. 2020;9(10):641. <http://dx.doi.org/10.3390/antibiotics9100641>
- (43) Kalliantas D, Kallianta M, Kordatos K, Karagianni S. The nanostructure character of *Arnica montana* as ultra high diluted succussed solution medicinal product. Recent advances and prospects. *Journal of Nanomedicine*. 2020;3(1):1021. <https://B2n.ir/kf4524>
- (44) Casillas-Vargas G, Ocasio-Malavé C, Medina S, Morales-Guzmán C, Del Valle RG, Carballeira NM, et al. Antibacterial fatty acids: An update of possible mechanisms of action and implications in the development of the next-generation of antibacterial agents. *Progress in lipid research*. 2021;82:101093. <https://doi.org/10.1016/j.plipres.2021.101093>
- (45) Blasio M, Balzano S. Fatty acids derivatives from eukaryotic microalgae, pathways and potential applications. *Frontiers in Microbiology*. 2021;12:718933. <https://doi.org/10.3389/fmicb.2021.718933>
- (46) Alagawany M, Elnesr SS, Farag MR, Abd El-Hack ME, Khafaga AF, Taha AE, et al. Omega-3 and omega-6 fatty acids in poultry nutrition: effect on production performance and health. *Animals*. 2019;9(8):573. <https://doi.org/10.3390/ani9080573>
- (47) Yagmur A, Ghayas S, Jan H, Kalaycioglu GD, Moghimi SM. Omega-3 Fatty Acid Nanocarriers: Characterization and Potential Applications. *Current Opinion in Colloid & Interface Science*. 2023:101728. <https://doi.org/10.1016/j.cocis.2023.101728>
- (48) Jumina J, Lavendi W, Singgih T, Triono S, Steven Kurniawan Y, Koketsu M. Preparation of monoacylglycerol derivatives from Indonesian edible oil and their antimicrobial assay against *Staphylococcus aureus* and *Escherichia coli*. *Scientific reports*. 2019;9(1):10941. <https://doi.org/10.1038/s41598-019-47373-4>
- (49) Čižinauskas V, Elie N, Brunelle A, Briedis V. Fatty acids penetration into human skin ex vivo: A TOF-SIMS analysis approach. *Biointerphases*. 2017;12(1). <https://doi.org/10.1116/1.4977941>
- (50) Shapiro S. The inhibitory action of fatty acids on oral bacteria. *Oral microbiology and immunology*. 1996;11(5):350-5. <https://doi.org/10.1111/j.1399-302X.1996.tb00193.x>
- (51) Prasath KG, Sethupathy S, Pandian SK. Proteomic analysis uncovers the modulation of ergosterol, sphingolipid and oxidative stress pathway by myristic acid impeding biofilm and virulence in *Candida albicans*. *Journal of proteomics*. 2019;208:103503. <https://doi.org/10.1016/j.jprot.2019.103503>
- (52) Prasath KG, Tharani H, Kumar MS, Pandian SK. Palmitic acid inhibits the virulence factors of *Candida tropicalis*: Biofilms, cell surface hydrophobicity, ergosterol biosynthesis, and enzymatic activity. *Frontiers in microbiology*. 2020;11:864. <https://doi.org/10.3389/fmicb.2020.00864>
- (53) Boubaker J, Ben Toumia I, Sassi A, Bzouich-Mokded I, Ghoul Mazgar S, Sioud F, et al. Antitumoral potency by immunomodulation of chloroform extract from leaves of *Nitraria retusa*, Tunisian medicinal plant, via its major compounds  $\beta$ -sitosterol and palmitic acid in BALB/c mice bearing induced tumor. *Nutrition and cancer*. 2018;70(4):650-62. <https://doi.org/10.1080/01635581.2018.1460683>
- (54) Chen W, Ma L, Zhou P-p, Zhu Y-m, Wang X-p, Luo X-a, et al. A novel feedstock for biodiesel production: the application of palmitic acid from *Schizochytrium*. *Energy*. 2015;86:128-38. <https://doi.org/10.1016/j.energy.2015.03.110>