Physiological Response of Halophilic Bacteria

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by

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DECLARATION BY STUDENT

I hereby declare that the data presented in this Dissertation entitled,**PhysiologicalResponse of Halophilic Bacteria"** is based on the results of investigations carried out by me in the Marine Biotechnology at the School of Biological Sciences and Biotechnology, Goa University under the supervision of Ms. Dviti Mapari and the same has not been submitted elsewhere for the award of a degree or diploma by me. Further, I understand that Goa University or its authorities will not be responsible for the correctness of observational or other findings given the dissertation.

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COMPLETION CERTIFICATE

This is to certify that the dissertation **Physiological Response of Halophilic Bacteria"** is a bonafide work carried out by **Mr. Viraj Naresh Govekar** under my mentorship in partial fulfilment ofthe requirements for the award of the degree of Master's in the Discipline of Marine Biotechnology at the School of Biological Sciences and Biotechnology, Goa University.

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ABBREVIATIONS

PSU	Practical Salinity Unit
mL	Millilitre(s)
M	Molar
GCMS	Gas Chromatography-Mass Spectroscopy
℃	Degree Celsius
hr	Hour
mins	Minutes
rpm	Revolutions Per Minute

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CHAPTER 1:

INTRODUCTION

Life on the earth is divided into three domains viz Bacteria, Archaea, and Eukarya. All the ecosystems on the earth are inhabited by organisms from these domains. Some ecosystems are considered extreme environments with conditions in which humans cannot thrive. These environments are mainly habited by bacteria and archaea, which are considered extremophiles.

In nature, the largest saline water bodies comprise oceans with average salinities ranging from 32-35psu. The hypersaline environments formed because of the evaporation of the saline water bodies, whose salinity can be higher than normal saline water. As every environment is inhabited by microorganisms, these hypersaline environments are colonized by "salt-loving" organisms, i.e., halophiles. Prokaryotes and eukaryotes are both domains of life that consist of halophilic organisms (Kerkar, n.d.).

In all cells, the selective barrier between the inner cellular environment and the outer environment is the plasma membrane which mediates the in-flow and out-flow of nutrients and waste. The current understanding of the structure and dynamics of the plasma membrane comes from the "Fluid mosaic" model proposed by Singer and Nicolson in 1972. Lipids are the precursors of the plasma membrane. The amphipathic nature of the lipids (i.e., they pose both hydrophobic and hydrophilic moiety) is the reason for the bilayer nature of the plasma membrane. When in the aqueous environment, the lipids organize themselves in a bilayer (Goñi, 2014).

The halophiles habiting the hypersaline environments have special modifications in their overall cell structure and composition which help them to thrive. Under salt stress, the morphology of the bacterial cells changes. Another such modification that occurs is in the plasma membrane of these bacteria. The lipid composition of this bacteria changes accordingly to the salt stress to modulate the fluidity of the membrane.

Fatty acids are the precursor molecules for the synthesis of lipids. Likewise, there are changes in the expression of the fatty acids which in turn will modulate the fluidity of the membrane.

CHAPTER 2:

AIMS AND OBJECTIVES

Aim:

To study the physiological responses of halophilic bacteria at varying concentrations of salt.

Objectives:

1. To study the effect of salinity stress on the morphology of halophilic bacteria.
2. To study the effect of varying salinity on the fatty acid profiles of halophilic bacteria.
3. To study salinity stress's effect on the halophilic bacteria's lipid composition.

CHAPTER 3:

REVIEW OF LITERATURE

Introduction:

Bacteria adapt to and deal with a diverse range of environmental fluctuations. The survival of the bacteria depends on its ability to cope with environmental factors such as temperature, pH, salinity, and pressure. Major metabolic reprogramming is frequently necessary for bacteria to respond to stress. This reprogramming includes coordinated changes in the cell's transcriptome, proteome, and metabolome and remodeling of the cellular membrane (Rowlett et al., 2017).

The current understanding of the structure and dynamics of the plasma membrane comes from the "Fluid mosaic" model proposed by Singer and Nicolson in 1972. Lipids are the precursors of the plasma membrane. The amphipathic nature of the lipids (i.e., they pose both hydrophobic and hydrophilic moiety) is the reason for the bilayer nature of the plasma membrane. When in the aqueous environment, the lipids organize themselves in a bilayer (Goñi, 2014).

2.1 Hypersaline Environment and Halophiles:

The environments that are characterized by an average salinity greater than that of seawater are defined as hypersaline environments (Mcgenity, 2012). Hypersaline ecosystems, such as saline lakes, salt pans, salt marshes, or saline soils, are widely dispersed in many diverse geographical regions of the planet. Halophiles can be found there. The three domains of life i.e., Archaea, Bacteria, and Eukarya consist of halophiles and are assorted by their requirement for the salinity conditions (Amoozegar et al., 2019).

2.2 Physiology of halophilic bacteria:

2.2.1 Requirement of salt:

An organism with an optimal growth requirement of high salt concentration is termed a halophile, whereas an organism that can tolerate high salt conditions is termed a halotolerant organism (Kanekar et al., 2012).

On the basis of how they react to NaCl, halophiles can be divided into three groups:

Types of Halophiles	Optimum NaCl Range (%)
Slight Halophiles	2-5
Moderate Halophiles	5-20
Extreme Halophiles	20-30

Table 1. Classification of Halophiles based on NaCl Concentration (%) (Kerkar, n.d.).

2.2.2 Enzymatic Adaptations in Halophiles:

Working of the cellular machinery is a prerequisite for the bacteria growing at high salt concentrations; hence halophiles are known to have modifications in their enzymes. An enzyme of halophilic bacteria has a protein that consists of more acidic amino acids than basic amino acids as some suggestions indicate the high concentration of ions might affect the confirmation of the enzyme (Dundas, 1977). The acidic nature of the halophilic proteins also reduces the hydrophobic content and also the presence of some specific ion interaction aids in the solubility and stability at high salt concentrations (Munawar & Engel, 2013).

2.3 Osmoadaptation Strategies:

Osmoadaptation is defined as the changes in the physiological and genetic compositions of a bacterium in response to stress (Kanekar et al., 2012). When it comes to high salt conditions or hypersaline environments, the microbial flora inhabiting there phylogenetically varies greatly.

The halophilic and halotolerant organisms that inhabit the hypersaline environments represent all three domains of life, i.e., Archaea, Bacteria, and Eukarya. The mechanisms of osmoadaptation of these life forms are different, but some of the general principles of adaptations are

I. A biological membrane can let water through. As a result, water enters and exits cells due to variations in water activity between the cytoplasm and the surrounding medium.

II. When the cells are suspended in the hypersaline environment the water from the cytoplasm moves out of the cell. Therefore, an active pumping of the water inside of the cell is not practicable and requires a lot of cellular energy. Hence, the cells maintain an isosmotic intracellular environment with the outer.

III. All microorganisms possess a positive turgor pressure except Halobacteria, a halophilic Archaea. The maintenance of positive turgor pressure helps in cell expansion.

IV. The "salt-in" strategy involves building up large levels of inorganic salts in the medium to establish osmotic equilibrium. The "salt-in" process is based on KCl rather than NaCl as the major intracellular salt since Na+ ions are minimized in cells in all three domains of life.

V. The “low-salt-in” method involves using compatible solutes. Compatible solutes are molecules that help microorganisms carry out normal metabolic functions at high concentrations. Numerous chemical molecules from various classes, including polyols, sugars, amino acids, betaines, ectoine, acetylated diamino acids, and N-derivatized carboxamides of glutamine, have been demonstrated to function as compatible solutes in various groups of microbes. The synthesis of compatible solutes is more energy-consuming than the “salt-in” method. Hence, microorganisms can sequester these from the environment instead of synthesizing them (Gunde-Cimerman et al., 2018).

2.5 Effect of salt stress on cell morphology:

The morphological changes observed in the bacteria when exposed to salinity stress are elongation of the cells, irregularity of cell structure, and cell membrane deformities. The coagulation of cytoplasmic content of bacterial cells is also observed widely (Gandhi & Shah, 2016).

Survival and adaptive strategies of Listeria monocytogenes to different stress factors such as low temperature, pH variations, and salt variations were studied using other techniques. The techniques used in the study were light microscopy and Scanning Electron Microscopy (SEM), GC-MS, and SDS-PAGE. Microscopy techniques were used for determining morphological changes in the cells of L. monocytogenes. There was no change in morphology under pH and temperature stress but a change was observed with respect to salt stress. The cells were elongated under salt stress because of improper cell division. The reason for improper cell division is that the protein-coding gene (Fts) required for the formation of septate between cells is inhibited by the product of the Min gene (Satyajit Babasaheb Kale, 2017).

2.6 Effect of salt stress on membrane composition:

Like eukaryotic cell membranes, bacterial cell membrane composes of lipids and proteins in roughly equal proportion. The bacterial cytoplasmic membrane attains a bilayer structure because of the lipid. The broad chemical classes into which the lipids fall are relatively few, although most of these groups have molecular variations that differ in size and isomeric shape. The primary source of the precursor compounds is central metabolism. The cell membrane acts as a barrier between the outside environment and the cell cytoplasm. It directs the export and import of an untold number of solutes and ions across the cell. The transmembrane potential formed by ion transportation is responsible for many metabolic activities (Strahl & Errington, 2017).

When halotolerant and moderately halophilic bacteria grow in environments with increasing salt concentrations, the phospholipid content of their membrane changes. The most significant alterations appear to be a net increase in the proportion of negatively charged phospholipids and, in rare situations, a net rise in the proportion of branched-chain fatty acids or cyclopropanoid fatty acids (in some Gram-positive bacteria) (in some Gram-negative bacteria). The changes that occur because of salt stress is thought to affect the signal-transducing pathways. The lipids may also involve activating an enzyme or cause changes in the rate of biosynthetic or degradative pathways (Kates, 1986).

Phosphatidylglycerol (PG), cardiolipin (CL), phosphatidylcholine (PC), and phosphatidylethanolamine (PE) were the main constituents of the polar lipids and very little Phosphatidic acid (PA) was observed in the halophilic phototrophic Ectothiorhodospira species. The principal lipids that were regulated during osmoadaptation in Ectothiorhodospira are PG and PE. The increase in salt concentration leads to an increase in the PG phospholipid and a decrease in PE. Simultaneously, during the shit-down experiment, there was an increase in PE and a reduction in PG in Ectothiorhodospira species. The study also suggested a net increase in negatively charged phospholipids to stabilize the plasma membrane. PG and PE phospholipids help stabilize and form a plasma membrane bilayer (Thiemann & Imhoff, 1991).

The membrane fatty acid content of the Listeria monocytogenes was analyzed by converting fatty acid into FAME and subsequently detection using GS-MC. It was observed that there was an increase in C13:0, C14:0, and C16:0 in response to pH stress. As these fatty acids have a straight chain, they maintain the membrane fluidity to maintain pH. Under cold temperatures, C16:0 fatty acid was observed and also the presence of unsaturated fatty acids (C18:0, C18:1, C18:1) was observed as an unsaturation stabilized membrane under cold conditions.

Lastly, under salt stress, there was a decline in C16:0 to increase membrane fluidity for the accumulation of compatible solutes (Satyajit Babasaheb Kale, 2017).

2.7 Effect of salt stress on membrane proteins:

It has been observed that extremely and moderately halophilic organisms have a more significant proportion of acidic amino acids than basic amino acids compared to non-halophilic microorganisms. Some species of moderately halophilic organisms requiring extreme salt concentration have the same amino acid composition. Therefore, it has been speculated that this change of membrane amino acids is a genotypic modification of halophilic organisms.

Pseudomonas halosaccrolytica, the moderately halophilic organism, has more acidic amino acids than basic ones in its outer membrane. This concentration is at least as similar as to extremely halophilic organisms and greater than non-halophilic organisms. This adaptation is genotypic and not phenotypical because this composition of the amino acid is not changed when the salinity of the medium is increased or decreased.

Extreme halophiles are relatively deficient in non-polar amino acids compared to non-halophilic proteins. The hydrophobic bonds in the protein are strengthened by the increasing concentration of salts. Hence, the relatively low concentration of non-polar residues avoids the tight folding of the proteins, making them functionally active. This same effect is observed when the surface acidic residues are increased (Russell, 1989).

Survival in the marine environment exerts much physiochemical stress which a microbe has to overcome for its survival. The stress can be temperature, pH, salinity, or pressure. To overcome this, many marine microbes produce EPS, which helps the cells to thrive in an environment with frequent fluctuations in temperature, salinity, pressure, or pH (Poli et al., 2010).

The response to stress is also seen in changes in the morphology of cells. There are chemical changes observed inside the cell and also modifications in the cell plasma membrane.

The present study gives insight into the effect of salt stress on the membrane composition of the halophilic bacteria, the morphology of the cell, and the fatty acid composition of the bacteria.

CHAPTER 4:

METHODS AND MATERIALS

3.1 Cultivation of Bacterium:

The isolate PSDM 20 used for the experimental studies was revived from a glycerol stock. The isolate was maintained on Nutrient agar supplemented with 8% NaCl at 28ºC.

3.2 Gram-staining of the bacterial isolate for the morphological analysis:

To examine the effect of salinity on the morphology of the isolate, the culture was grown in Nutrient Broth (HiMedia, Mumbai, India) supplemented with the different concentrations of salt (NaCl) (0%, 0.5%, 2%, 8%, 12%, and 16%) at 28ºC for 48hrs. After incubation, the cell pellet was harvested by centrifugation and suspended in PBS. A thin smear was prepared on a grease-free glass slide, and Gram-staining was performed as described by (Satyajit Babasaheb Kale, 2017). After staining, the slides were observed under the oil-immersion lens to determine the gram character and observe the morphology.

3.3 Fatty Acid Methyl Ester (FAME) of bacterial isolate for the fatty acid profiling:

To study the Fatty acid profiles of the halophilic bacteria, the isolate was grown in Nutrient Broth supplement with different concentrations of NaCl (0%, 0.5%, 2%, 8%, 12%, and 16%). The cells were harvested by centrifuging at 10000 rpm for 10 mins. The obtained pellet was washed twice with PBS and further processed for FAME analysis.

The washed cell pellet was hydrolyzed by adding 1 mL of 1M KOH prepared in 70% ethanol and incubated for 1 hr at 90°C. The reaction mixture was then acidified by adding 0.2 mL of 6N HCl, and 1 mL of water. The free fatty acids (FFA) were released by adding 1 mL of hexane. The hexane was allowed to evaporate, and 1 mL of 10% boron trifluoride in methanol was added to methylate the FFA and incubated at 37°C for 20 mins. One mL of water was added to the solution, followed by the extraction of fatty acid methyl esters (FAME) by adding

1 mL hexane. The FAMEs were analyzed using SHIMADZU GCMS-QP2020 (Satyajit Babasaheb Kale, 2017).

3.4 Thin-Layer Chromatography (TLC) analysis of the bacterial isolate for lipid analysis:

The harvesting of the cells for the TLC analysis proceeded by the same process as mentioned in section "3.3". The harvested cell pellet was then processed for TLC analysis by a slight modification in the protocol of (Sturt et al., 2004) and (Imhoff,' et al., 1982). 5 mL of chloroform-methanol-water (2:1:0.8) was added to each tube and sonicated for 5-10 mins. After sonication, 1 mL each of chloroform and water was added to bring the ratio to chloroform-methanol-water (1:1:0.9) and kept overnight for phase separation. Afterward, the lower chloroform layer was collected.

The extracted lipids were separated on TLC plates (Silica G-60, Merck) in the solvent system containing, chloroform-methanol-acetic acid-water (85:15:10:3.5) and for identifying the plates were sprayed with the following reagents, -naphthol, iodine vapor, and Dragendroff reagent.

CHAPTER 5:

RESULT AND DISCUSSION

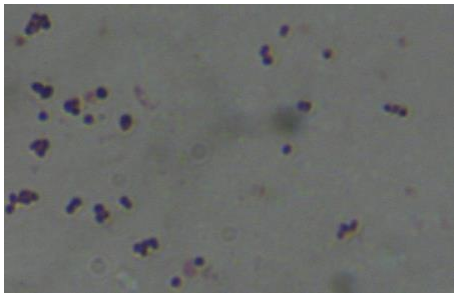
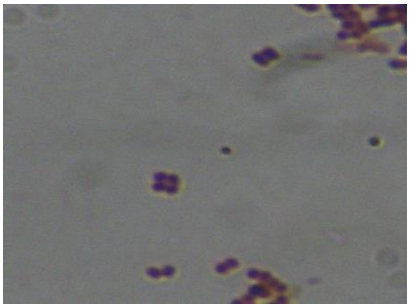
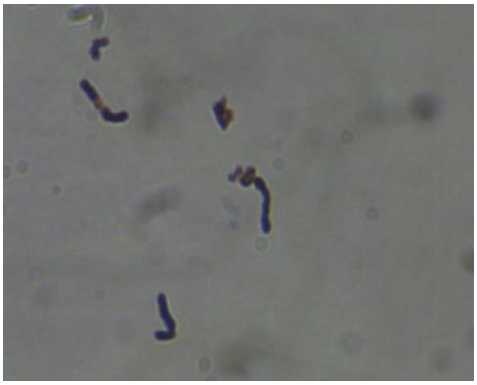
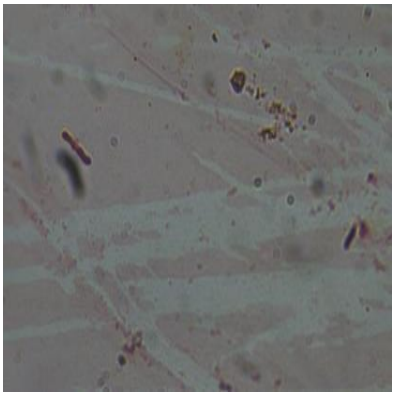
4.1 Effect of salinity on the morphology of halophilic bacteria

Gram-staining was performed to study the morphological changes occurring in halophilic bacteria during salinity variations. The gram staining revealed the gram character of the isolate to be gram-positive. The isolate, when grown in NB without NaCl (0%) and ameliorated with NaCl concentrations of 0.5% showed Gram-positive cocci as morphology. However, the rod shape morphology was dominant when grown in NB supplemented with NaCl concentrations of 2%, 8%, and 16% NaCl concentrations. Short rods were observed in NB with 2% NaCl and long rods with 16% NaCl. The results are summarized in Figure No. 1.

Cells, when grown in their optimum salt concentrations conditions, showcase their actual morphology, however, when they are grown at salt stress which is higher than their required optimum concentrations, they tend to form long filaments or show elongation. The reason for the same can be that the cell division restricts because of which proper septate are not formed (Satyajit Babasaheb Kale, 2017).

The bacteria when under salt stress will show drastic changes in their morphological characteristics i.e., when grown at low salt stress they appear as shorter than actual morphology while when grown at higher salt stress the bacteria appear elongated and disordered cells observed (Li et al., 2021).

Figure No. 1. Effect of salinity on the morphology of the isolate PSDM 20.

	
a. Isolate grown in Nutrient Broth composition without NaCl (0% NaCl)	b. Isolate grown in Nutrient Broth with 0.5% NaCl
	
a. Isolate grown in Nutrient Broth with 2% NaCl.	b. Isolate grown in Nutrient Broth with 8% NaCl.
b.	c.
c. Isolate grown in Nutrient Broth with 12% NaCl.	d. Isolate grown in Nutrient Broth with 16% NaCl.

4.2. Effect of salinity on the fatty acid composition of halophilic bacteria:

The fatty acids profiles of the isolate grown on different concentrations of NaCl (0%, 0.5%, 2%, 8%, 12%, and, 16%) were identified using GCMS. The obtained results are summarized in the figures below. Figure 2.1 shows the chromatogram obtained for the fatty acid profile of isolate grown on Nutrient Broth containing 0% NaCl.

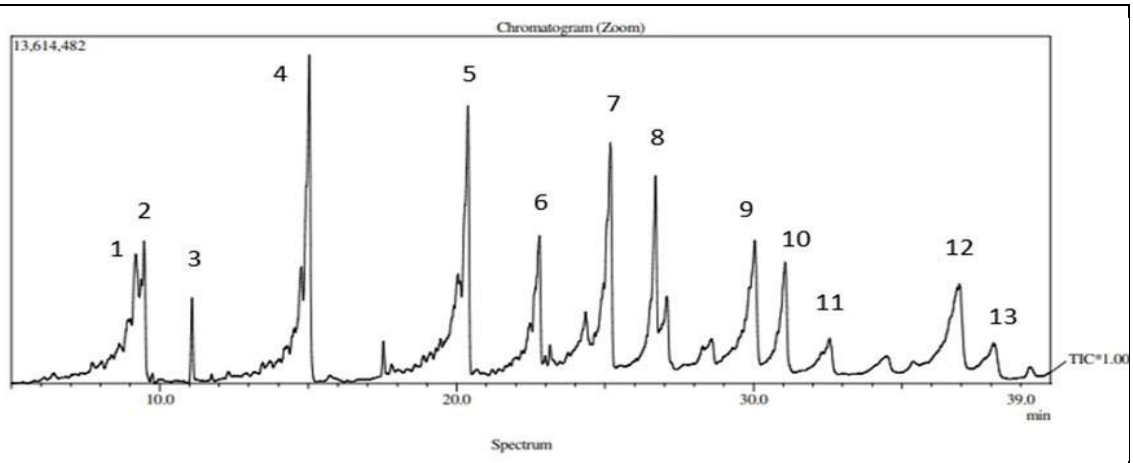


Figure 2.1. Fatty acid chromatogram of the isolate grown on Nutrient Broth Containing 0.5% NaCl.

Table 2.1. Similarity search results of the chromatogram of the fatty acid profile after growing the isolate at 0.5% NaCl.

Peak No.	Compound Name	Formula
1	Cyclononasiloxane, octadecamethyl-	C18H54O9Si
3	1,1,3,3,5,5-Hexamethyl-1,5-diphenyl-trisiloxane	C18H28O2Si
4	1,1,3,35,5,7,7,9,9,11,11,13,13,15,15-HEXAD	C16H50O7Si
5	1,1,3,35,5,7,7,9,9,11,11,13,13,15,15-HEXAD	C16H50O7Si
7	Cyclononasiloxane, hexadecamethyl-	C16H48O0Si

Figure 2.1 shows the chromatogram obtained for the fatty acid profile of isolate grown on Nutrient Broth containing 0.5% NaCl.

Table 2.1 gives the peak identification of the chromatogram of the isolate. Many peaks showed some similarity search according to the library while peak No. 2,6,8,9,10,11,12, and 13 requires further identification. The peaks identified are derivatives of C:16 and C:18 compounds.

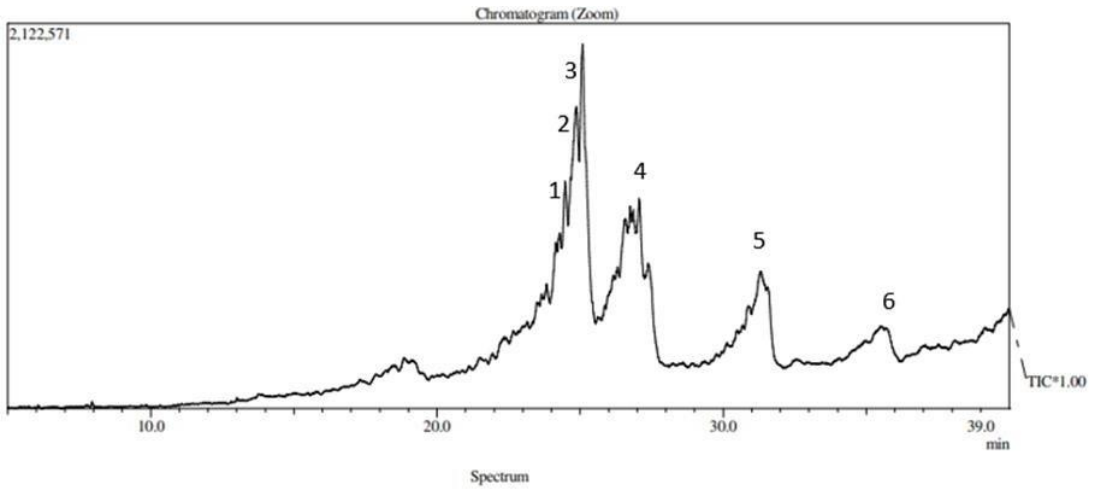


Figure 2.2. Fatty acid chromatogram of the isolate grown on Nutrient Broth Containing 2% NaCl.

Table 2.2. Similarity search results for Figure 2.2. chromatogram.

Peak No.	Compound Name	Formula
1	Cyclononasiloxane, octadecamethyl-	C18H54O9Si
2	Cyclononasiloxane, octadecamethyl-	C18H54O9Si
3	Cyclononasiloxane, octadecamethyl-	C18H54O9Si

Figure 2.2 shows the chromatogram obtained for the fatty acid profile of isolate grown on Nutrient Broth containing 2% NaCl.

Table 2.2 gives peak identification of the chromatograph in Figure 2.2. The identified peaks were of the same compound derivate of C:16. There were unidentified peaks (Peak No. 4,5 and 6) that did not show hits to the compounds present in the library. The identified compounds are saturated.

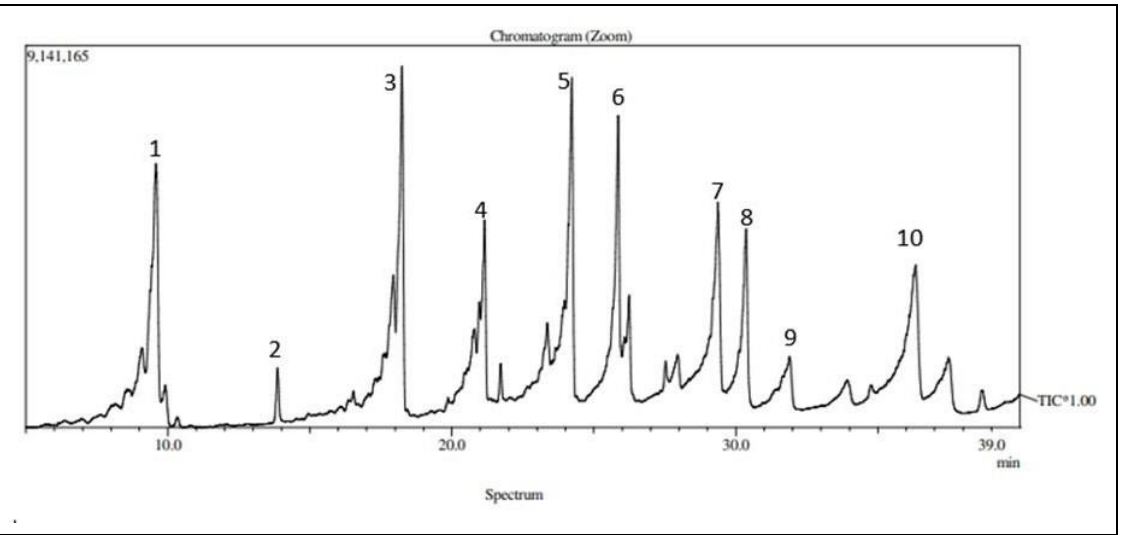


Figure 2.3. Fatty acid chromatogram of the isolate grown on Nutrient Broth Containing 8% NaCl.

Table 2.3. Similarity search results for Figure 2.3. chromatogram.

Peak No.	Compound Name	Formula
1	1,1,3,3,5,5,7,7,9,9,11,11,13,13-TETRADECA	C14H44O6Si
3	1,1,3,3,5,5,7,7,9,9,11,11-DODECAMETHYL-	C12H38O5Si
4	Octasiloxane	C16H50O7Si
9	1,1,3,3,5,5,7,7,9,9,11,11,13,13-TETRADECA	C14H44O6Si
10	Octasiloxane	C16H50O7Si

Figure 2.3 shows the chromatogram obtained for the fatty acid profile of isolate grown on Nutrient Broth containing 8% NaCl.

Table 2.3 gives information on the Figure 2.3 chromatogram. A total of 10 peaks were obtained, of which five peaks showed a hit with the library. Peak No. 2, 5,6,7, and 8 are yet to be identified. The compounds identified showed derivates of compounds C:16, C:18, and, C:12.

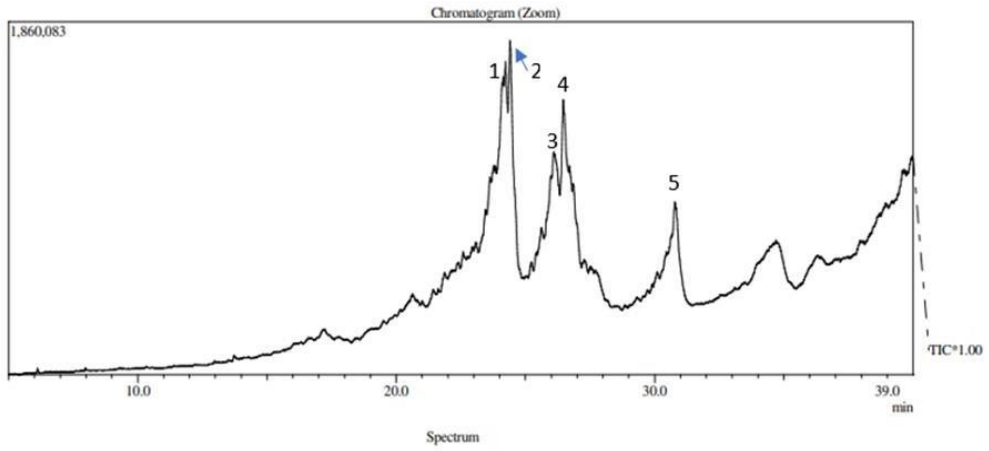


Figure 2.4. Fatty acid chromatogram of the isolate grown on Nutrient Broth Containing 12% NaCl.

Table 2.4. Similarity search results for Figure 2.4. chromatogram.

Peak No.	Compound Name	Formula
2	Cyclononasiloxane, octadecamethyl-	C18H54O9Si
3	1,1,3,3,5,5,7,7,9,9,11,11-Dodecamethyl	C12H38O5Si
5	Octasiloxane	C16H50O7Si

Figure 2.4 shows the chromatogram obtained for the fatty acid profile of isolate grown on Nutrient Broth containing 12% NaCl.

Table 2.4 gives information on Figure 2.4. chromatogram. The total peaks obtained were 5 out of which Peak No. 2, 3, and 5 were identified that showed hit with the library. These compounds are derivatives of C:12, C:16, and C:18 with a saturated nature of the compounds.

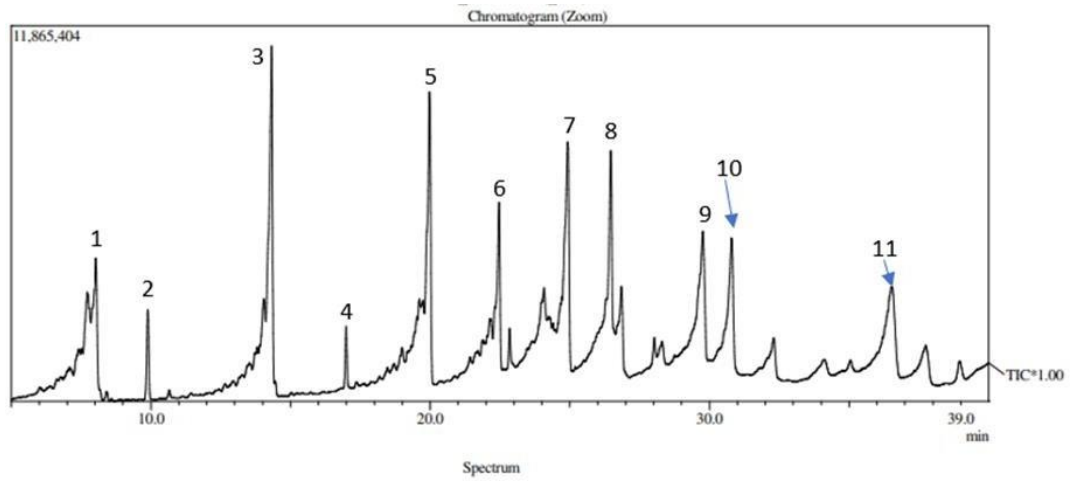


Figure 2.5. Fatty acid chromatogram of the isolate grown on Nutrient Broth Containing 16% NaCl.

Table 2.5. Similarity search results for Figure 2.5. chromatogram.

Peak No.	**Compound Name**	**Formula**
2	1,1,3,3,5,5-Hexamethyl-1,5-diphenyl-trisiloxane	C18H28O2Si
3	Octasiloxane	C16H50O7Si
5	1,1,3,3,5,5,7,7,9,9,11,11-Dodecamethyl	C12H38O5Si
7	Cyclononasiloxane, hexadecamethyl-	C18H48O8Si
9	Cyclononasiloxane, octadecamethyl-	C18H54O9Si
11	Octasiloxane	C16H50O7Si

Figure 2.5 shows the chromatogram obtained for the fatty acid profile of isolate grown on Nutrient Broth containing 16% NaCl.

Table 2.5 give peak information that was obtained in Figure 2.5. chromatogram. Peaks No. 2, 3, 5, 7, 9, and 11 showed hit with the library and were identified as derivates of C:12, C:16, and C:18. The unidentified peaks need further characterization.

The fatty acids U/S ratio obtained in the study carried did not show any significant amount of the U/S ratio, all the peaks that were identified showed saturated fatty acids. There can be a possibility that the unidentified peaks might be of fatty acids which would have provided speculation about the U/S ratio of the samples.

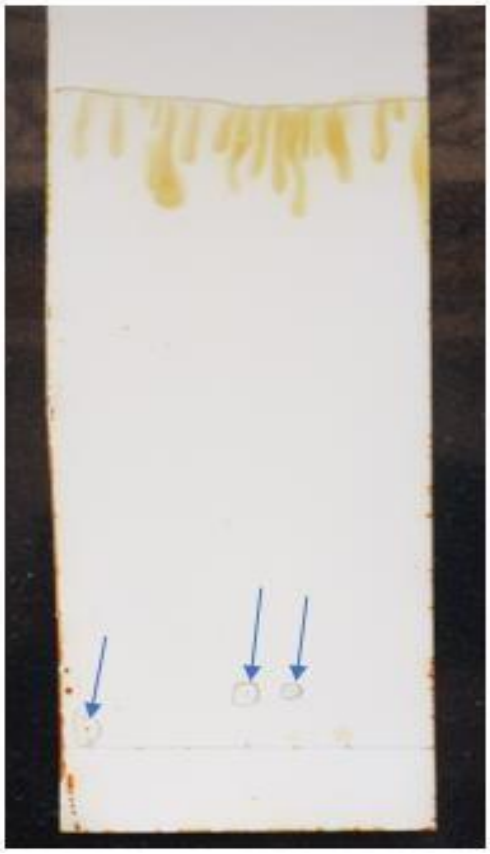
The fatty acid profiling of the isolate grown at different NaCl concentrations indicated that the main fatty acids which were identified in the cells were derivates of C:12, C:14, C:16, and C:18, out of which C:16 and C:18 were the major fatty acids found in the cells. This suggests that any modulation or fluctuations that have to be done in the cell in response to salinity stress are observed in these fatty acids.

A study carried out by (Gandhi & Shah, 2016) suggests that during salt stress the bacterialcells' unsaturation to saturation (U/S) ratio changes so as to manage or overcomethe environmental stress. These changes in the U/S ratio stabilize the plasma membrane by directly affecting its fluidity.

4.3. Effect of salinity on the lipid composition of the halophilic bacteria:

The phospholipid profile of the isolate at different concentrations of NaCl was evaluated by thin-layer chromatography, and the developed plates with stains mentioned in section "3.4" were examined for the spots. The results are summarised in Figure. 2.

Figure No. 2. Separation of lipid extraction on TLC plates.



a. Staining with iodine vapours. NaCl concentration (%) right to left (0, 0.5, 2, 8, 12, 16).

b. Staining with Dragandroff reagent. NaCl concentration (%) right to left (0, 0.5, 2, 8, 12, 16).

After exposing the plate to iodine vapours spots were obtained in Nutrient broth with NaCl concentrations of 0%, 8%, and 12% NaCl, and the Rf values of the spots were 0.04, 0.08, and 0.10 respectively. Similarly, the other plate sprayed with Dragandroff reagent obtained spots in NaCl concentration of 0%, 0.5%, 2%, 8%, and 16%. The spot obtained in 0% and 16% were comparatively migrated from the spotting region, while the other spots remained in the spotting region. The Rf values of spots obtained in 0%, and 16% NaCl concentrations were 0.04, and 0.51 respectively. The results cannot be conclusive because the Rf values obtained did not co-relate with standard studies.

The phospholipids in the cells are modified under salt stress so as to modulate the cell membrane fluidity. Plasma membrane possesses net negative hence the negative charge of the plasma membrane and positive charge of salt molecules can cause electrostatic forces which can de-stabilizes the membrane. To overcome this stress the lipid machinery of the cells reduces the synthesis of anionic phospholipids which in turn will lower these electrostatic forces and stabilizes the membrane and helps in the survival of the cell (Gandhi & Shah, 2016).

CHAPTER 6:

SUMMARY

The present study focuses on the physiological factors of halophilic bacteria, which encompass factors like morphological characteristics, lipid analysis, and fatty acid profiles under varying salinity conditions.

The isolate's physiology was studied by cultivating it in salinity stress and analyzing the adaptive mechanism and effect of salt stress on it. The halophilic bacterium has to overcome the salinity stress exerted by the surrounding hypersaline environments and to do so it brings about some physio-chemical changes in it. The cell in the salinity stress will show different morphology such as the formation of long filaments or short cocci because of the alterations in the cell division compared to its optimum salinity.

The cell membrane, which is an integral part of the cell structure, also varies toward the salinity stress to modulate the passage of sorting osmolytes and salt molecules. The reason behind the variation of the plasma membrane lies in the core or precursor molecules that build the plasma membrane, i.e., phospholipids and fatty acids. The phospholipid changes in the membrane happen to stabilize the membrane by regulating the synthesis of charged phospholipids, which in turn will interact with the charges of molecules present in the vicinity of the cell. Also, the modulation of the fatty acid helps in the adaptation of the plasma membrane. The salinity stress is mainly overcome by upregulating the synthesis of unsaturated fatty acids which will help in the structural integrity of the plasma membrane.

The cell membrane of any cell is an essential structural component that acts as a barrier for solute molecules and maintains the cell structure; therefore, studying its regulation is essential, which in turn will determine the cell viability under any stress.

CHAPTER 7:

FUTURE PROSPECTS

The isolate's morphology, fatty acid content, and lipid content were studied concerning salt stress. These studies provided a piece of information about the adaptive changes a cell can bring about in its physiochemical properties during stress. Further investigations can be carried out to identify the unidentified fatty acids in the present study. This will provide more detailed insight into the modulation of membranes during stress. Also, like membrane modulation that happens to take place during salt stress, further investigation can be done on the membrane proteins regulation during the same.

CHAPTER 8:

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APPENDIX

❖ **Media:**

Nutrient Agar (pH 7.4)

Ingredients	Grams/Litre
Peptone	5
Sodium chloride	5
Yeast extract	2
Agar powder	15

Nutrient Broth (pH 7.4)

Ingredients	Grams/Litre
Peptone	5
Sodium chloride	5
Yeast extract	2
Beef extract	1

❖ **Reagents and Solutions:**

- **Gram's staining kit:**
- Crystal violet
- Gram's iodine
- Decolourizer
- Safranin

❖ Methanol

❖ MiliQ water

❖ Dragendroff reagent

❖ Phosphate Buffer Saline