EFFECT OF PHAGE INFECTION AND SELECTED PHYSICO-CHEMICAL FACTORS ON GROWTH PATTERNS OF PROTEOBACTERIA AND CYANOBACTERIA IN LAKE MAGADI, KENYA

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Declaration

I, Bancy N. Muruga, hereby declare that this is my original work, and has not been presented for a degree in any other University.

Bancy N. Muruga

Sign --

Date --

We confirm that the work reported in this thesis was carried out by the candidate under our supervision and has been submitted with our approval as the University supervisors

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Date --

Dedication

I dedicate this work to my late father and late eldest brother for being life pacesetters for me and for many others.

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Table of Contents

Declaration ii

Dedication iii

Acknowledgements iv

Table of Contents v

List of Tables ix

List of Figures x

List of Abbreviations and Acronyms xi

List of Chemical Symbols xii

Abstract xiii

CHAPTER ONE: GENERAL INTRODUCTION 1

1.1 Dynamics of Lake Magadi ecosystem 1

1.2 Problem statement 2

1.3 Justification of study 3

1.4 Objectives 4

1.4.1 Broad objective 4

1.4.2 Specific objectives 4

CHAPTER TWO: LITERATURE REVIEW 5

2.1 Overview of lake ecosystems 5

2.2 Physicochemical characteristics of hypersaline environments 6

2.3 Overview of phages 11

2.3.1 Bacteriophages 11

2.3.2 Cyanophages 13

2.4 Overview of cyanobacteria 14

2.5 Taxonomy of cyanobacteria 16

2.6 Economic importance of cyanobacteria 17

2.7 Impact of cyanobacteria on ecosystem 19

2.8 Previous studies on phages in alkaline-saline lakes 22

2.8.1 Studies at Lake Nakuru 22

2.8.2 Studies at Mono Lake 25

CHAPTER THREE: GENERAL MATERIALS AND METHODS 27

3.1 Study area 27

3.2 Sampling of water from Lake Magadi 30

3.3 Types of media used for isolation of cyanobacteria, proteobacteria and phages 32

3.3.1 Aiba and Ogawa medium for marine cyanobacteria 32

3.3.2 The medium modified for haloalkaliphilic bacteria 32

3.3.3 Phage growth media 32

CHAPTER FOUR: DIVERSITY OF PROTEOBACTERIA AND CYANOBACTERIA SPECIES IN LAKE MAGADI WATER 33

Abstract 33

4.1 Introduction 34

4.2 Materials and methods 36

4.2.1 Isolation and characterization of bacteria from Lake Magadi water 36

4.2.2 Identification of bacterial isolates by analysis of 16S rRNA gene 37

4.2.3 Isolation and identification of cyanobacteria 39

4.3 Results 39

4.3.1 Morphological and biochemical characteristics of bacterial isolates 39

4.3.2 Identity of bacteria from Lake Magadi based on analysis of 16S rRNA gene 40

4.3.3 Identity of cyanobacterial isolates sampled from Lake Magadi water 43

4.4 Discussion 49

4.5 Conclusions 56

CHAPTER FIVE: EFFECT OF PHYSICOCHEMICAL FACTORS ON GROWTH PATTERNS OF CYANOBACTERIA ISOLATES 58

Abstract 58

5.1 Introduction 59

5.2 Materials and methods 60

5.2.1 Determination of growth patterns of unicellular cyanobacterial isolates 60

5.2.2 Determination of optimum temperature for growth of cyanobacterial isolates 61

5.2.3 Determination of optimum pH for growth of cyanobacterial isolates 61

5.2.4 Determination of optimum salinity for growth of cyanobacterial isolates 62

5.3 Results 62

5.3.1 Growth patterns of unicellular cyanobacteria species 62

5.3.2 Effect of temperature on growth of cyanobacterial isolates 63

5.3.3 Effect of pH on growth of cyanobacterial isolates 66

5.3.4 Effect of salinity on growth of cyanobacterial isolates 68

5.4 Discussion 70

5.5 Conclusions 71

CHAPTER SIX: EFFECT OF PHAGE INFECTION ON GROWTH PATTERNS OF PROTEOBACTERIA AND CYANOBACTERIA HOSTS 73

Abstract 73

6.1 Introduction 74

6.2 Materials and methods 75

6.2.1 Determination of phage host range by plaque assay technique 75

6.2.2 Determination of effect of phage infection on growth of their bacterial hosts 77

6.2.3 Determination of effect of variation in temperature and pH on cyanophage infectivity 78

6.3 Results 79

6.3.1 Host range of phage 79

6.3.2 Effect of phage infection on growth of proteobacterial and cyanobacterial hosts 81

6.3.3 Effect of variation of temperature and pH on phage infectivity 84

6.4 Discussion 85

6.5 Conclusions 90

CHAPTER SEVEN: GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS 92

7.1 General discussion 92

7.2 General conclusions 94

7.3 Recommendations 95

REFERENCES 97

List of Tables

Table 2.1: Concentration of ions in hypersaline brines---8

Table 2.2: Physicochemical conditions of sites 1 to 4 at Lake Magadi-------------------------------9

Table 2.3: Physicochemical conditions of sites 5 to 8 at Lake Magadi------------------------------10

Table 4.1: Characteristics of proteobacterial isolates from Lake Magadi---------------------------40

Table 4.2: Bacteria isolates and their similarity to their closest match on NCBI GenBank -----42

Table 4.3: Morphological characteristics of cyanobacteria isolated from Lake Magadi-- -------48

Table 5.1: Growth characteristics of extremophiles ---60

List of Figures

Fig. 3.1: Parts of Lake Magadi colored pink by haloalkaliphilic archaea----------------------------28

Fig. 3.2: Semi arid environs of Lake Magadi--29

Fig. 3.3: The environs of Lake Magadi showing Loita Hills--29

Fig 3.4: Water sampling sites on the shoreline of Lake Magadi-------------------------------------31

Fig. 4.1: Colonies of proteobacterial isolates sampled from Lake Magadi-------------------------40

Fig. 4.2: Ethidium bromide-stained electrophoresis gel of PCR product on 1 % agarose --------41

Fig. 4.3: Phylogenetic tree of proteobacterial isolates---43

Fig. 4.4: Unicellular cyanobacterial isolates---44

Fig. 4.5: Filamentous cyanobacterial isolates---45

Fig. 4.6: Colonies of cyanobacterial isolates sampled from Lake Magadi------------------------46

Fig. 5.1: Growth patterns of unicellular cyanobacterial isolates-------------------------------------63

Fig. 5.2: Growth of cyanobacterial isolates in varying temperature conditions---------------------65

Fig. 5.3: Growth of cyanobacterial isolates in varying pH conditions-------------------------------67

Fig. 5.4: Growth of cyanobacterial isolates in varying salinity conditions--------------------------69

Fig. 6.1: Cyanobacterial isolates used to determine phage host range ------------------------------77

Fig. 6.2: Plaques formed on proteobacterial isolate --80

Fig.6.3: Plaques formed on cyanobacterial isolate and carboxysomes------------------------------81

Fig. 6.4: Growth of phage - infected and non-infected proteobacteria -----------------------------83

Fig. 6.5: Growth of phage - infected and non- infected cyanobacteria------------------------------84

Fig. 6.6: Growth of cyanobacterial isolate inoculated with phage at varied temperature and pH--85

List of Abbreviations and Acronyms

CICBN---------- Committee on the International Code of Botanical Nomenclature

CFU--------------Colony Forming Unit

DNA--------------Deoxyribonucleic Acid

EDTA-------------Ethylene Diamine Tetraacetic Acid

GIS----------------Geographic Information Systems

NCBI--------------National Centre for Biotechnology Information (USA)

PFU----------------Plaque Forming Unit

PPT-----------------Parts per Thousand

rRNA--------------Ribosomal Ribonucleic Acid

TAE----------------Tris Acetate EDTA

TN-----------------Total Nitrogen

TP------------------Total Phosphates

LL------------------Low Light

HL------------------High Light

Vp/l----------------Viral particles per liter

List of Chemical Symbols

Ca^{2+} ----------------Calcium ions

$CaCO_3$ ------------Calcium carbonate

Cl^- ------------------Chloride ions

CO_2 ----------------Carbon IV oxide

CO_3^{2-} --------------Carbonate ion

HCO_3^- -------------Hydrogen carbonate ion

K_3PO_4 -------------Potassium phosphate

KH_2PO_4 ---------- Potassium hydrogen phosphate

Mg^{2+}--------------- Magnesium ion

Na^+ ---------------- Sodium ion

Na_2CO_3 ---------- Sodium carbonate

Na_2HPO_4 --------- Sodium hydrogen phosphate

NaCl ----------------Sodium chloride

NaCN --------------Sodium cyanide

NH_4^+ ---------------Ammonium ion

N_2 ------------------Nitrogen gas

NH_4Cl -------------Ammonium chloride

NO_2^- ----------------Nitrite ion

NO_3^- ----------------Nitrate ion

Abstract

Lake Magadi is a hypersaline soda lake in the Rift Valley of Kenya. It harbors diverse species of photosynthetic bacteria and cyanobacteria which contribute significantly to primary production. Factors that impact on the growth of photosynthetic bacteria affect the primary productivity of the lake. This study aimed at investigating the effect of selected physicochemical conditions and phage infection on the growth patterns of proteobacteria and cyanobacteria. Water samples were collected from Lake Magadi shoreline in January of 2012 and 2013. Proteobacteria and cyanobacteria were isolated on medium modified for haloalkaliphiles and Aiba and Ogawa medium modified for marine cyanobacteria. To identify the proteobacterial isolates, 16S rRNA gene was analyzed by extraction of genomic DNA, amplification by polymerase chain reaction (PCR) using 16S primer, followed by gene sequencing and phylogeny. Identification of cyanobacterial isolates was based on cell and colony morphology. The cyanobacterial isolates were subjected to varying physicochemical conditions of temperature, pH and salinity and their growth monitored. Growth of proteobacterial isolates was determined by measurement of optical density (680 nm) of culture grown in liquid medium while that of cyanobacteria isolates was determined by cell enumeration using a Neubauer chamber. Phage growth medium was used for phage isolation and host range determined by plaque assay technique. The effect of infection by phage on the growth of proteobacteria and cyanobacteria hosts was determined. Two DNA sequences from the bacterial isolates were obtained and aligned to sequences in the National Centre for Biotechnology Information (NCBI) database. One of the bacterial isolates had over 99 % similarity to *Idiomarina* sp. of the class Gammaproteobacteria while the second had 99 % similarity to *Rhodobacter* sp. of the class Alphaproteobacteria. Phylogenetic analysis showed close clustering of each isolate to members of their respective classes. Cyanobacterial isolates

included colony forming, unicellular genera such as *Synechococcus* and *Microcystis* and colony forming, filamentous ones such as *Anabaena* and *Arthrospira*. Optimum growth of cyanobacteria isolates was found to occur at room temperature ($23 \pm 2\ ^{o}C$) in adequate light, pH 6 to pH 7 and salinity of between 0.0 to 0.3 % w/v. Plaques of about 1 - 2 mm in diameter developed on the proteobacterial isolate (*Rhodobacter* sp.) and 1.2×10^{5} PFU/ml were counted in two days of incubation. Growth of the proteobacteria host with phage infection was significantly ($p = 0.009$) lower than the one without phage infection. Plaques of 5 mm in diameter were observed on cyanobacterial isolate (*Synechococcus*) and 1.18×10^{4} PFU/ml were counted in 10 days. The population of the cyanobacterial host inoculated with phage lysate decreased from 1.5×10^{7} cells/ml to about 0.6×10^{7} cells/ml in 10 days, a decrease of about 50%. There was a difference of about 4.9×10^{7}cells/ml between the phage - infected and the uninfected cyanobacteria isolate in 10 days. Infection by phage caused a significant ($p<0.001$) decline on the growth of the cyanobacteria host. The findings of this study may be used by ecologists to correlate diversity and population sizes of proteobacteria and cyanobacteria species to presence of infectious phages and prevailing physicochemical factors in the lake. The findings may also be useful to environmentalists when there is need to vary primary productivity of the lake and other aquatic ecosystems.

Key words: Soda Lake, physicochemical conditions, proteobacteria, cyanobacteria, phage.

CHAPTER ONE: GENERAL INTRODUCTION

1.1 Dynamics of Lake Magadi ecosystem

Lake Magadi is a closed lake basin in the Southern part of the Gregory Rift, a branch of Africa's Great Rift Valley. The Magadi-Natron basin had been occupied by a larger body of water on several occasions during late Pliocene and Pleistocene times. Both Lake Magadi and Lake Natron are saline, but Lake Magadi is the most saline of the soda lakes in the Gregory Rift (Grant, 1992).

The closed system exhibits complete nutrient cycling under anaerobic and aerobic conditions. Cyanobacteria notably *Arthrospira* are the major primary producers. Primary productivity is also contributed by anoxygenic phototrophic bacteria such as *Ectothiorhodospira* (*Halorhodospira*) (Jones *et al,* 1998) *and Rhodobaca.* Chemoorganotrophs such as the genus *Halomonas* and *Natronococcus* produce hydrolytic enzymes including proteinases, cellulases, lipases and amylases in order to utilize the products of primary production (Milford *et al.,* 2000).

Nitrogen cycling involves production of ammonia by fermentative bacteria such as *Tindalia magadii*. Ammonia is used by nitrifiers to produce nitrate which is used by the autotrophs. Sulfate reducing bacteria provide sulfide as the electron donor for the sulfur oxidizing bacteria, anaerobic autotrophs and heterotrophs (Kevbrin *et al*., 1998). Methane produced by methanogenic bacteria is not lost from the system as methane oxidizers such as the *Methylobacter* sp. may be present in the soda lake (Jones *et al.*, 1998).

Intraspecific and interspecific interactions range from cooperative and competitive to antagonistic (such as predator-prey interactions). An important consequence of these interactions is coevolution (Fuhrman, 1999). Phages occurring in these ecosystems regulate species composition of bacterial and phytoplankton populations hence impact on biological production (Fuhrman, 1999). Haloalkalophilic bacteria, archaea and cyanobacteria present in the lake act as hosts for the numerous bacteriophages and cyanophages that inhabit the waters.

Lake Magadi is a popular destination for many animals due to the fact that it is situated between Maasai Mara and Amboseli National Parks, but very few animals actually have any contact with, or live in the lake itself. The lake is also a popular destination for wading birds during the dry season including flamingos, heron, pelicans, and spoonbills. The birds congregate in the seasonal streams of fresh water that run into the lake because they bring in large amounts of diverse food. Lagoons formed by the hotsprings support a thriving colony of fish, the *Alcolapia grahami* species, which can thrive on pH of 10.5 and temperature of 39 ^{o}C (Tindall, 1988).

1.2 Problem statement

Some of the impediments to a global assessment of inland waters have diminished, leading to the emergence of a sub-discipline that could rightly be referred to as global limnology. The wide availability and improving quality of GIS information has facilitated enumeration and measurement of water bodies (Wetzel, 1990). Studies that focus on individual lakes or lake clusters can be supplemented with analyses that take into account the influences of climate, hydrology and morphometry of the water bodies as well as the populations of organisms that

thrive in them. Attempts to generalize globally about lakes or other inland waters will strengthen the conceptual basis of limnology and promote a better understanding of the connection of lakes with other ecosystems (Lehner and Döll, 2004).

Phages play important roles in aquatic ecosystems through carbon cycling in food webs, gene transfer by transduction and conversion of hosts by lysogeny. However little is known about the natural distribution of viruses that infect the photosynthetically important group of marine prokaryotes, the cyanobacteria. Environmental factors and the physiological state of cyanobacteria that affect cyanophage-cyanobacterial interactions remain poorly understood in many aquatic ecosystems (Suttle, 2007).

1.3 Justification of study

Phages are of critical importance in that they cause substantial lysis of bacteria and cyanobacteria. This in turn exerts an extensive effect on the dynamics of carbon flow in many of the aquatic environments (Martin and Kokjohn, 1999). It is important to study the impact of viruses on processes such as nutrition and mortality of microorganisms, maintenance of species diversity and cycling of organic matter in aquatic ecosystems (Sime-Ngando *et al*., 2003).

Photosynthetic proteobacteria and cyanobacteria are important in aquatic ecosystems for primary production. Heterocyst-forming cyanobacterial species have a major role, as they are able to fix nitrogen gas into ammonia, nitrites or nitrates which can be absorbed by plants and converted to protein and nucleic acids. Appropriate cyanophages can be used by environmentalists as a means of controlling cyanobacterial blooms.

1.4 Objectives

1.4.1 Broad objective

To investigate the effect of phage infection and selected physicochemical factors on growth patterns of proteobacteria and cyanobacteria species in Lake Magadi.

1.4.2 Specific objectives

i. To determine the diversity of proteobacteria and cyanobacteria species in water samples from Lake Magadi.

ii. To establish the effect of selected physicochemical factors on growth patterns of cyanobacterial isolates.

iii. To determine the effect of phage infection on growth patterns of proteobacterial and cyanobacterial hosts.

CHAPTER TWO: LITERATURE REVIEW

2.1 Overview of lake ecosystems

Lentic waters range from ponds to lakes and wetlands as opposed to lotic ecosystems which involve flowing terrestrial waters such as rivers and streams. Lakes have three regions, the pelagic (open) water zone, the benthic comprising the shore and bottom regions and the profundal region, the deep bottom regions not exposed to light (Kalff, 2002). These three areas can have very different abiotic conditions and hence host species that are specifically adapted to live there. Lakes are also divided into photic and aphotic regions, the prior receiving sunlight and latter being below the depths of light penetration, making it void of photosynthetic capacity. In relation to lake zonation, the pelagic and benthic zones are considered to lie within the photic region, while the profundal zone is in the aphotic region (Brönmark and Hansson, 2005).

Bacteria are present in all regions of lentic waters. Free-living forms are associated with decomposing organic material, biofilm on the surfaces of rocks and plants, suspended in the water column, and in the sediments of the benthic and profundal zones. Other forms are also associated with the guts of lentic animals as parasites or in commensal relationships. Bacteria play an important role in system metabolism through nutrient recycling (Kalff, 2002).

Benthic invertebrates can be filter feeders, grazers feeding on algae, deposit feeders that consume sediment, digesting any organic material it contains or predators that capture and consume living animals (Jónasson, 2005). The profundal zone is home to a unique group of filter feeders that use small body movements to draw a current through burrows that they have created

in the sediment. This mode of feeding requires the least amount of motion, allowing these species to conserve energy (Brown, 1987). A small number of invertebrate taxa are predators in the profundal zone. These species are from other regions and only come to these depths to feed. The vast majority of invertebrates in this zone are deposit feeders, getting their energy from the surrounding sediments (Jónasson, 2005). Fish can graze on periphyton and macrophytes or pick phytoplankton out of the water column, feed on zooplankton in the water column, insects at the water's surface, other fish or detritus (Brönmark and Hansson, 2005).

The organisms in aquatic ecosystems are loosely associated at specific trophic groups as primary producers, herbivores, primary carnivores and secondary carnivores. Generally, top-down processes dictate that the abundance of prey taxa is dependent upon the actions of consumers from higher trophic levels while bottom-up processes function when the abundance or diversity of members of higher trophic levels is dependent upon the availability or quality of resources from lower levels (Brönmark and Hansson, 2005).

2.2 Physicochemical characteristics of hypersaline environments

Hypersaline environments have been defined as environments with total salt concentrations exceeding that of seawater (Grant, 2004). Most hypersaline aquatic ecosystems are classified into thalassic waters that describe marine waters with high concentrations of NaCl and athalassic waters that refer to non-marine waters with an appreciable salt content (Maturrano *et al*., 2006). A typical example of thalassohaline environments are solar salterns, where sea salt is produced as a result of the evaporation of seawater (Grant, 2004). Athalassohaline hypersaline

environments contain ionic compositions markedly different from that of thalassohaline environments. Potassium, magnesium and sodium are the most dominant in athalassic waters (non-marine waters) systems and are frequently the sources of potash, magnesium metal, soda and borax if the waters also contain high concentration of boron (Gillevet, 2002). However, the chemical composition of seawater brines (thalassohaline) is chiefly characterized by having chloride as its major anion (Galinski and Trüper, 1994).

In addition to ionic compositions, pH is important in determining the nature of microbial populations in hypersaline water (Grant, 2004). In the neutral saline lakes (pH 6 to 8.5) sodium and magnesium form the major cations and chloride and sulphate represent the major anions with low buffering capacity (Jones *et al*., 1998).

Well studied ecosystems such as Mono Lake (USA), the Dead Sea, the alkaline soda lakes in Egypt (Wadi Natrun) and Lake Magadi are typical examples of athalassohaline hypersaline systems (Gillevet, 2002). Athalassohaline soda brines lack the divalent cations of magnesium and calcium because of their low solubility at alkaline pH (DasSarma, 2006). All these ecosystems are populated by microorganisms, which must be very well adapted to the conditions. Table 2.1 compares ion concentration of thalassohaline (seawater) and athalassohaline (close to neutral saline water, Dead Sea, and alkaline soda lakes, Lake Magadi and Wadi Natrun) systems.

Table 2.1: Concentration of ions (g/l) in thalassohaline (sea water) and athalassohaline brines

Ion	Sea water	Dead Sea	Lake Magadi	Wadi Natrun
Na^+	10.8	339.1	161.0	142.0
Mg^{2+}	1.3	42.4	0.0	0.0
Ca^{2+}	0.4	17.2	0.0	0.0
K^+	0.4	7.6	2.3	2.3
Cl^-	19.4	219.0	11.8	154.6
SO_4^{2-}	2.7	0.4	16.8	22.6
CO_3^{2-}/HCO_3^-	0.3	0.2	23.4	67.2
pH	8.2	6.3	11.0	11.0

Source: Grant (2004)

Saltwater can evaporate leaving evaporite deposits consisting of salts such as sodium chloride (halite) and calcium sulfate (gypsum). Within evaporites are fluid inclusions (small trapped pockets of water) that can provide a refuge for microbes for at least six months (DasSarma, 2006). Cyanobacteria trapped within dry evaporite crusts can continue to have low levels of metabolic function such as photosynthesis. These deposits also form fossils of the organisms trapped within them. Although highly controversial, it has been claimed that bacteria might survive for millions of years in the fluid inclusions of salt deposits (DasSarma, 2006). Some physical chemical parameters measured at Lake Magadi in January 2009 (Table 2.2) showed water temperature ranging from 30 °C to 47 °C and pH ranging from pH 10 to pH 11.

Table 2.2: Physicochemical conditions of hot springs at Lake Magadi in January 2009

Parameter	Site 1	Site 2	Site 3	Site 4
Coordinates	1˚59.320S	1˚59.320S	01˚59.542S	01˚57.240S
	36˚15.87E	36˚15.896E	36˚15.890E	36˚13.880E
Elevation	606 m	606 m	603 m	625 m
Temp (°C)	37°C	34°C	42°C	47°C
pH	pH11.0	pH10.0	pH10.3	pH10.5
H_2O_2 (ppm)	<0.50	<0.50	<0.50	<0.50
NO_3^- (ppm)	0.00	1.00	0.00	0.00
NO_2^- (ppm	0.00	0.20	0.00	0.00
ORP (mV)	-47.70	-80.90	-29.10	-43.70
DO (%)	30.50	51.10	22.70	25.70
DO (mg/l)	1.85	2.88	1.07	1.55
EC (mS/cm)	37.68	20.26	39.8	14.07
TDS (mg/l)	20.40	10.03	21.49	9.61
Salinity	25.55	11.73	24.15	7.89

Source: Muruga (2010)

H_2O_2, - Hydrogen peroxide, NO_3,- Nitrate, NO_2 – Nitrite, all in parts per million, ORP (mV) - Oxidation-Reduction Potential in milliVolts, DO-Dissolved Oxygen, EC (mS/cm) - Electrical conductivity in millisiemens per centimeter, TDS -Total dissolved solutes

Table 2.3 Physicochemical conditions of three hot springs and along the Western causeway at Lake Magadi in January 2009

Parameter	Site 5	Site 6	Site 7	Site 8
Coordinates	1˚55.880S 36˚13.880E	1˚51.026S 36˚13.030E	1˚51.392S 36˚14.200E	1˚51.800S 36˚14.552E
Elevation	601 m	601 m	605 m	605 m
Temp(°C)	44°C	30°C	43°C	34°C
pH	pH 10.0	pH 10.0	pH 10.0	pH 10.0
H_2O_2 (ppm)	<0.500	<0.50	<0.50	<0.50
NO_3^- (ppm	0.00	0.00	0.00	0.00
NO_2^- (ppm)	0.00	0.00	0.00	0.00
ORP (mV)	-40.80	-40.50	-41.22	-43.60
DO (%)	24.50	24.20	25.50	24.59
DO (mg/l)	1.57	1.32	1.61	1.70
EC (mS/cm)	32.98	34.17	36.32	36.88
TDS (mg/l)	16.49	18.19	18.48	18.61
Salinity	20.54	23.46	18.19	20.30

Source: Muruga (2010)

H_2O_2, - Hydrogen peroxide, NO_3,- Nitrate, NO_2 – Nitrite, all in parts per million, ORP (mV) - Oxidation-Reduction Potential in milliVolts, DO-Dissolved Oxygen, EC (mS/cm) - Electrical conductivity in millisiemens per centimeter, TDS -Total dissolved solutes

2.3 Overview of phages

2.3.1 Bacteriophages

Bacteriophages are viruses that infect and replicate within bacteria. They are composed of proteins that encapsulate a DNA or RNA genome, and may have relatively simple or elaborate structures. Their genomes may encode as few as four genes, and as many as hundreds of genes. Bacteriophage are among the most common and diverse entities in the biosphere (McGrath and van Sinderen, 2007). They are classified into families with regard to their morphology and size. About 96% of them are tailed, but there are filamentous and pleomorphic ones as well (Ackermann, 2007). Generally, the phage virion consists of two basic components, the nucleic acid (double or single-stranded RNA or DNA) and a protein envelope. Some have lipids as components of the envelope (Ackermann, 2003).

Viruses often out number bacteria by 15- fold (Suttle, 2007). Algicidal bacteria become abundant during harmful algal blooms (Barlaan *et al*., 2007). Due to their rapid growth and high cell density, the algicidal bacteria become more vulnerable to phage infection and lysis (Wommack, 2000). Thus phage can also influence the growth and termination of bloom-forming algae by regulating the numbers of algicidal bacteria.

Nineteen families that infect bacteria (including cyanobacteria) and archaea are currently recognized. Of these, only two families have RNA genomes while the rest have DNA. Of the seventeen viral families with DNA genomes, only two have single-stranded genomes while the rest are double-stranded. Eight of the viral families with DNA genomes have circular genomes, while nine have linear genomes. Nine families infect bacteria only, nine infect archaea only, and

one (*Tectiviridae*) infects both bacteria and archaea. Of the nineteen, only five families are enveloped (Mc Grath and van Sinderen, 2007).

Since ancient times, reports of river waters having the ability to cure infectious diseases, such as leprosy, have been documented. In 1896, Ernest Hanbury Hankin reported that something in the waters of the Ganges and Yamuna rivers in India had marked antibacterial action against cholera. In 1917, French Canadian microbiologist Felix d'Herelle, discovered a virus parasitic on bacteria and called it a bacteriophage (from the Greek *phagein* meaning to eat) or bacteria-eater. He also recorded the healing of a man suffering from dysentery by the use of bacteriophages and henceforth introduced the concept of phage therapy (d'Hérelle, 1949).

Phage therapy has been used in Georgia and Central and Eastern Europe. Globalyz Biotech is an international joint venture that commercializes bacteriophage treatment and its various applications across the globe. The company has successfully used bacteriophages in treating bacterial infections, including *Staphylococcus, Streptococcus, Pseudomonas, Salmonella*, skin and soft tissue, gastrointestinal, respiratory, and orthopedic infections. Meanwhile, Western scientists are developing engineered viruses to overcome antibiotic resistance, and engineering the phage genes responsible for coding enzymes which degrade the biofilm matrix, phage structural proteins and also enzymes responsible for lysis of bacterial cell wall (BBC Horizon, 1997).

Bacteriophages have also been used in hydrological tracing and modeling in river systems, especially where surface water and groundwater interactions occur. The use of phages is

preferred to the more conventional dye marker because they are significantly less absorbed when passing through ground waters and they are readily detected at very low concentrations (Martin, 1988). Phages also served as model microorganisms for some of the most significant discoveries in the field of molecular biology, including deciphering the genetic code and the discovery of the transduction phenomenon (Aze and Pasternack, 2010).

Other uses include application as a food additive, to target and kill *Listeria monocytogenes* in ready-to-eat poultry and meat products (Steele, 2006), spray application in horticulture for protecting plants and vegetable produce from decay and spread of bacterial disease, as biocides for environmental surfaces in hospitals, and as preventative treatments for catheters and medical devices such as uniforms, curtains, or even sutures for surgery prior to use in clinical settings. Clinical trials show success in veterinary treatment of pet dogs with otitis (Wright *et al*., 2009). Bacteriophages are also important model organisms for studying principles of evolution and ecology. They make positive contributions to the fitness of bacteria hosts through phage conversion by transduction of genes from other bacteria (Fuhrman, 1999).

2.3.2 Cyanophages

Cyanophages are the viruses that attack cyanobacteria. They were first discovered by Safferman and Morris (1963) from a waste stabilization pond of Indiana University (USA). The first cyanophage studied was the cyanophage attacking *Lyngbya, Plectonema* and *Phormidium.* They named the virus as LPP-1 using the first letter of the three genera. Thereafter, several serological strains of LPP were isolated from different parts of the world and named LPP-1, LPP-2, LPP-3, LPP-4 and LPP-5. Besides LPP groups of cyanophages, other cyanophages such as SM-I, AS-I,

N-I, C-I, AR-I and AI have been reported (Safferman and Morris, 1963).

Phages are regarded as one of the major elements in the ocean regulating biogeochemical cycling, mediating gene transfer, influencing climate change, and modulating community structure. Phage ecology impacts on the biotic as well as the abiotic world, in particular the flow of energy between and through ecosystems, especially aquatic ones (Fuhrman, 1999). Bacteria consume, produce, and store nutrients and energy and also contribute to the decomposition of other organisms. Phage infections cause solubilization of bacteria cells, by host-cell lysis. Solubilized bacteria can no longer function as consumers, producers, or decomposers and are also less available as food to bacteria grazers (protists or animals). This results in reduction of productivity of bacteria populations as well as a delay in the movement up food chains of bacteria-contained nutrients and energy. Phage DNA and protein coats, following abortive infection, could also serve as a bacteria nutrient (Fuhrman, 1999).

2.4 Overview of cyanobacteria

Cyanobacteria also known as blue-green algae, blue-green bacteria, or Cyanophyta is a phylum of bacteria that obtain their energy through photosynthesis. The name "cyanobacteria" comes from the color of the bacteria (Greek: kyanós = blue) due to the pigment phycocyanin, which they use to capture light for photosynthesis. The ability of cyanobacteria to perform oxygenic photosynthesis is thought to have converted the early reducing atmosphere into an oxidizing one, which dramatically changed the composition of life forms on earth by stimulating biodiversity and leading to the near-extinction of oxygen-intolerant organisms (Vasishta, 1986).

Cyanobacteria can occur as planktonic cells or form phototrophic biofilms in fresh water. They include unicellular and colonial species. Colonies may form filaments, sheets or even hollow balls. Some filamentous colonies show the ability to differentiate into several different cell types: vegetative cells, the normal, photosynthetic cells that are formed under favorable growing conditions; akinetes, the climate-resistant spores that may form when environmental conditions become harsh; and the thick-walled heterocysts, which contain the enzyme nitrogenase, vital for nitrogen fixation. Heterocysts may also form under the appropriate environmental conditions (anoxic) when fixed nitrogen is scarce. Nitrogenase is inactivated by oxygen and the heterocyst must create a microanaerobic environment. It produces three additional cell walls including one of glycolipid that forms a hydrophobic barrier to oxygen (Wolk *et al.,* 1994).

Many cyanobacteria also form motile filaments, called hormogonia, that travel away from the main biomass to bud and form new colonies elsewhere. Each individual cell of a cyanobacterium typically has a thick, gelatinous cell wall. They lack flagella, but hormogonia and some species may move about by gliding along surfaces or by oscillation of filaments back and forth. In water columns some cyanobacteria float by forming gas vesicles (Walsby, 1980).

Cyanobacteria utilize the energy of sunlight to drive photosynthesis, a process where the energy of light is used to split water molecules into oxygen, protons, and electrons. Photosynthesis in cyanobacteria generally uses water as an electron donor and produces oxygen as a by-product, though some may also use hydrogen sulphide as occurs among other photosynthetic bacteria. In most forms the photosynthetic machinery is embedded into folds of the cell membrane, called thylakoids. Cyanobacteria, being generally obligate photoautotrophs, possess photosynthetic

pigments for this function. Such pigments are of three types namely chlorophylls, carotenoids and phycobilins (Prescott, 1969).

Chlorophyll pigments are fat soluble compounds of different types named chlorophyll a, b, c, d, and e. Carotenoids are fat soluble yellow colored pigments consisting of carotenes, xanthophylls and carotenoid acids. Phycobilins are water soluble blue (phycocyanin) and red (phycoerythrin) colored pigments. These two pigments are unique to cyanobacteria and are not found in any algae. Prescott (1969) added phycocyanin-r and allophycocyanin to the list. The cell envelope of cyanobacteria consists of an inner cell wall and an outer sheath. The sheath is a layer of extracellular mucilage made of pectic substances that holds the cells in colonies together. Its slimy nature gives it great water absorbing and water retaining capacity (Vasishta, 1986).

2.5 Taxonomy of cyanobacteria

The Committee on the International Code of Botanical Nomenclature (CICBN) has recommended certain suffixes for use in classification of algae. These are -phyta for division, -phyceae for class, -phycideae for subclass,-ales for order, -inales for sub order, -aceae for family, -oideae for sub family. The Greek name is used for genus and the Latin name for species. Classification of algae is based on certain morphological and physiological features. These include pigments in the cells, chemical nature of stored food material, chemical composition of cell wall, presence or absence of an organized nucleus and relative length of flagellum (Vasishta, 1986).

Modern algologists have divided algae into eleven divisions namely, xanthophyta, chrysophyta, bacilliarophyta, pyrrophyta, cryptophyta, euglenophyta, cyanophyta, chlorophyta, charophyta, phaeophyta and rhodophyta. The division cyanophyta (blue green algae) has a prokaryotic cell structure and is now placed in the domain prokarya, kingdom bacteria. It has a single class the Cyanophyceae, comprising of five orders namely the Chroococales such as the *Chroococcus* sp. which are unicellular and reproduce by binary fission or budding, Pleurocapsales that reproduce by multiple fission, Oscillatoriales (*Oscillatoria* sp.) that reproduce by trichome fragmentation or formation of motile homorgonia, Nostocales (*Nostoc* or *Anabaena* sp.) that have special cells called heterocysts and akinetes and reproduce by trichome fragmentation forming homorgonia, Stigonematales (*Stigonema* sp.) that may display true branching forming uniseriate and multeseriate trichomes (Ahoren, 2004).

2.6 Economic importance of cyanobacteria

Cyanobacteria account for 20 - 30% of the earth's photosynthetic productivity and exert an extensive effect on the dynamics of carbon flow in many of the marine environments (Martin and Kokjohn, 1999) as well as other aquatic ecosystems. They also produce a variety of compounds that have shown potential application in major disease management such as cancer, asthma, arthritis, diabetes and HIV. They have also shown immense potential in wastewater and industrial effluent treatment, bioremediation of aquatic and terrestrial habitats, biofertilizers, biofuel and cosmetics. Species such as *Arthrospira* constitutes the only food source for the vast flocks of lesser flamingo (*Phoeniconaias minor*) that graze on some African soda lakes. The carotenoids in *A. maxima* give flamingoes their pink color (Fatma, 1999).

Some species such as *Aphanizomenon flos-aquae* and *Arthrospira platensis* are used as food (Spolaore *et al.,* 2006). Dried spirulina contains about 60 % protein. It is a complete protein containing all essential amino acids, though with reduced amounts of methionine, cysteine and lysine when compared to the proteins of meat, eggs and milk. It is, however, superior to typical plant protein, such as that from legumes (Cifferi, 1983). Spirulina has a lipid content of about 7% by weight, and is rich in gamma-linolenic acid (GLA), and also provides alpha-linolenic acid (ALA), linoleic acid (LA), stearidonic acid (SDA),eicosapentanoic acid (EPA), docosahexanoic acid (DHA) and arachidonic acid (AA) (Tokusoglu and Unal, 2003). *Microcytis aeruginosa* is the subject of research into the natural production of butylated hydroxytoluene (BHT) an antioxidant, food additive, and industrial chemical (Babu and Wu, 2008).

Researchers have shown the possibility of application of cyanobacteria in the generation of Clean and Green Energy via converting sunlight directly into electricity. The use of cyanobacteria to produce hydrogen and oxygen from solar radiation is a promising new technology for the production of non-fossil fuel based energy (Ananyev *et al.,* 2008). Biologically produced fuel is gaining popularity as fossil fuels are becoming more expensive and scarce (Jacobson, 2009). Efforts have been made to commercialize algae-based fuels such as diesel, gasoline and jet fuel (Radmer, 1994). Nitrogen fixing cyanobacteria species are seeded into rice fields to increase fertility. *Oscillatoria* can be used as an indicator of water quality or eutrophication of a water source. As a body of water becomes more eutrophic or polluted, *O. rubescens* increases in population as *O. agardhii* decreases in population (Babu and Wu, 2008).

Certain cyanobacteria produce cyanotoxins including anatoxin-a, anatoxin-as, aphlysiatoxin, domoic acid, microcystin LR, nodularin R (from *Nodularia* sp.), or saxitoxin. These toxins can be neurotoxins, hepatotoxins and cytotoxins and can be toxic and dangerous to humans and animals. Several cases of human poisoning have been documented but a lack of knowledge prevents an accurate assessment of the risks. Studies suggest that significant exposure to high levels of cyanobacteria causes amylotrophic lateral sclerosis (ALS) (Carmichael, 1991). Waste stabilization ponds, eutrophic lakes and polluted water support the luxuriant growth of cyanobacteria. These can become obnoxious blooms in the water reservoirs and result in mortality of fish and other organisms.

2.7 Impact of cyanobacteria on ecosystem

Environmental factors influence cyanobacterial growth rates and formation of blooms. Proliferation often occurs at water temperature above 20 ^{o}C resulting in depletion of dissolved inorganic N and free CO_2 from the water (Murrell and Lores, 2004). In extremely shallow lakes (mean depth < 2 m), dominance of cyanobacteria may persist for years if the ratio of photic depth to mixed depth never falls to levels that prevent net growth of low-light adapted taxa such as *Oscillatoria agardhii* (Berger, 1989), but remains low enough to exclude other plankton.

High irradiance in the surface mixed layer and low wind velocities result in short-lived hyper-scums of *Microcystis* a*eruginosa* (Zohary *et al.,* 1995). The bloom is often followed by a population crash during periods of high outflow volume, when washed out to downstream systems, at which time other algae can become dominant. Observational and experimental studies have shown that cyanobacteria become increasingly dominant at low TN: TP (Total

Nitrogen: Total Phosphate) ratios (Smith and Bennett, 1999), yet some studies discount this as coincidental rather than causal (Reynolds, 1999). Downing *et al.* (2001) noted that concentration of TP is a better predictor of cyanobacteria dominance than TN: TP ratios.

Studies have suggested that other potential causal factors include pH and scarcity of free CO_2 (Dokulil and Teubner, 2000). Others have suggested that greater resistance to zooplankton grazing may favor cyanobacteria, and that certain cyanobacteria produce allelopathic chemicals that inhibit growth of other algae. Accessory pigments that allow net growth to occur at low irradiance (Scheffer *et al.,* 1997) and buoyancy that allows certain taxa to bloom at the water surface (Reynolds *et al.,* 1987) are also considered important to cyanobacteria dominance.

In shallow, mixed lakes with high TP, there is extreme and highly predictable dominance by *Oscillatoria* sp. whereby a low ratio of photic to mixed depth allows these cyanobacteria to out compete other algae (Scheffer *et al.,* 1997). In contrast, predominance of high light requiring nitrogen fixers such as *Anabaena circinalis* and *Aphanizomenon flos aquae* is predictably linked to stable water columns, depletion of dissolved inorganic nitrogen and high temperature. Philips *et al.* (1997) documented that changes in biomass of nitrogen fixing cyanobacteria and density of heterocysts were strongly coupled with depletion of dissolved inorganic nitrogen.

Cyanobacteria and phytoplankton can utilize both NO_3^- and NH_4^+ nitrogen, and the dominant form of Nitrogen can be a selective force in determining which plankton dominates in the water. Blomqvist *et al.* (1994) proposed that a high NO_3^- environment is suitable for the development of phytoplankton, while non nitrogen fixing cyanobacteria prefer high NH_4^+ environments.

Under such conditions, species such as *Microcystis*, a non nitrogen fixing cyanobacteria, are able to become dominant due to their high affinity for NH_4^+ nitrogen. Low concentrations of both NO_3^- and NH_4^+ occur after *Microcystis* population density becomes high due to increased uptake. An increase in NO_2^- concentration occurs when the *Microcystis* sp. population density begins to increase (Yoshida *et al.,* 2007).

Studies have suggested that anoxic conditions are suitable for the growth of *Microcystis* sp. (Trimbee and Harris, 1984). When nitrogen is fixed it is incorporated into amino acids and proteins and on death the nitrogenous materials undergo decomposition forming ammonia which is later converted into nitrates by nitrifying bacteria. Due to their ability to fix nitrogen in aerobic conditions, the nitrogen fixing cyanobacteria are often found as symbionts with a number of other groups of organisms such as fungi (lichens), corals, pteridophytes (Azolla) and angiosperms (Gunnera) (Enrique-Flores, 2008). Cyanobacteria are the only group of organisms that are able to reduce nitrogen and carbon in aerobic conditions, a fact that may be responsible for their evolutionary and ecological success (Schultz, 2009).

Toxin production occurs in particular taxa of cyanobacteria that include *Anabaena circinalis*, *Aphanizomenon flos–aquae*, *Cylindrospermopsis raciborskii*, *Microcystis aeruginosa,* and species of *Oscillatoria*, *Nostoc*, and *Aphanocapsa*. However, factors controlling the amount of toxin produced during a bloom are not well understood. Studies have suggested links between toxin concentrations and ratios of particulate to dissolved nutrients (Oh *et al*., 2001), concentrations of soluble P (Jacoby *et al.,* 2000), TP (Rapala *et al.,* 1997), TN and irradiance (Rolland *et al.,* 2005).

Toxin production by cyanobacteria may lead to a wide array of biological impacts including: allelopathic effects on other phytoplankton (Suikkanen *et al.,* 2004), suppression of zooplankton grazing, leading to reduced growth and reproductive rates and changes in dominance (Ghadouani *et al*., 2003), hepatotoxic effects on fish (Anderson *et al.,* 1993) and accumulation of toxins in tissues of invertebrates (Lehtiniemi *et al.,* 2002). There are reports of impacts on waterfowl, such as the mass mortality of ducks that coincided with a *Microcystis* bloom in a Japanese lake (Matsunga *et al.,* 1999).

2.8 Previous studies on phages in alkaline-saline lakes

2.8.1 Studies at Lake Nakuru

Lake Nakuru is an alkaline - saline lake in the Rift Valley of Kenya where the Lesser Flamingo, *Phoeniconaias minor*, is the dominating and characteristic bird species (Krienitz and Kotut, 2010). The species has been classified as 'near-threatened' (IUCN Red List, 2012). The dense population of these pinkish waterfowl (1.5 - 2.5 million in Eastern Africa, which is around 75 % of the worldwide occurrence) has been rated for decades as one of the most significant wildlife spectacles worldwide (Jenkin, 1929).

Flamingos are filter feeders of photoautotrophic algal primary producers, *Arthrospira fusiformis,* a very fast-growing alkaliphilic cyanobacterium. Low flamingo numbers of around only 100 individuals were detected at the beginning of the year 2009, followed by an increase in April, reaching a dense population of more than 1 250 000 in June. This pattern coincided with food availability. Together with the breakdown of the algal biomass, a drastic decrease in flamingo

abundance was observed, with a lowest number of 1500 individuals in October. The correlation between A. *fusiformis* biomass and *P. minor* numbers was significant (Peduzzi *et al.,* 2014). Fluctuations of the food resource were reported as the overwhelming factor influencing Lesser Flamingo distribution. The typically dense population *of A. fusiformis* collapses unpredictably. This is thought to be the major cause for episodes of disappearance or significant irregular mass movements of flamingo between the East African lakes (Krienitz and Kotut, 2010).

Causes for the sudden *A. fusiformis* breakdowns have remained a matter of speculation. Peduzzi *et al.* (2014) hypothesized that viruses (as most abundant biological entities) are capable of mediating a bottom-up cascade (Hunter and Price, 1992), thus being responsible for the frequent breakdowns in *A. fusiformis* biomass and, as a consequence, for the drastic drop in flamingo numbers at Lake Nakuru. This is an example of a virus-mediated bottom-up cascade in the short food chain from photoautotrophic prokaryotes to birds.

Water samples from Lake Nakuru revealed very high numbers of viruses of up to 7.0×10^9 vp/1. The heterotrophic bacterial abundance was high while the virus-to-bacteria ratio fluctuated but remained in the same order of magnitude (Peduzzi *et al.,* 2014). During the study period, *A. fusiformis* biomass fluctuated between low values of 0.44 mg/1 in January and a maximum of 217.3 mg/1 in June 2009. Here, at peak phytoplankton biomass, almost 100 % of the total algal biomass was comprised by *A. fusiformis* (Kaggwa *et al.,* 2013). After this maximum, *A. fusiformis* biomass dropped again to undetectable levels in October.

During the crash of *A. fusiformis*, visible signs of cyanophage infection were detected by TEM (Transmission Electron Microscopy) analyses. The highest frequency of visible infection (24.3 % of inspected cells) occurred at peak abundance of *A. fusiformis* host cells, which clearly marked the onset of this biomass breakdown. From early June until the complete vanishing of *A. fusiformis*, almost each sampling revealed a high percentage (on average 5.9 %) of visible infections (Peduzzi *et al.,* 2014).

The consumption activity of the flamingos on *A. fusiformis* is unlikely to be responsible for the breakdowns of the algal biomass consuming only 0.46 % of the standing stock per day (Krienitz and Kotut, 2010). *A. fusiformis* tolerates high alkalinity, salinity and elevated temperatures of more than 35 ^{o}C (Vonshak and Tomaselli, 2000). During the investigation period, all these parameters, together with soluble reactive phosphorus, remained within a range that does not limit *A. fusiformis* growth (Peduzzi *et al*., 2014). The results point to a virus attack on the highly specialized algal diet of the Lesser Flamingo.

Cyanophage infection is considered as a major controlling factor in phytoplankton successions (Muhling *et al*., 2005). The assumption was that the irregular and unpredictable collapses of *A. fusiformis* may be the result of dynamic interactions between resistant and non-resistant host strains and co-occurring, genetically diverse cyanophage populations. It was concluded that, at Lake Nakuru, a virus-mediated loss of the most important food source triggered the collapse in *P. minor* abundance in a bottom-up cascade (Peduzzi *et al*., 2014).

2.8.2 Studies at Mono Lake

Viral ecology has been studied in a wide range of aquatic habitats including rivers, lakes, oceans and seas, sea ice (Maranger *et al*., 1994), and solar salterns (Guixa *et al*., 1996). The results indicate that viruses are truly ubiquitous, though their impact on microbial communities can be variable.

The ecology of viruses was investigated in Mono Lake, an alkaline hypersaline lake in California. The lake has a simple food web, with the brine shrimp *Artemia monica* as the sole macro zooplankton (Dana and Lenz, 1986). Phytoplankton productivity is relatively high in comparison to other hypersaline lakes (Duckworth *et al*., 1996) and decreases immediately following the onset of episodes of high runoff (Jellison and Melack, 1993). The phytoplankton community contains relatively few species dominated by *Picocystis salinarum* (Lewin *et al*., 2000), unidentified small flagellates and coccoid cyanobacteria. There is evidence of metabolically diverse populations of prokaryotes in the lake including methanogens, methylotrophs, sulfate reducers, nitrate reducers, ammonia oxidizers, anoxygenic and oxygenic photoautotrophs (Cornell *et al*., 1997). Overall productivity is limited by nitrogen and, because external inputs of nitrogen are low, bacteria provide a key source by regenerating ammonia (Joye *et al*., 1999).

Studies of the abundance and diversity of viruses in Mono Lake revealed that viral abundance was high and comparable to concentrations observed in hyper saline evaporator ponds used to produce salt from seawater (Guixa, 1996). Bacterial abundance ranged from 0.3 to 4.4 x 10^7vp/l while viral abundances were generally more than one order of magnitude greater than those of

bacteria, ranging from 0.1 to 1 x 10^9 vp/l. Significant changes ($P < 0.01$) in bacterial and viral abundance along vertical profiles of the lake were detected (Guixa, 1996).

Viral and bacterial abundances in the anoxic layer were significantly higher ($P < 0.01$) than in overlying waters. There were no significant differences in viral and bacterial abundance between seasons (Jiang *et al*., 2003). Although high, the virus concentrations in Mono Lake were consistent with the observed bacterial concentrations in the lake, given that bacterial and viral abundance display a general positive correlation over a wide range of environments (Maranger, 1996).

Lack of a significant correlation between bacterial and viral abundance may simply reflect a more limited spatial and temporal scale during data collection. The simultaneous predator-prey dynamics for many virus-host systems may cause the correlation to appear chaotic. The peaks in viral abundance found in anoxic water could be attributed to viruses infecting anaerobic bacteria which then get transported to the bottom waters by adsorption to sinking particles. Measurements of viral infection and grazing rates in Lake Plubsee (Wenbauer and Hofle, 1998) suggested that viruses were a more important source of mortality in anoxic waters while protozoan grazing was more important in oxygenated surface waters.

CHAPTER THREE: GENERAL MATERIALS AND METHODS

3.1 Study area

Lake Magadi is situated in a closed lake basin about 100 km south of Nairobi and has a depth of about 1m. It is situated in the Rift Valley of Kenya and located about 2º S, 36º 20´E. There are no permanent rivers entering the Magadi basin but shallow lagoons in the Northern and Southern ends of the lake are fed by hot alkaline springs with temperature as high as 86 ^{0}C. In the vicinity is Ol Doinyo Lengai, which is considered to be the youngest volcano, presenting eruptions of carbonates consisting of sodium carbonate (Na_2CO_3) and calcium carbonate ($CaCO_3$). It is famous for being the only volcano known in the world to have erupted carbonatite lava in historical time (DasSarma, 2006). The lake consists of an almost solid deposit of sodium chloride and sodium carbonate, the latter existing as sodium sesquicarbonate or trona ($Na_2CO_3 \cdot NaHCO_3 \cdot 2H_2O$).

Gels of sodium-aluminum silicate as thick as 5 cm are found in Lake Magadi. These gels are formed through the interaction of hot alkaline spring waters with alkali trachyte flows. Algal mats from the lake protect the gels from erosion. It is believed that the gel was a precursor for the cherts of rocks in Lake Magadi (Eugster *et al*., 1968). The cherts have high fluoride concentration due to the weathering of volcanic rocks, found in the same area as Lake Magadi, that are enriched in fluoride and alkalis. This has affected the local community that has customarily used the chert as a meat tenderizer and has inadvertently ingested high levels of fluorine (Joan, 1999).

Flooding of Lake Magadi often takes place in March and April, dries by June and July, and rains again between November and December (Ahoren, 2004). The hot, dry conditions expose the lake to high evaporation rates. It is now largely a dry bed that fills up with water only after rains when water enters the northern part of the lake via temporary watercourses. Sections of the lake waters are often colored red (Fig. 3.1) by the dense microbial communities of pigmented halophilic archaea such as *Halobacterium.* The environs of Lake Magadi have semi arid and arid conditions (Fig. 3.2). Loita Hills in the environs of Lake Magadi are located to the West of the Nguruman escarpment (Fig. 3.3).

Fig.3.1: Parts of Lake Magadi colored pink by halophilic archaea

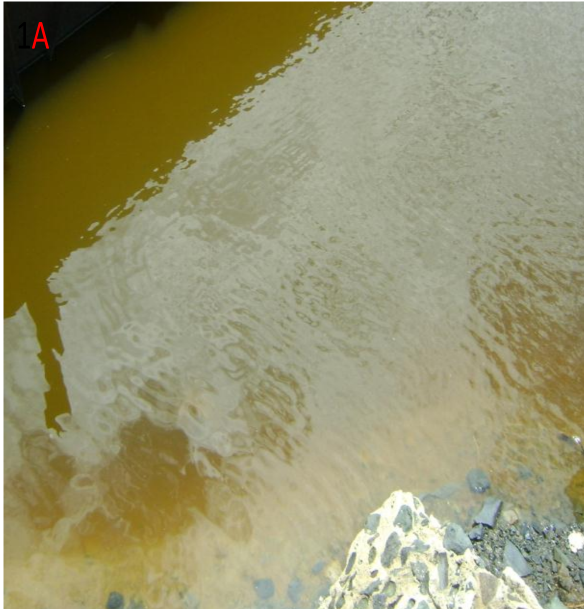
Source: Muruga (2010)

Fig 3.2: Semi arid environs of Lake Magadi with some few settlements

Fig. 3.3: The environs of Lake Magadi showing part of Loita Hills in the background

3.2 Sampling of water from Lake Magadi

Sampling was done at the shores of the lake in the month of January 2012 and January 2013. Water samples were collected randomly from the lake at four sampling sites with different water coloration ranging from brownish, bluish and green to clear (Fig. 3.4A to D). About 30 ml of sample water was collected from each site in three sterile 150 ml culture bottles. Each sample was labeled at the time and a photograph taken at each sampling site. Samples were transported to the Genetics Laboratory at the University of Nairobi in sterile culture bottles wrapped in foil in a cool box and stored at 4 ^{0}C.



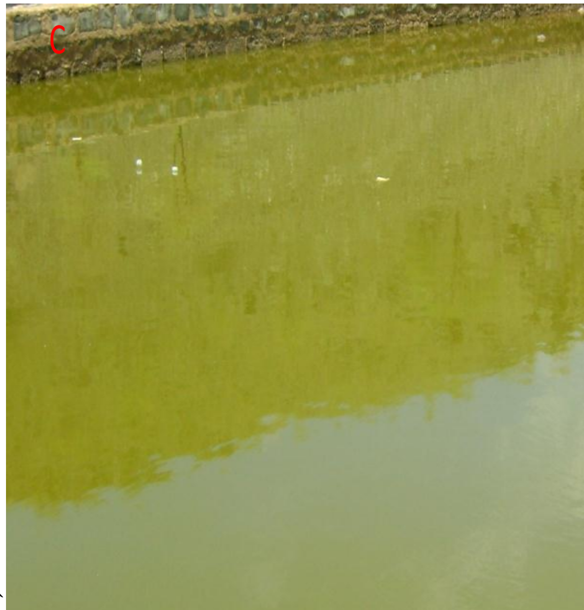


Fig 3.4: Water sampling sites on the shoreline of Lake Magadi with different coloration. (A) brown coloration of water; (B) bluish water; (C) green coloration of water; (D) colorless water.

3.3 Types of media used for isolation of cyanobacteria, proteobacteria and phages

3.3.1 Aiba and Ogawa medium for marine cyanobacteria

The medium used for cyanobacteria and bacteria isolation was prepared as two separate solutions to avoid precipitation:

Solution A: - consisted of 2.0 g/l NaCl, 0.4 g/l $MgSO_4 \cdot 7H_2O$, 2.0 g/l K_2SO_4, 80 mg/l $CaCl_2 \cdot 2H_2O$, 5g/l $NaNO_3$, 20 mg/l $FeSO_4 \cdot 7H_2O$ and 2.0 ml/l Gaffron micronutrients

Solution B: - consisted of 1.0 g/l K_2HPO_4, 8.06 g/l Na_2CO_3, and 27.22 g/l $NaHCO_3$.

After autoclaving, solution A was mixed with solution B aseptically after cooling to about 60°C giving a final pH of 9.4 - 9.8 (Cyanosite, 2012).

3.3.2 The medium modified for haloalkaliphilic bacteria

This medium was used for proteobacteria isolation and consisted of 53.0 g/l Na_2CO_3, 42.0 g/l $NaHCO_3$, 29.5 NaCl (sea salt), 1.5 g/l TSB, and 15.0 g/l Agar. Salts were sterilized in an autoclave at 121 ^{0}C for 20 min separately then mixed with the rest of the sterilized ingredients at 60 ^{0}C just before pouring onto plates (Designed by Prof Duboise, personal communication).

3.3.3 Phage growth media

The medium consisted of 1.5 g/l KH_2PO_4, 3 g/l Na2HPO4, 1.0 g/l NH_4Cl, 0.2 g/l $MgSO_4.7H_2O$, 10.0 g/l glycerol, 5.0 g/l acid hydrolysed casein, 0.01 g/l dl-tryptophan, 0.02 g/l gelatin. The media was sterilized in the autoclave at 121 ^{0}C at 100 KPa for about 5 mins (Brown, 2005).

CHAPTER FOUR: DIVERSITY OF PROTEOBACTERIA AND CYANOBACTERIA SPECIES IN LAKE MAGADI WATER

Abstract

Lake Magadi harbors a large population of proteobacteria and cyanobacteria and there is need to isolate and identify them as well as study their diversity. They contribute significantly to primary production by adding carbon and nitrogen into the ecosystem through carbon dioxide and nitrogen fixation respectively. Cyanobacteria are also important as they can be used as biofertilizers, food and feed while others produce compounds that have potential biotechnological application. Not much research has been carried out on bacteria, particularly cyanobacteria, in Lake Magadi. This study aims at isolating and identifying bacteria from samples of water collected from Lake Magadi. Proteobacteria and cyanobacteria were isolated on media modified for haloalkaliphiles and on Aiba and Ogawa medium for cyanobacteria. Identification of proteobacteria was done by carrying out biochemical tests and analysis of 16S rRNA followed by phylogeny. Extraction of DNA was done using Quiagen DNA Kit, followed by amplification by PCR (Polymerase Chain Reaction). Gene sequences obtained were compared to NCBI (National Center for Biotechnological Information) GenBank database using BLAST (Basic Local Alignment Search Tool). The sequences of the bacterial isolates together with sequences of their close relatives were aligned and differences in nucleotides were converted to distance matrices using neighbor joining method. A phylogenetic tree was generated on MEGA 5 (Molecular Evolutionary Genetics Analysis) software. Identification of cyanobacteria species was done by cell size and shape, motility of cells and colony morphology. Two proteobacterial isolates were obtained. One was identified as *Idiomarina* sp. a Gram negative, motile and aerobic

rod that had optimum growth in the medium for haloalkaliphiles and the second on Aiba and Ogawa medium was identified as *Rhodobacter* sp. an autotrophic, Gram negative rod that was non-motile and aerobic. Phylogeny showed clustering with members of the classes Gammaproteobacteria and Alphaproteobacteria for *Idiomarina* sp. and *Rhodobacter* sp. respectively. Lake Magadi therefore harbors a genetically diverse bacterial population. Seven cyanobacterial isolates were obtained consisting of four colony forming unicellular genera; *Synechococcus*, *Microcystis, Synechocystis* and *Prochlorococcus* and three colony forming filamentous genera; *Arthrospira*, *Anabaena* and *Oscillatoria*. Culturing microorganisms is a major hurdle for the complete understanding of the microbial diversity and different types of media should be utilized during isolation. Population sizes of such photosynthetic bacteria species should be maintained at certain critical levels in order to sustain sufficient energy flow and nutrient cycling in the lake.

4.1 Introduction

Taxonomically diverse bacterial populations consisting mainly of aerobic and anaerobic autotrophic and organotrophic prokaryotes are found in soda lakes. Lake Magadi harbors a dense and diverse population of halophilic, halotolerant, alkaliphilic and alkalitolerant representatives of major bacterial and archaeal phyla. The majority of Gram negative species are the members of the gamma subdivision of Proteobacteria, including many proteolytic organisms related to members of the genus *Halomonas* (Duckworth *et al*., 2000).

Halophiles thrive well in environments with very high concentrations of salt. They have the pigment bacteriorhodopsin for photosynthesis and carotenoids for ultraviolet protection. They are

adapted to their environment by producing large amounts of compatible solutes or by concentrating a solute from their environment. Enzymes that function inside the cells of *Halobacterium* have evolved to require large doses of K^+ for catalytic activity. Membranes or cell wall-positioned proteins in *Halobacterium* require Na^+ and are typically stable only in the presence of high Na^+ concentration (Horikoshi and Grant, 1991). Extreme alkaliphiles such as *Natronobacterium magadii*, isolated from Lake Magadi, grow optimally at a pH of about 10. Proteins found in the cell wall and cell membrane are stable at high pH (Grant and Jones, 2000).

When cyanobacterial blooms occur, irradiance is reduced leading to reduction in the growth of producers that cannot maintain a position near the surface of the water, including epiphyton, benthic algae and rooted vascular plants. Thus, lakes with very dense blooms, especially if they are frequent or long lasting, may not support large populations of other producers (Scheffer *et al.,* 1993).

Intense blooms with high photosynthetic activity also deplete free CO_2 from lake water. This may stimulate formation of surface scum and dominance by cyanobacteria taxa that can move to the air-water interface where CO_2 is available, shading other algae in the process (Paerl and Ustach, 1982). There is evidence that high pH during intense cyanobacteria blooms may be toxic to certain species of fish (Kann and Smith, 1999). Oxygen depletion that occurs in the water during bloom senescence can also lead to the death of organisms in the water. There is also the impact of high levels of ammonia during bloom senescence that can cause mortality of snails and other macro invertebrates (Jones, 1987).

It is important to understand the dynamics of the lake by keeping track of bacterial species inhabiting the lake. This should be done regularly in order to understand temporal and spatial variation of the ecosystem. This study aimed at determining the diversity of proteobacteria and cyanobacteria species in Lake Magadi. Proteobacterial isolates from samples of water collected from Lake Magadi were identified by analysis of the 16S rRNA gene. Identification of cyanobacterial isolates was based on cell and colony morphology and mode of motility. The data obtained could be put to use when there is need for bacteria species with potential biotechnological application.

4.2 Materials and methods

4.2.1 Isolation and characterization of bacteria from Lake Magadi water

Two types of culture media were used for bacterial isolation. These were the Aiba and Ogawa medium for marine cyanobacteria and the modified medium for haloalkaliphilic bacteria. Proteobacteria were cultured using the enrichment culture method by mixing water samples with each liquid medium separately in 1:1 ratio. This was followed by the spread plate technique on agar plates of each medium. One set of each medium culture was incubated at room temperature with plenty of sunlight, while the other was incubated at 37 ^{o}C. Discrete colonies that developed were streaked on fresh plates to obtain pure isolates. The pure cultures obtained were sub cultured in tubes containing each liquid medium and were stored at 4 ^{o}C.

Gram stain reaction was done by spreading a thin film of each isolate over the surface of the slide. It was then heat fixed and flooded with crystal violet for about 60 sec then washed for 5 sec with water and flooded with iodine solution for about a minute. It was rinsed with water for 5

sec and ethanol was added drop wise until the blue-violet color was no longer eluted from the specimen. It was then rinsed with water for 5 sec, counterstained with safranin, allowed to stand for about a minute then rinsed with water for 5 sec to remove excess dye (Brown, 2005). Gram positive cells remained blue-violet in appearance while gram negative bacteria took on the pink color of the counter stain. The slide was allowed to dry before viewing under the microscope (Leica dm 500 model).

Motility test was done with motility medium, a semisolid medium containing 7 g/l of agar (Brown, 2005), was inoculated with the bacterial isolates. This was done using an inoculating needle, stabbing the motility medium in a tube in a straight line and then incubating at 37 ^{0}C for 12-24 hr. Migration away from the original line of inoculation meant that the test organism was motile while lack of migration indicated a lack of motility.

Catalase test was done by smearing a small amount of each isolate onto a clean glass slide using an inoculation loop. A drop of 70 % hydrogen peroxide was then added to the smear. Visible bubbles of oxygen indicated catalase positive while lack of bubbles indicated catalase negative (Brown, 2005).

4.2.2 Identification of bacterial isolates by analysis of 16S rRNA gene

Bacterial cells were harvested in the early log phase of growth in liquid broth (about 24 hr after inoculation). Total DNA of the microbial isolates was extracted using a DNEasy Blood and Tissue kit, from Qiagen, according to the manufacturer's instructions (Molecular Biology methods-Qiagen, 2013). The genomic DNA obtained was stored at -25 ^{o}C.

Poymerase chain reaction was used to amplify the 16S rRNA gene for sequencing. Base sequences of the primers used for amplification were: 16S F 5' - AGAGTTTGATCHTGGCTYAG - 3'; 16SR 5'- ACGGNTACCTTGTTACGACTT - 3'. The reaction mixture contained the following components; 38.25 µl PCR water; 4 µl DNA template diluted 1: 10 in PCR water; 5 µl thermopol – 10 x buffers; 1.25 µl dNTP mix; 0.5 µl each of forward and reverse 16S primer diluted 1: 10 with PCR water; 0.5 µl Taq polymerase, totaling 50 µl. The thermocycler program used was 95 ^{o}C for 3 min, 95 ^{o}C for 30 sec, 55 ^{o}C for 30 sec, 72 ^{o}C for 1 min for extension of the PCR product, 30 cycles were performed with a final extension of 3 min at 72 ^{o}C. The reaction was held at 4 ^{o}C and the product removed and stored at -25 ^{o}C. To purify the PCR products a QIAquick Purification KIT from Qiagen was used according to instructions (Molecular Biology methods-Qiagen, 2013). About 50 µl of eluate was collected and stored at -25 ^{o}C and some was used for gel electrophoresis.

To run gel electrophoresis 1 % agarose gel was made by dissolving 1.0 g agarose in 100 ml TAE (Tris acetate EDTA) and stained with ethidium bromide. Exactly 6 µl of each PCR product mixed with 1.5 µl of loading dye (bromothymol blue) was loaded into the wells of the agarose gel. A DNA molecular marker (0.5-10 kbp) was loaded in the first well. The electrophoresis was left to run for about 20 min at 5 V/cm. The gel was observed in a U.V. transilluminator and band images taken using a digital camera and the Doc-It LS Image Analysis Software.

The PCR product obtained was sequenced and Gene sequences obtained were compared to NCBI (National Center for Biotechnological Information) GenBank database using BLAST (Basic Local Alignment Search Tool). The sequences of the bacterial isolates together with sequences

of their close relatives were aligned using multialin program (Multalin, 2013). The differences in nucleotides were converted to distance matrices using Jukes and Cantor (1969) neighbor joining method. A phylogenetic tree was generated on MEGA 5 (Molecular Evolutionary Genetics Analysis) software (Takamura *et al*., 2011).

4.2.3 Isolation and identification of cyanobacteria

To build up the population of the cyanobacteria, the water samples were enriched with Aiba and Ogawa liquid medium in the ratio of 1: 1 and incubated at room temperature in (23 ± 2 °C) next to a window allowing enough sunlight for 10 days. The cyanobacterial suspension obtained was subcultured by the spread plate technique using plates of Aiba and Ogawa media containing 15 g/l of agar. Lawns of cyanobacteria were observed under the microscope and each discrete colony was picked using an inoculating needle and put in fresh liquid medium in culture bottles. The cultures were left to grow for ten days. Subculturing in solid medium, then in liquid medium was repeated until pure cultures were obtained. Images of lawns and colonies of cyanobacteria isolates were taken by digital camera (Sony cyber-shot model) and by optical microscope (Leica dm 500 model) respectively. Isolates were identified by their cell size and shape, cell motility and colony morphology.

4.3 Results

4.3.1 Morphological and biochemical characteristics of bacterial isolates

White and cream colored colonies were obtained from samples of water from Lake Magadi (Fig. 4.1). Two proteobacteria species were isolated and given the code names MB3 and MB5.

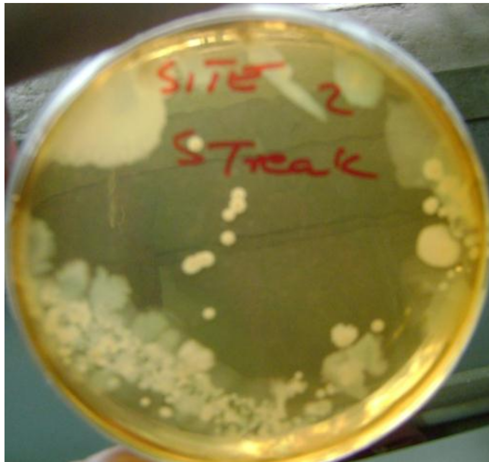


Fig. 4.1: Colonies of proteobacteria from samples of water collected from site two at Lake Magadi cultured on the medium for haloalkaliphiles.

Table 4.1: Characteristics of proteobacterial isolates from Lake Magadi

Examination by light microscope showed that the isolates were both catalase positive, Gram negative rods. Isolate MB3 was motile while isolate MB5 was non- motile (Table 4.1).

Isolate	Colony color	Gram stain	Morphology	Motility	Catalase
MB3	White	Negative	Rod	Present	Positive
MB5	Cream	Negative	Rod	Absent	Positive

4.3.2 Identity of bacteria from Lake Magadi based on analysis of 16S rRNA gene

Gel electrophoresis

Two bands were observed on the gel image (Fig. 4.2). Lane 1 and 11 were band images of PCR product of genomic DNA of bacterial isolate MB3 and MB5 respectively. The first lane had bands of

1 kb DNA ladder of between 0.5 to 10 kbp. Lanes 2 to lane 10 had PCR product from bacterial isolates that failed to produce bands.

Fig 4.2: Ethidium bromide-stained electrophoresis gel of PCR product on 1 % agarose.

Comparison of bacteria isolates with their closest matches on NCBI GenBank

Two bacterial isolates were identified based on 16S rRNA gene sequences. The gene sequence of isolate MB3 was 99 % similar to *Idiomarina* sp. of accession number JX434741.1 while isolate MB5 was 99 % similar to *Rhodobacter veldkampii* of accession number NR_043405.1 (Table 4.2).

Table 4.2: Bacteria isolates and their similarity to their closest match on NCBI GenBank based on 16S rRNA gene sequences

Isolates	Accession number	Names of comparative isolates	Maximum identity %
MB3	JX945780.1	GammaProteobacteria	99
	JX434741.1	*Idiomarina* sp.	99
	JX415314.1	Idiomarinaceae bacterium	98
MB5	NR_043405.1	*Rhodobacter veldkampii*	99
	NR_042212.1	*Rhodobacter* sp.	99
	NR_044285.1	*Rhodobaca barguzinensis*	98

Phylogenetic tree

Phyogenetic tree was based on 16S rRNA gene sequences obtained from isolate MB3 and MB5 together with their closest matching organisms (Fig 4.3). The closest matching organism for MB3 was *Idiomarina* sp. while MB5 clusters closely with *Rhodobcter veldikampii*. Bootstrap values at the nodes of the branches were based on 1,000 replications.

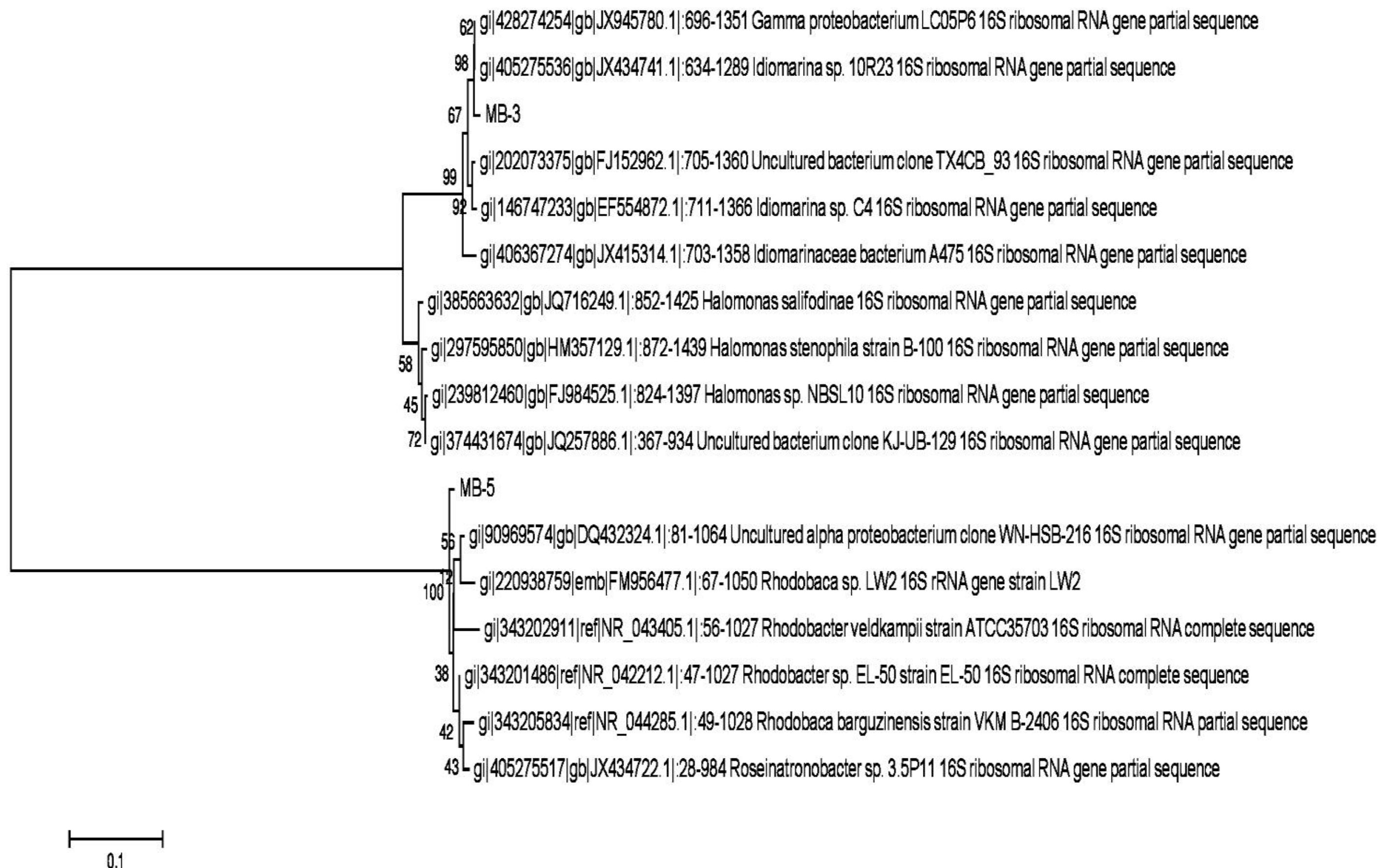


Fig 4.3: Phylogenetic tree showing the relationship between 16S rRNA gene sequences of isolates MB3, MB5 and their closely related organisms from NCBI GenBank.

4.3.3 Identity of cyanobacterial isolates sampled from Lake Magadi water

Four cyanobacterial isolates observed under the optical microscope (x 1000) as wet mounts were unicellular (Fig. 4.4). The cells of isolate MCy1 had wavy motion and had a length of about 1.5 μm. The cells had undergone binary fission and remained loosely attached (Fig. 4.4A). Cells of isolate MCy2 were spherical with a diameter of about 2 μm and had dark streaks (Fig. 4.4B) while cells of isolate MCy3 were relatively small with a diameter of about 1 μm and had gliding

motion. Some had undergone binary fission and remained attached (Fig. 4.4C). Cells of isolate MCy4 were very small and measured about 0.7μm (Fig. 4.4D).

Three cyanobacterial isolates were filamentous (Fig. 4.5). Isolate MCy5 had heterocystous filaments (Fig. 4.5A). Filaments of isolate MCy6 were closely spiralling (Fig. 4.5B) while filaments of isolate MCy7 were straight and exhibited oscillation movement (Fig. 4.5C).

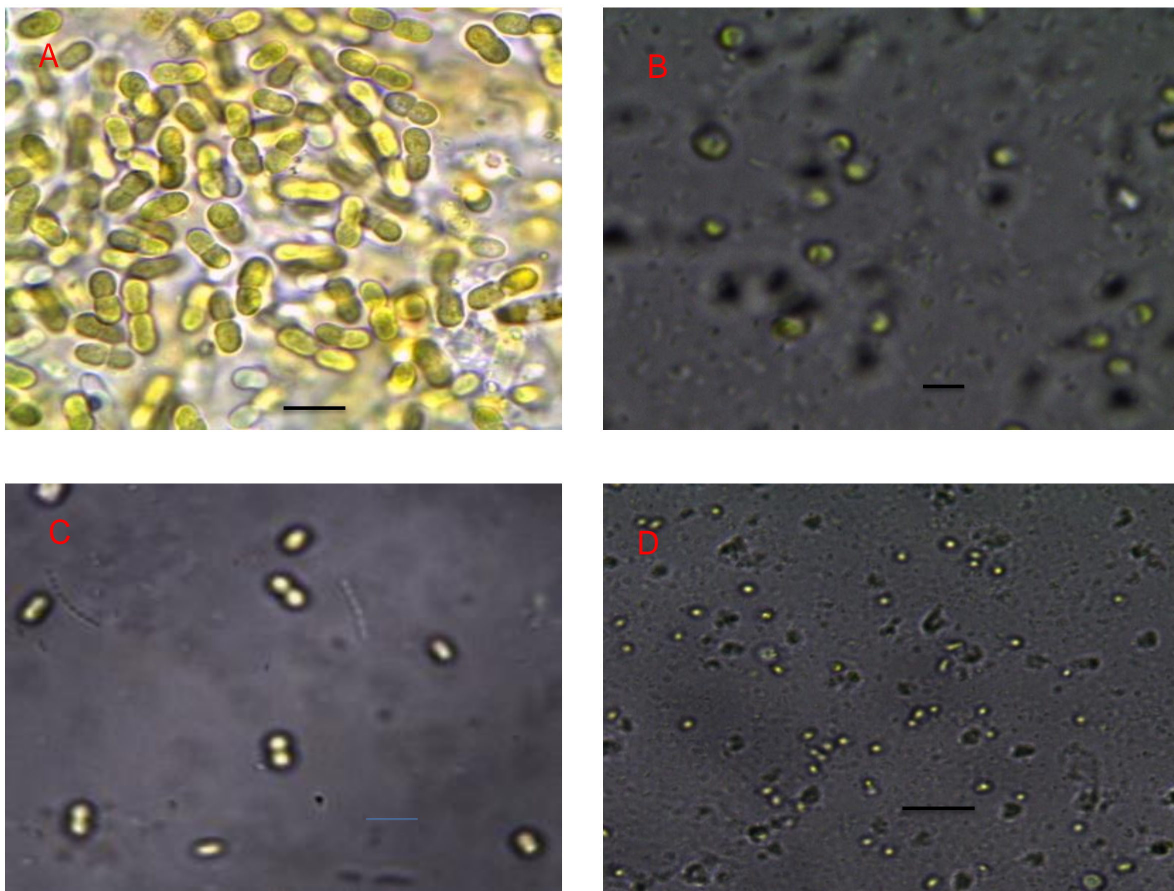


Fig. 4.4: Cells of unicellular cyanobacteria isolated from water samples from Lake Magadi, (A) Isolate MCy1; (B) Isolate MCy2; (C) Isolate MCy3; (D) Isolate MCy4 (Bar = 2 μm).

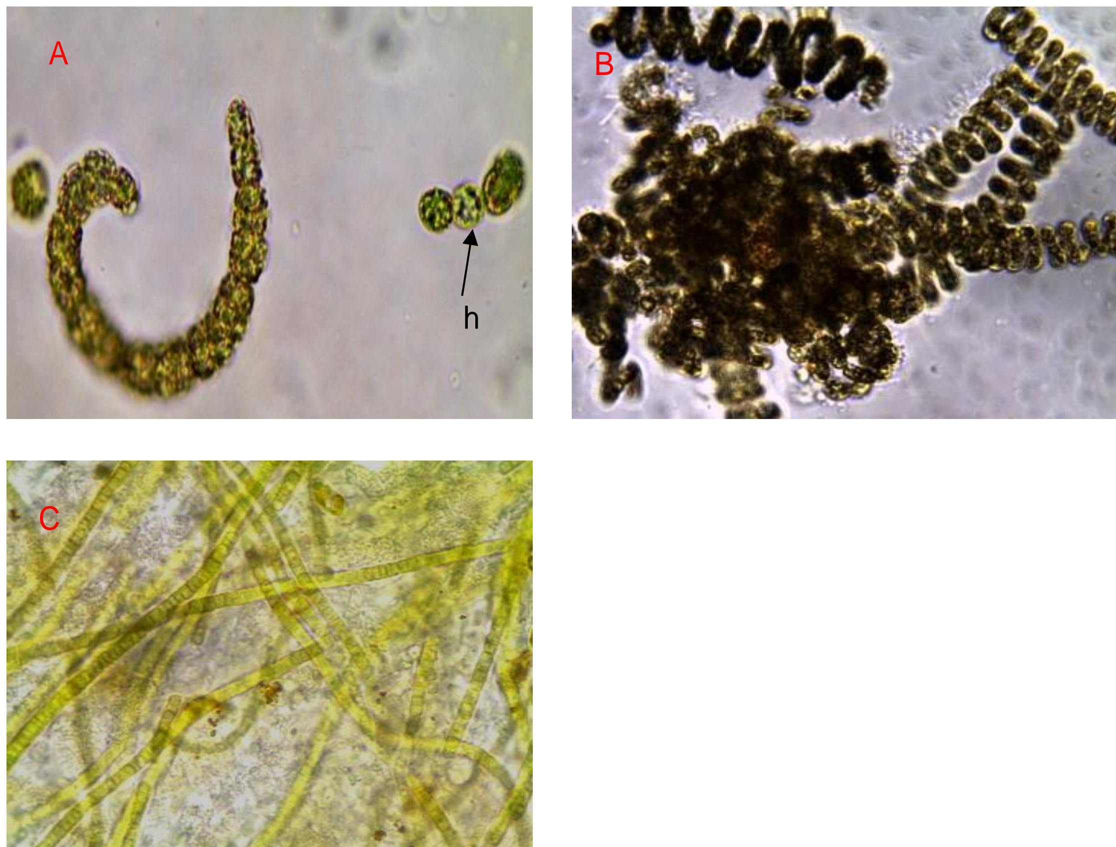


Fig. 4.5: Filamentous cyanobacterial isolates from samples of water from Lake Magadi. (A) Isolate MCy5; (B) Isolate MCy6; (C) Isolate MCy7 (h = heterocyst).

Colonies of cyanobacterial isolates from Lake Magadi water

Lawns and colonies of isolates cultured in Aiba and Ogawa agar medium formed in about ten days and were given code names MCy1 to MCy7. Isolate MCy1 and MCy4 formed dark green lawns (Fig. 4.6A). When observed under the light microscope, isolate MCy2 appeared as small, rounded colonies in early growth (Fig. 4.6B) while older colonies of about two weeks were dark and segregated (Fig. 4.6C). Isolate MCy3 formed small rounded discrete colonies (Fig. 4.6D) while isolate MCy7 formed filamentous colonies (Fig. 4.6E). Other filamentous isolates, MCy5 and MCy6, did not exhibit growth of colonies on solid media.

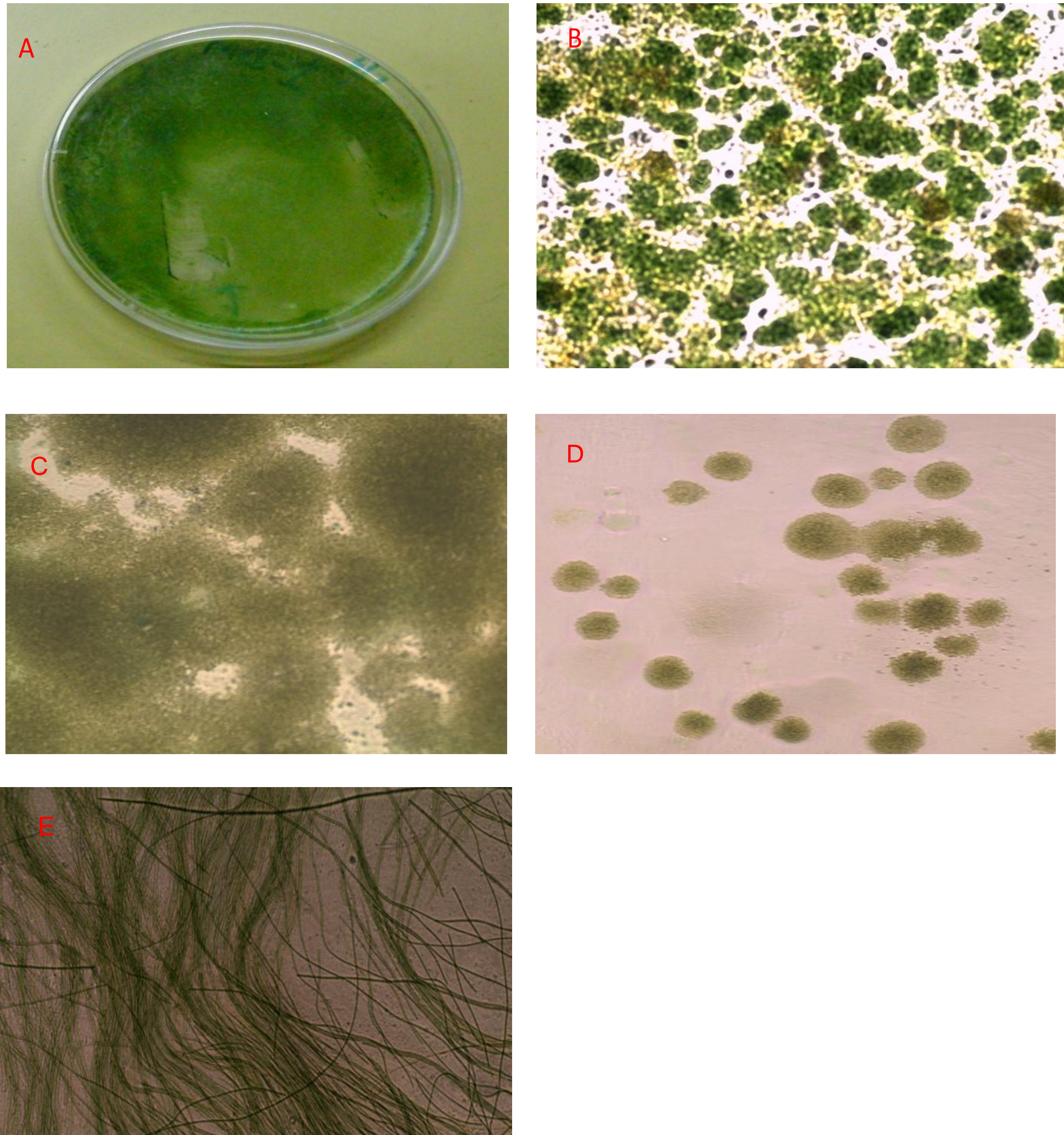


Fig. 4.6: Colonies of cynobacteria isolated from samples of water from the shoreline of Lake Magadi. (A) Lawn of isolate MCy1; (B) Isolate MCy2 in 7 days (x 400); (C) Colonies of isolate MCy2 in 14 days (x 400); (D) Isolate MCy3 (x 400); (E) Colonies of isolate MCy7 (x 400).

Summary of morphological characteristics of cyanobacteria isolates from Lake Magadi

Seven isolates with distinct morphology were obtained (Table 4.3). Unicellular colonial and filamentous colonial cyanoabacteria were isolated from Lake Magadi. Cell sizes (ranging from about 0.5 µm to 2 µm), cell shapes (five isolates had unique cell and filament structures), motility (three isolates were motile) and colony shapes (two isolates had unique colonies), were used for identification of the species.

Table 4.3: Morphological characteristics of cyanobacteria species isolated from Lake Magadi

Isolate	Morphology	Reproduction	Cell size and shape and nature of colony
MCy1	Colonial, unicellular	Binary fission	Cells with cylindrical shape, 1.5 µm in length, wavy motility, remain loosely attached after binary fission, broth culture appears dark green
MCy2	Colonial, unicellular	Binary fission	Spherical cells with dark streaks, about 2 µm in diameter, dark colonies under the optical microscope, yellowish green liquid cultures
MCy3	Colonial, unicellular	Binary fission	Cells are spherical, about 1 µm in diameter, gliding motion, small rounded colonies
MCy4	Colonial, unicellular	Binary fission	Cells are tiny, less than 1 µm and spherical
MCy5	Colonial, filamentous	Trichome fragmentation	Coiled filaments, spirals not close, heterocysts present, colonies float on the surface of liquid medium
MCy6	Colonial, filamentous	Growth of chain of cells	Closely spiraling filaments, colonies float within the medium
MCy7	Colonial, filamentous	Growth of chain of cells	Straight filaments forming a membranous sheet of colonies that floats at the bottom of the liquid medium, oscillation movement of filaments

4.4 Discussion

Isolate MB3 was found to be 99 % similar to *Idiomarina* sp. belonging to class Gamma proteobacteria. Phylogeny showed the isolate clustered closely with Gamma proteobacterium and *Idiomarina* sp., a Gram-negative, aerobic, flagellated bacterium which has been isolated from seawater, including oceanic water, coastal sediments, and submarine hyper thermal fluids. It grows optimally at pH 7.0 - 8.0 and at 30 - 37 ^{o}C (Jean *et al*., 2009). Isolate MB5 was found to be 99 % similar to *Rhodobacter veldkampii* and clustered closely with *Rhodobacter veldkampii* strain ATCC3503 belonging to class alphaproteobacteria, a purple non-sulfur bacterium that is able to carry out anoxigenic photosynthesis.

Cells of isolate MCy1 were similar to cells of *Synechococcus*, a unicellular cyanobacterium whose size varies from 0.8 µm to 1.5 µm that prefers well lit surface waters. Cells are known to be motile by a gliding type method and a novel uncharacterized, non-phototactic swimming method that does not involve flagella motion (Waterbury *et al*., 1985). All marine *Synechococcus* strains appear to be obligate photoautotrophs that are capable of supporting their nitrogen requirements using nitrate, ammonia or in some cases urea as a sole nitrogen source. The main photosynthetic pigment in *Synechococcus* is chlorophyll a, while its major accessory pigments are phycobilliproteins. The five commonly recognized phycobilins are phycocyanin, allophycocyanin, allophycocyanin b, phycoerythrin and zeaxanthin (Stanier and Cohen-Bazire, 1977). They exhibit a G+C content ranging from 39 % to 71 % (Waterbury *et al*., 1979).

Bergey's Manual (Herdman *et al.* 2001) divides *Synechococcus* into five clusters (equivalent to genera) based on morphology, physiology and genetic traits. Cluster one includes relatively large

(1–1.5 µm) non-motile obligate photoautotrophs that exhibit low salt tolerance. Cluster two also is characterized by low salt tolerance. Cells are obligate photoautrotrophs, lack phycoerythrin and are thermophilic. Cluster three includes phycoerythrin lacking marine *Synechococcus* that are euryhaline, capable of growth in both marine and fresh water environments. Cluster four contains a single isolate, is an obligate marine and contains phycoerthrin. The last cluster contains strains that are obligate photoautrophs and are around 0.6 - 1.7 µm in diameter.

Synechococcus has been observed to occur at high abundances in environments with low salinities and/or low temperatures. *Synechococcus* is usually far outnumbered by *Prochlorococcus* in all environments, where they co-occur. Marine *Synechococcus* group occupy an important position at the base of the marine food web: they are abundant in the world's oceans and as a result are major primary producers on a global scale and one of the most numerous genomes on earth (Waterbury *et al*., 1986). Members of this group are adapted to life in the ocean. They are obligately marine, having elevated growth requirements for Na^{+}, Cl^{-}, Mg^{2+} and Ca^{2+}; they have the ability to acquire major nutrients and trace metals at the submicromolar concentrations found in the oligotrophic open seas (Carr and Mann, 1994), and their light-harvesting apparatus is uniquely adapted to the spectral quality of light in the ocean (Wood, 1985).

A third of the *Synechococcus* possess a unique type of swimming motility not seen in any other type of microorganism, they propel themselves through seawater at speeds of upto 25 diameters/sec in the absence of any demonstrable external organelle (Waterbury *et al*., 1985). They use their motility to respond to extremely small gradients (10^{-9} to 10^{-10} Mol/dm^{3}) of

nitrogenous compounds (Wiley and Waterbury, 1989). Members of the marine *Synechococccus* group are closely related at the level of 16S rRNA and form a monophyletic cluster within the group (Toledo *et al*., 1999).

Isolate MCy2 was similar to *Microcystis,* a genus of freshwater cyanobacteria species characterized by small cells of about 2.5 to 5.5 µm in diameter. The cells are usually organized into colonies that begin in a spherical shape, but losing their coherence to become perforated or irregularly shaped over time. The coloration of the protoplast is a light blue-green, appearing dark or brown due to optical effects of gas-filled vesicles which are useful as a distinguishing characteristic when using light microscopy (Lalita *et al*., 2009). The presence of the gas-filled structures can keep *Microcystis* cells close to the surface of water body, where there is optimal light and oxygen for growth. Thus, when the water column is stable, the colonies can accumulate at the water surface and form surface water blooms (Mlouka *et al*., 2004).

Surface water blooms can cause anaerobic conditions below the surface in the water and thus make other phytoplankton including *Microcystis aeruginosa* themselves live in an unfavorable environment. However, *M. aeruginosa* appears to be more tolerant to dark anaerobic conditions, which helps it to dominate in eutrophic lakes (Shi *et al*., 2007). The species is favored by warm temperatures, but toxicity and maximal growth rates are not totally coupled, as the cyanobacterium has highest laboratory growth rates at 32 ^{o}C, while toxicity is highest at 20 ^{o}C. Growth has been found to be limited below 15 ^{o}C and *is* inhibited by the aquatic plant *Myriophyllum spicatum* that produces allelopathic polyphenols including ellagic, gallic and pyrogallic acids (Nakai *et al*., 2000).

Isolate MCy3 with unicellular cells of about 1µm that were spherical, exhibited gliding motion and formed small discrete colonies characteristic of *Synechocystis*. The species has a photosynthetic apparatus very similar to the one found in plants and exhibits phototactic movement (Anderson and McIntosh, 1991). Among all cyanobacterial species, *Synechocystis* sp. PCC 6803 is one of the most extensively studied species since it was initially isolated from a freshwater lake in 1968. The entire genome, including four endogenous plasmids, was sequenced, and over 3000 genes have been annotated to date (Kaneko *et al*., 2003). *Synechocystis* 6803 demonstrates versatile carbon metabolisms, growing under photoautotrophic, mixotrophic and heterotrophic conditions (Vermas, 1996). Additionally, biochemical similarities between the plant chloroplasts and *Synechocystis* 6803 make the latter an ideal system for studying the molecular mechanisms underlying stress responses and stress adaptation in higher plants (Los, 2010).

Isolate MCy4 with minute cells of about 0.5 µm similar to *Prochlorococcus* the smallest known photosynthetic organism whose cell size varies from 0.45 to 0.75 µm (Lindell, 1998). It possesses a pigment complement, which includes divinyl derivatives of chlorophyll (Chl *a* and Chl *b)*, which are referred to as Chl *a*2 and Chl *b*2 that are unique to this genus (Neveux, 1993). One of the most intriguing ecological characteristics of *Prochlorococcus* sp., besides its capacity to grow over a very wide range of irradiances in nature, is its ability to colonize extremely oligotrophic areas. Under these conditions, the small cell size and the resulting high surface-to-volume ratio are adaptative advantages for nutrient uptake (Chisholm, 1992).

Prochlorococcus has been found to be abundant in the euphotic zone of the world's tropical oceans (Chisolm *et al.,* 1992). It is possibly the most plentiful species on earth; a single millilitre of surface seawater may contain 100,000 cells or more. *Prochlorococcus* is ubiquitous and dominates in the oligotrophic (nutrient poor) regions of the oceans (Partensky *et al.,* 1999). It is mostly found in a temperature range of 10 – 33 ºC and some strains can grow at depths with low light (<1 % surface light) (Munn, 2011). The bacterium accounts for an estimated 20% of the oxygen in the earth's atmosphere, and forms part of the base of the ocean food chain (Munn, 2011). It is the only known wild-type oxygenic phototroph that does not contain chlorophyll 'a' as a major photosynthetic pigment, and is the only known prokaryote with α-carotene (Goericke and Repeta, 1992).

Prochlorococcus occupies two distinct niches, leading to the nomenclature of the low light (LL) and high light (HL) groups which vary in pigment ratios (LL has a high ratio of chlorophyll b2: a2 and HL has a low ratio of b2: a2), light requirements, nitrogen and phosphorus utilization, copper and virus sensitivity (West and Scanlan,1999). High-light adapted strains inhabit depths between 25 and 100 m, while low-light adapted strains inhabit waters between 80 and 200 m (Martiny *et al*., 2009). Recently the genomes of several strains of *Prochlorococcus* have been sequenced. Twelve complete genomes have been sequenced which reveal physiologically and genetically distinct lineages of *Prochlorococcus marinus* that are 97% similar in the 16S rRNA gene (Martiny *et al.,* 2009).

The heterocystous filaments of isolate MCy5 that formed a slime layer at the surface of the medium were similar to those of *Anabaena* which has gas vacuoles that inflate or deflate with air

to provide upward or downward movement. This adaptation positions *Anabaena* at a favorable depth, determined by available sunlight, water temperature, or oxygen concentration. With optimal environmental conditions, *Anabaena* grows unchecked, forming large blooms on the surface of the water (Herrero and Flores, 2008).

The filaments of *Anabaena* have numerous vegetative cells responsible for photosynthesis. During periods when combined nitrogen (ammonia or nitrate) is unavailable, *Anabaena* forms heterocysts, larger, round, nitrogen-fixing cells found in every ten to twenty cells or so on the filament. Heterocysts function to convert environmental nitrogen (N_2) into compounds such as ammonia or nitrate. Nitrogenase, an oxygen-sensitive enzyme, is essential for this conversion. For the proper functioning of nitrogenase, the intracellular environment of the heterocyst should be anaerobic, a task achieved by the oxygen-impermeable structure of the heterocyst wall. Although functioning independently from each other, vegetative cells and heterocysts are both essential to the survival of the organism, vegetative cells providing energy-rich sugars to the organism, while heterocysts fix nitrogen for cellular biosynthesis (Herrero and Flores, 2008).

In harsh conditions, *Anabaena* forms spore-like cells called akinetes. *Anabaena* forms symbiotic relationships with certain plants, such as the mosquito fern. They are one of four genera of cyanobacteria that produce neurotoxins, which are harmful to local wildlife, as well as farm animals and pets. Production of these neurotoxins is assumed to be an input into its symbiotic relationships, protecting the plant from grazing pressure (Herrero and Flores, 2008).

Isolate MCy6 with closely twisted filaments was similar to *Arthrospira* (*Spirulina*) which is an oxygenic phototroph that can tolerate high levels of bicarbonate, carbonate, salinity and survives in water with a pH as high as 11. *Arthrospira platensis* is a multicellular, filamentous cyanobacterium. Each unbranched, cylindrical filament of cells is called a trichome and is approximately 5µm in diameter. The trichomes vary greatly in length and are typically spiral-shaped, though they can take on a left-hand helical structure in liquid media (Cifferi, 1983). Individual cells within the filament are wider than they are long and are separated by transverse cross-walls. The cell wall contains four layers; an innermost fibril layer, a second peptidoglycan layer, a third layer composed of proteins, and an outermost layer analogous to the cell wall of all gram-negative bacteria (Cifferi, 1983).

Cells of *Arthrospira platensis* contain chlorophylls, carotenoids, and phycobiliproteins, which are pigments capable of absorbing light energy and funneling it to the reaction centers where photosynthesis takes place. The cytoplasm contains gas vacuoles, carboxysomes, and thykaloid membranes as adaptations to being phototrophic. The gas vacuoles within the cells increase the cell's buoyancy so that it remains at the surface of its aqueous environment where the presence of light is greatest. The carboxysomes are protein-enclosed compartments that contain the enzyme, RuBisCo (ribulose-bisphosphate carboxylase), necessary for fixing carbon dioxide, and the thylakoid membranes contain the pigments previously mentioned. *Arthrospira platensis* lacks heterocyst and can therefore not carry out the process of nitrogen fixation (Slonczewski and Foster, 2009).

Isolate MCy7 had long un-branching filaments that were motile by oscillation motion and was identified as *Oscillatoria*. It uses the mobility to move towards light in order to conduct

photosynthesis and forms a mass of colonies at the bottom of culture bottle. Källqvist (1981) investigated growth of *Planktothrix (Oscillatoria)* and found that it was able to grow at very low light intensities below 3 m depth. The ability of *Planktothrix* sp. to grow at low light intensities and to harvest certain specific light qualities enables it to grow in the shadow of other phytoplankton. They have cylindrical or sometimes slightly tapering, unbranched filaments (trichomes), often with a rounded or capitate apical cell. Other cells are discoid, with further developing cross-walls. Trichomes leave a thin mucilaginous trail as they glide, but there is no sheath of mucilage as is present in the related genus, *Lyngbya*.

Species of *Oscillatoria* occur in a diverse range of conditions, in damp soil or on dripping rocks, in freshwater, in the sea and in hot springs. Some are tolerant of high levels of organic pollution and some are shade-tolerant and able to survive in water below blooms of green algae. In water they may be benthic or planktonic. *Oscillatoria rubescens* is a red species that can form conspicuous red blooms in eutrophicated lakes. It is implicated in the irritation of skin and mucous membranes sometimes suffered by people swimming off tropical coastlines (Hoek *et al*., 1995).

4.5 Conclusions

Lake Magadi provides an environment that is favorable for growth of microbes due to the unlimited supply of carbon dioxide and high temperature. Thus despite the apparent unfavorable hypersaline, alkaline conditions, a rich diversity of microbial life inhabits the lake. There is presence of heterotrophic proteobacteria such as *Idiomarina* sp. of class gammaproteobacteria and also autotrophic proteobacteria such as *Rhodobacter* sp. of class alphaproteobacteria.

Chemoorganotrophs such as the *Idiomarina* sp. are known to produce hydrolytic enzymes including proteinases, lipases and carbohydrases in order to utilize the products of primary production.

There is presence of cyanobacteria forming visible blooms during rainy seasons such as the unicellular *Synechococcus, Microcystis, Synechocystis, Proclorococcus and* filamentous *Anabaena, Arthrospira and Oscillatoria*. The cyanobacteria were generally small-celled which increases their surface area allowing sufficient diffusion of gases and nutrients. They possess protective slimy sheaths covering their cells enabling them to form colonies and to attach onto substrates. They possess photosynthetic pigments that enable them to absorb and use more of green, red and yellow wavelengths of light during photosynthesis. The relative proportion of the pigments accounts for the many hues of the lake water ranging from dark green to bluish, olive-green, brown and red. The *Anabaena* sp. possesses heterocysts for nitrogen fixation impacting on the ecosystems primary productivity. The *Synechococcus*, *Synechocystis*, *Oscillatoria* and *Microcystis* exhibited some form of motility enabling them to move towards light or nutrients. These adaptations enable the cyanobacteria to survive in the extreme soda lake environment.

Much of the cyanobacteria population resides in some parts of the shoreline and in the lagoons formed by hot springs. This is likely due to presence of considerably higher levels of nutrients brought by runoffs during the rainy season and lower salinity and alkalinity. More isolation of cyanobacteria species needs to be done in order to have an adequate record that can be used to track new members colonizing the ecosystem and those disappearing.

CHAPTER FIVE: EFFECT OF PHYSICOCHEMICAL FACTORS ON GROWTH PATTERNS OF CYANOBACTERIA ISOLATES

Abstract

Despite the extreme physicochemical conditions in Lake Magadi, microorganisms that are well adapted to these conditions are able to thrive there. Distribution of these microorganisms is likely to follow patterns related to the physicochemical conditions in the lake. It is necessary to understand the effect of physicochemical conditions on the growth of cyanobacteria that are important for primary productivity in Lake Magadi. This was done by determining the effect of selected physicochemical factors on the growth of unicellular cyanobacterial isolates. These factors included salinity between 0.0 % w/v and 0.9 % w/v, pH 6 to pH 10 and temperature starting with room temperature (23 ± 2 ^{o}C) to 60 ^{o}C. Population growth was measured as optical density of liquid cyanobacterial cultures at a wavelength of 680 nm. The optimum growth for five out of six isolates was at room temperature (23 ± 2 ^{o}C) next to a window allowing adequate light but growth was also present at 50 ^{o}C. Growth in four out of six isolates was optimum at pH 6 but growth was also registered at pH 10. All six isolates investigated had optimum growth at 0.0 to 0.3 % w/v NaCl concentrations but there was growth at 0.9 % w/v. The six unicellular cyanobacteria isolates investigated therefore exhibited tolerance to environmental extremes and could be described as thermotolerant, alkalitolerant and halotolerant. The data obtained may be useful to ecologists during studies of distribution of cyanobacteria species in the lake.

5.1 Introduction

A common observation is that cyanobacteria dominance of aquatic communities is greater when water temperatures are warmer (Brock, 1975). Waterbury *et al*. (1979) outlined the factors controlling the abundance of cyanobacteria species. He included grazing, viral mortality, genetic variability, light adaptation, temperature as well as nutrients and suggested that there is a relationship between ambient nitrogen concentrations and *Synechococcus* abundance. The factors controlling the abundance of cyanobacteria still remain poorly understood and need to be investigated on a rigorous and global scale.

This study aimed at investigating the effect of selected physicochemical factors on the population growth and abundance of cyanobacteria species. Growth of the isolates was subjected to varying conditions of temperature, pH and salinity. Cyanobacteria isolates were grouped based on growth characteristics for extremophiles (Table 5.1). The data obtained in this chapter could be used by environmentalists when making management decisions regarding soda lake ecosystems.

Table 5.1: Growth characteristics of extremophiles

Growth characteristics	Minimum	Optimum	Maximum
Halotolerant	–	$[Na^+]$ <0.2 m	$[Na^+]$ >0.2 m
Halophilic	$[Na^+]$ ≥0.2 m	$[Na^+]$ 0.2-1.7 m	–
Extreme halophile	$[Na^+]$ ≥0.2 m	$[Na^+]$ ≥ 1.7 m	–
Alkalitolerant	≥pH 6	< pH 8.5	>pH 9.0
Facultative alkaliphilic	<pH 7.5	≥pH 8.5	–
Obligate alkaliphilic	≥ pH 7.5	≥ pH 8.5	≥pH 10.0
Thermotolerant	–	< 50 ºC	> 50 ºC
Thermophilic	–	50 ºC	55 ºC

Source: Wiegel (1998)

pH should be measured at optimum growth temperature

(-) Indicates no boundary at this limit; $[Na^+]$ concentration of Na^+

"m" represents concentration of Na^+ in mols/liter

5.2 Materials and methods

5.2.1 Determination of growth patterns of unicellular cyanobacterial isolates

Fifty milliliters of Aiba and Ogawa liquid medium was inoculated with each pure unicellular cyanobacterial isolate growing on Aiba and Ogawa agar medium using a sterile inoculating needle. This was replicated thrice. The cultures were incubated at room temperature (23 ± 2 ºC) next to a window allowing adequate sunlight, for about 40 days. Growth was determined by counting the number of cells of the unicellular isolates using the Neubauer chamber after every two days. This was done by use of a micropipette to place 0.1 µl into the well of the chamber and

counting the cyanobacteria cells at x1000 magnification of the optical microscope (Leica dm 500 model).

5.2.2 Determination of optimum temperature for growth of cyanobacterial isolates

Five milliliters of stock broth cultures of each cyanobacterial isolate was added to 45 ml of freshly prepared Aiba and Ogawa nutrient broth. The cultures were incubated at the following temperatures: room temperature (23 ± 2 ᵒC) next to a window allowing enough sunlight, 30 ᵒC, 40 ᵒC, 50 ᵒC and 60 ᵒC in an incubator. This temperature range included the optimal temperature for enzyme-controlled metabolism (25 ᵒC to 40 ᵒC) and the optimal temperature for thermophiles (50 ᵒC to 60 ᵒC). The length of daylight did not vary substantially, staying within 12 hrs and 12 mins throughout the year. Light was therefore not likely to be a limiting factor for growth. The optical density of each broth culture was measured using a spectrophotometer (Beckmann Coulter Du 530 model) at wavelength of 686 nm (Brown, 2005) at incubation and after every three days for 12 days. The cultures at each temperature were replicated thrice.

5.2.3 Determination of optimum pH for growth of cyanobacterial isolates

Using concentrated hydrochloric acid and universal paper strips to monitor pH, 200 ml of broth medium for halophilic cyanobacteria had the pH adjusted from 10 to 7 and 6. This range of pH conditions was chosen to determine the alkaliphilic or alkalitolerant nature of the isolates. The media were filter sterilized using 0.22 µm pore size membrane filter. Forty- five milliliters was poured into sterile culture flasks and inoculated with 5 ml stock broth cultures of each isolate. The cultures were incubated at room temperature (23 ± 2 ᵒC) next to a window allowing enough sunlight and optical densities measured at the beginning and after every three days for

fifteen days. The cultures at each pH condition were replicated three times.

5.2.4 Determination of optimum salinity for growth of cyanobacterial isolates

Aiba and Ogawa medium was prepared with different NaCl concentrations (w/v) of 0 %, 0.1 %, 0.3 %, 0.6 % and 0.9 % w/v. This range of salinities used was to determine the halophilic or halotolerant nature of the isolates. Five milliliters of each broth culture was added to 45 ml of the liquid medium with different salt concentrations and incubated at room temperature (23 ± 2 ^{o}C) next to a window allowing sunlight for 14 days. The optical density of each broth culture was measured at the beginning and after every 4 days. The cultures at each salinity level were replicated thrice.

5.3 Results

5.3.1 Growth patterns of unicellular cyanobacteria species

Growth curves of four unicellular cyanobacteria species isolated from Lake Magadi showed lag phases ranging from 10 days with isolate MCy1 (*Synechococcus*) to 26 days with isolate MCy4 (*Prochlorococcus*). Isolate MCy3 (*Synechocystis*) had the least population growth with a maximum of about $4x10^6$ cells/ml while isolate MCy1 had the highest growth with maximum of about $1.4x10^7$ cells/ml followed by isolate MCy2 (*Microcystis*) with a maximum of about 8.5 $x10^6$ cells/ml (Fig. 5.1).

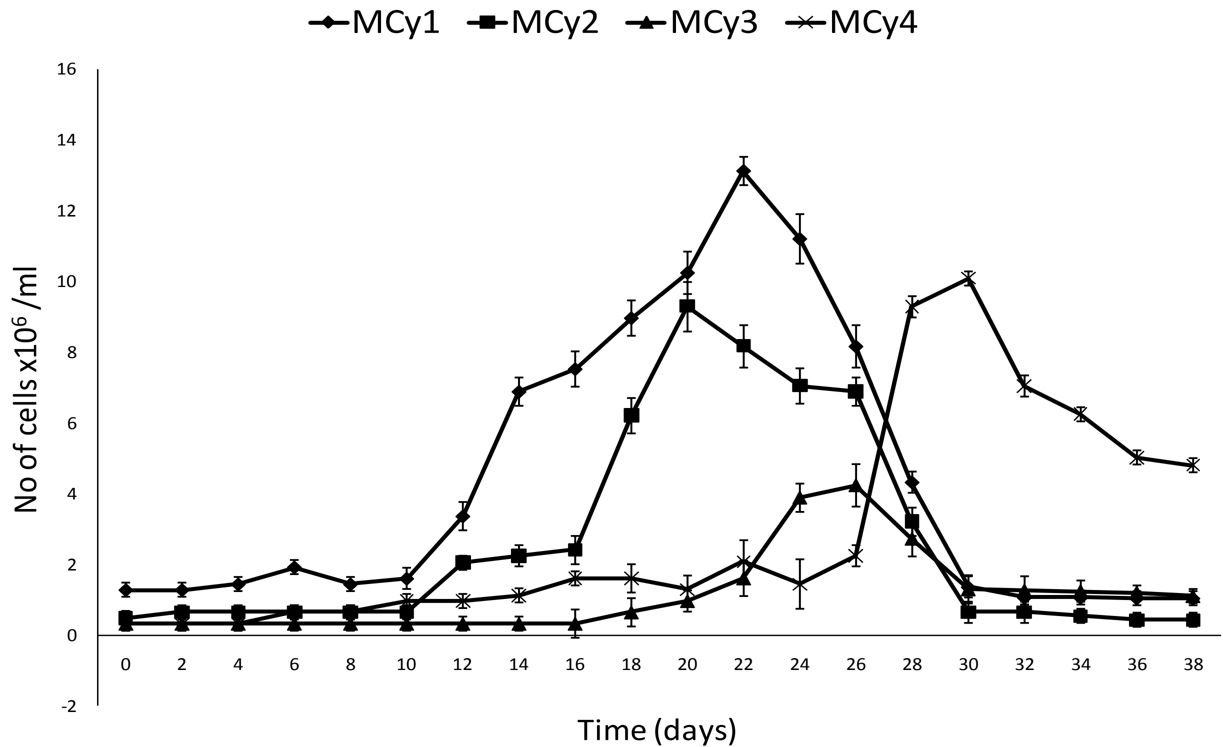


Fig.5.1: Population growth (number of cells/ml) of unicellular cyanobacteria species isolated from water sampled from Lake Magadi. Bars represent Standard error of the means.

5.3.2 Effect of temperature on growth of cyanobacterial isolates

Population of cyanobacteria incubated at 50 ᵒC (in the incubator) and at room temperature (23 ± 2 ᵒC) next to a window allowing plenty of sunlight generally increased with time. Population growth in the incubator at 30 ᵒC, 40 ᵒC and 60 ᵒC generally declined with time (Fig. 5.2). At 50 ᵒC the cells had less chlorophyll, indicated by a much lighter shade of green, and had less aggregation of cells into colonies. The measurement of optical density of isolate MCy7 was difficult as it formed membrane like masses of colonies that were difficult to homogenize.

Temperature had a significant ($p<0.001$) effect on the population growth of isolate MCy1, MCy2, MCy4, MCy5 and MCy6 (*Synechococcus*, *Microcystis*, *Prochlorococcus*, *Anabaena* and *Arthrospira* respectively). The highest population growth was at room temperature with means of optical density going up to 1.181 for all the isolates followed by 50 ^{0}C. The lowest growth rate was at 60 ^{o}C, 40 ^{o}C and 30 ^{o}C with means of only up to 0.5282. The latter three temperatures did not have a significant difference in population growth between them. Temperature did not have a significant ($p = 0.471$) effect on the population growth of isolate MCy3 (*Synechocystis*) (Fig. 5.2C). Isolate MCy4 (Fig. 5.2D) had higher growth at 50 ^{0}C in comparison to growth in the other temperatures.

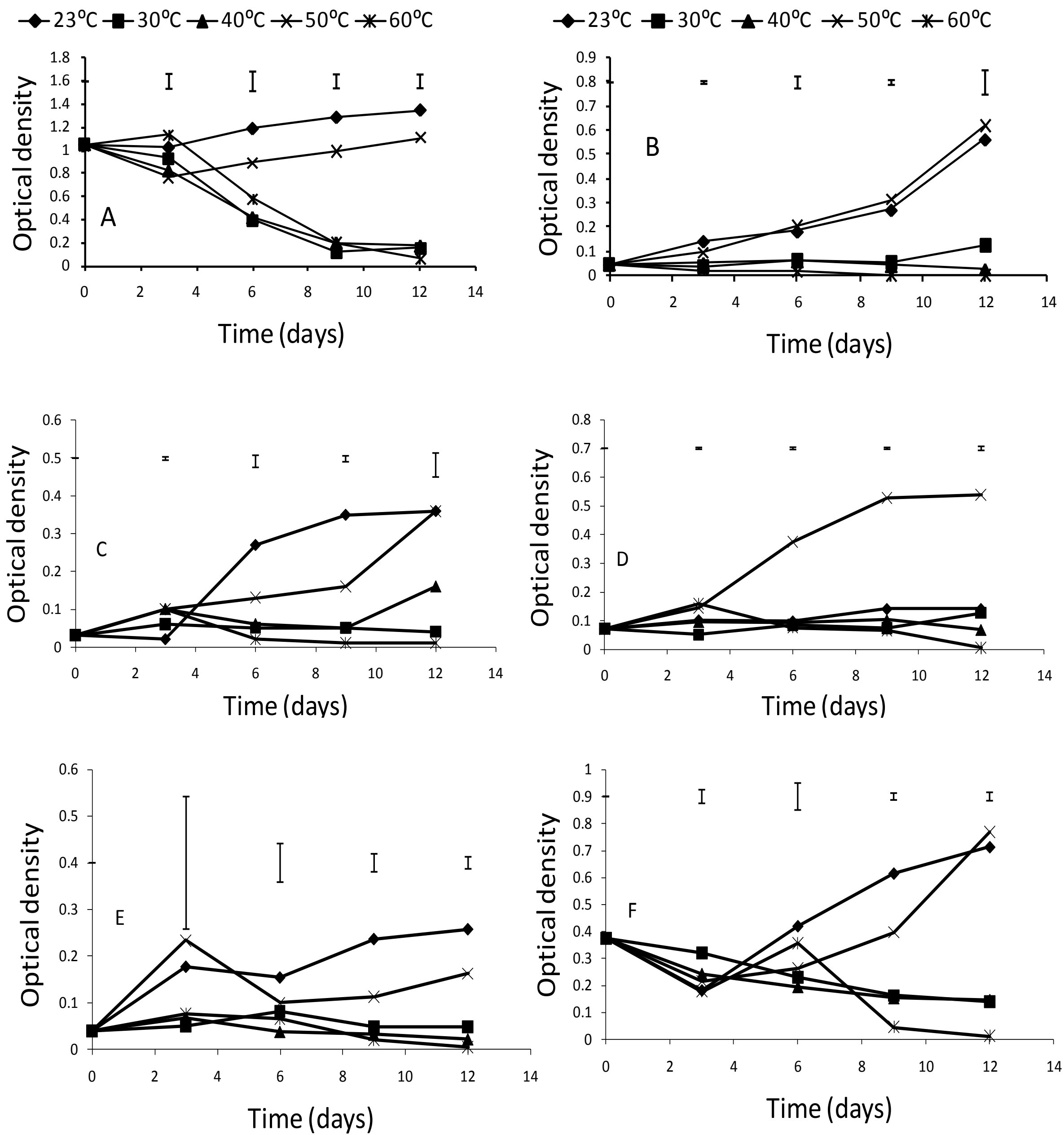


Fig. 5.2: Population growth (cell concentration measured in terms of optical density) of cyanobacteria from Lake Magadi cultured at varying temperatures. Each data point represents the average result of three counts with the least significant difference bars. (A) Isolate MCy1; (B) Isolate MCy2; (C) Isolate MCy3; (D) Isolate MCy4; (E) Isolate MCy5; (F) Isolate MCy6.

5.3.3 Effect of pH on growth of cyanobacterial isolates

Population growth of cyanobacteria isolated from samples of water from Lake Magadi was higher at pH 6 for four of the isolates (Fig. 5.3A, Fig. 5.3C, Fig. 5.3D and Fig. 5.3E). Two of the isolates had higher growth at pH 7 (Fig. 5.3B and Fig. 5.3F) though they appeared to grow relatively well in all the pH conditions. The pH value had a significant ($p<0.001$) effect on population growth of cyanobacterial isolate MCy1, MCy3, MCy4 and MCy5 (*Synechococcus*, *Synechocystis*, *Prochlorococcus* and *Anabaena* respectively). Variation of pH did not have a significant ($p = 0.377$) effect on growth of isolate MCy2 (*Microcystis*) and also isolate MCy6 (*Arthrospira*). Growth at pH 6 had the highest mean of 0.8158 followed by pH 7 which had a mean of 0.6138.

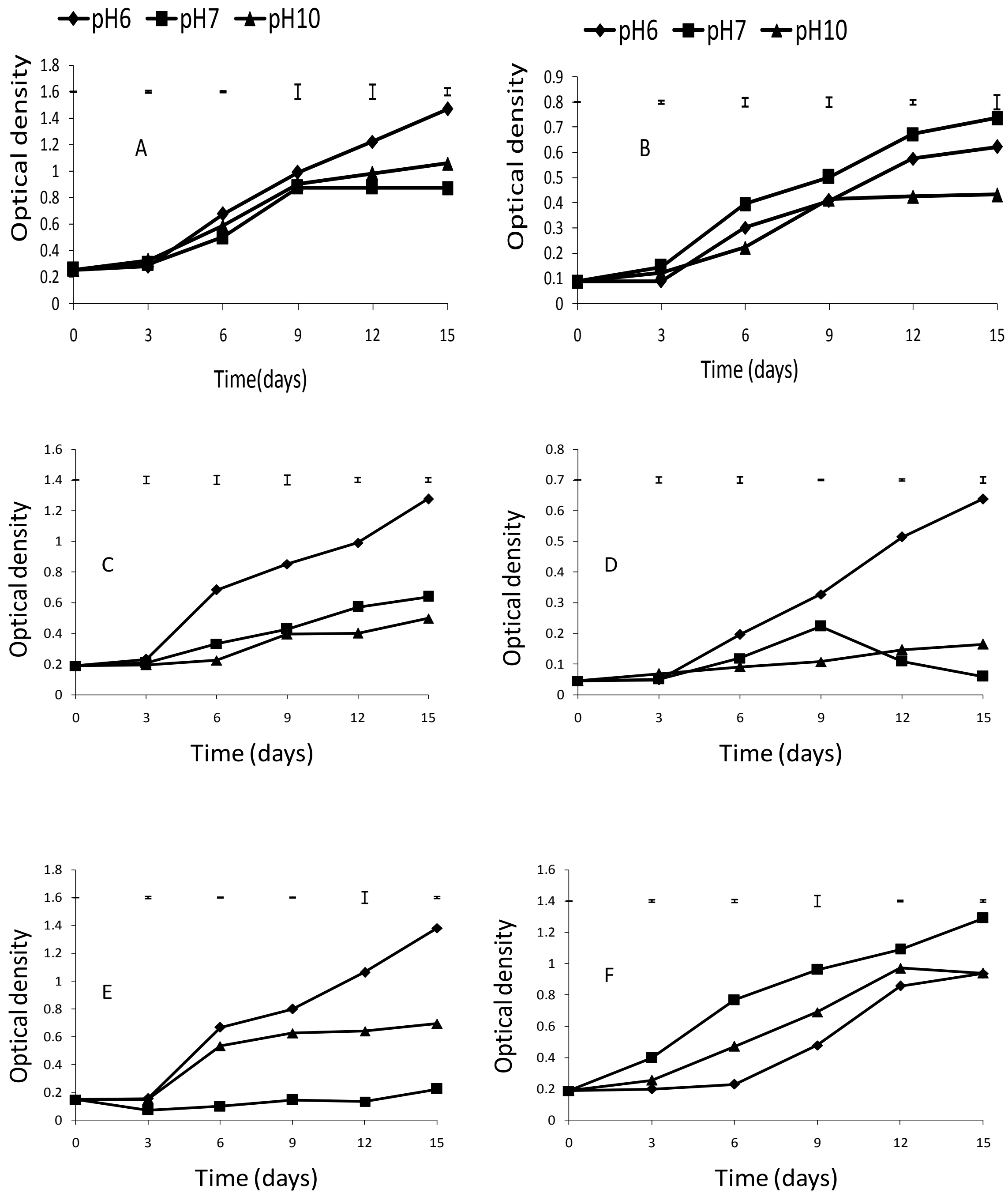


Fig. 5.3: Population growth (measured in terms of optical density) of cyanobacterial isolates from water sampled from Lake Magadi at different pH conditions. Each data point represents the average result of three counts with the least significant difference bars. (A) Isolate MCy1; (B) Isolate MCy2; (C) Isolate MCy 3; (D) Isolate MCy4; (E) Isolate MCy5; (F) Isolate MCy6.

5.3.4 Effect of salinity on growth of cyanobacterial isolates

Growth of cyanobacteria species isolated from Lake Magadi generally increased with time (Fig. 5.4). Salinity had a significant ($p=0.008$) effect on the growth of isolate MCy1 (Fig. 5.4A) with salinity of 0.3 % w/v having the highest mean (79.72) while other salinities were not significantly different from each other. Salinity also had a significant ($p<0.001$) effect on population growth of isolates MCy2, MCy4, MCy5 and MCy6 (*Microcystis*, *Prochlorococcus*, *Anabaena* and *Arthrospira* respectively) but did not have a significant ($p=0.423$) effect on the growth of isolate MCy3 (*Synechocystis*) (Fig. 5.4C). Isolates MCy4, MCy5 and MCy6 (*Prochlorococcus*, *Anabaena* and *Arthrospira* respectively) had their highest means of growth at salinity of 0.0 % w/v.

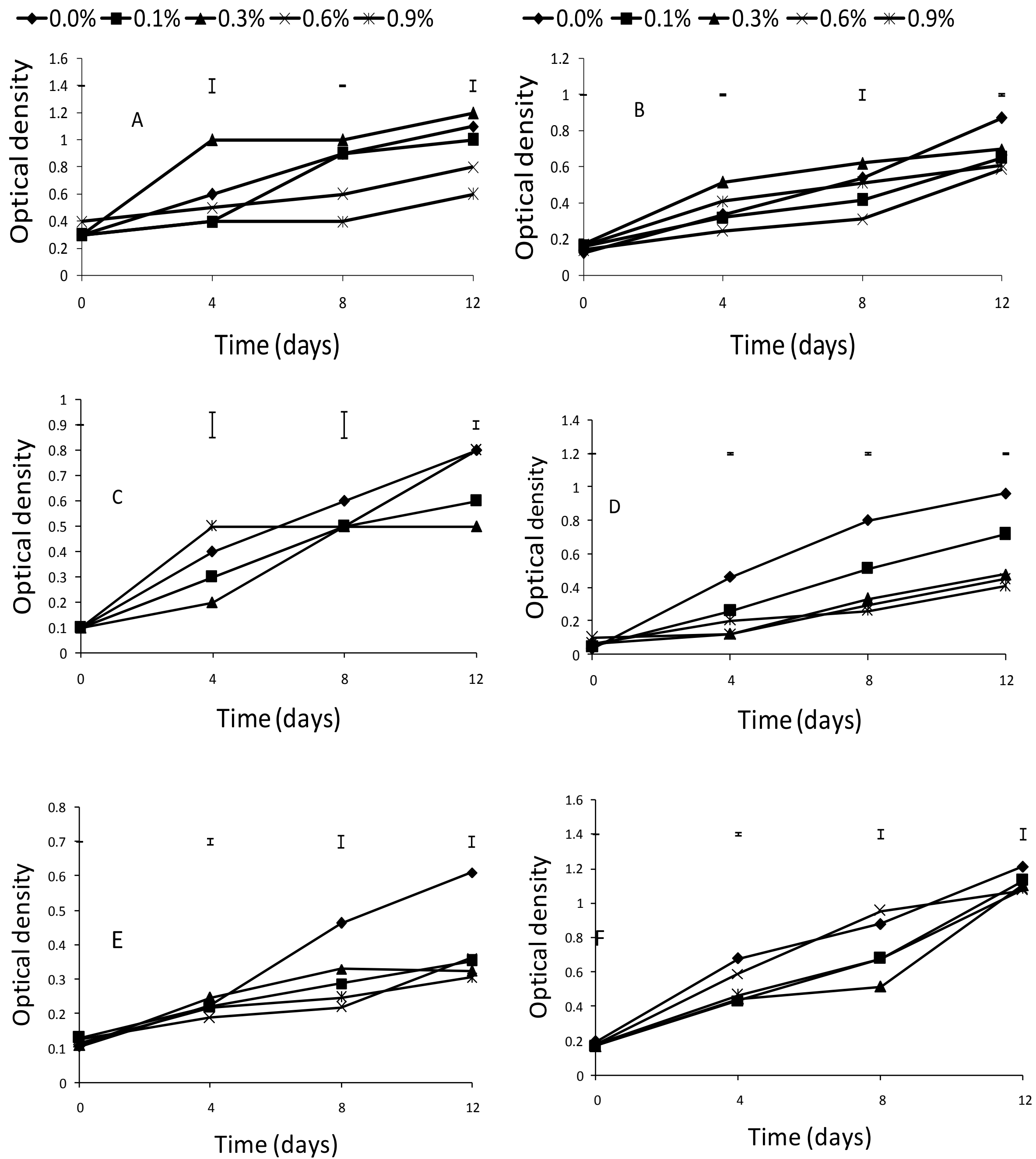


Fig.5.4: Growth (measured in terms optical density) of cyanobacterial isolates from water sampled from Lake Magadi at different salinity conditions. Each data point represents the average result of three experiments with the least significant difference bars. (A) Isolate MCy1; (B) Isolate MCy2; (C) Isolate MCy 3; (D) Isolate MCy4; (E) Isolate MCy5; (F) Isolate MCy6.

5.4 Discussion

Growth of cyanobacteria in culture media reaches maximum in about twenty four days after inoculation. All the isolates had optimal growth at room temperature (23 ± 2 °C) when placed in plenty of sunlight next to a window. Growth also occurred on incubation at 50 °C but lack of light may have led to less amount of chlorophyll. These findings were in agreement with those of Reynolds, (1984) that many phytoplankton cellular processes are temperature dependent, their rates accelerating exponentially with increasing temperature, with maximal values occurring between 25 °C and 40 °C. The photosynthetic and specific maximal growth rate responses of different species to temperature can be compared provided other factors, such as illumination and nutrients, remain saturating. Responses are, however, highly variable from one species to another (Reynolds, 1987). Co-existing communities of photosynthetic micro-organisms in lakes display both temporal and spatial patterns often associated with co-variations in temperature (Tilman and Kiesling, 1984). Renolds (1987) has pointed that apart from availability of nutrients, the other environmental factors controlling phytoplankton growth are temperature and light fluctuations.

The growth rate of isolate MCy6 (*Arthrospira*) was relatively high in all pH conditions. This agrees with studies on pH requirements for certain cyanobacterial species (Bano and Siddiqui, 2004) that showed *Spirulina major* to have high growth rates at all pH values though on the basis of chlorophyll content, best growth was obtained at pH 6.5, whereas highest growth was at pH 8.0 when protein content was taken into account.

Lower salinity of between 0.0 and 0.3 % w/v NaCl concentration favored the growth of all isolates. These results are supported by studies by Kevin *et al.* (1987), done on a bloom of the

cyanobacterium *Microcystis aeruginosa* in the upper Potomac River. It had densities of 193 $\times 10^6$ cells 1^{-1} and 83 % of total cells in the surface mixed layer. However, in regions typified by salinities of 1 - 2 ppt immediately down-river, the cyanobacteria disappeared from the phytoplankton assemblage, never contributing more than 17 % of total phytoplankton numbers. Bloom samples collected from the river were exposed to daily salinity increases of 1 - 2 ppt through the addition of NaCl. Relative to samples receiving no salt supplement, densities of *Microcystis* spp. declined in salinity-stressed samples. Chlorophyll concentrations declined slightly relative to assemblages receiving no salt additions while carbon fixation was reduced in salinity-stressed assemblages. These results suggest that salinities from 0.5 to 7 ppt could limit the distribution of *Microcystis*-dominated blooms.

The lake water is saturated with CO_2 and the molar concentration of HCO_3^- and CO_3^{2-} ions greatly exceeds that of Ca^{2+} and Mg^{2+}. As a result of evaporation, saturation of these alkaline earth cations is rapidly achieved and they precipitate out of solution as insoluble carbonates. This leaves Na^+, Cl^-, HCO_3^- and CO_3^{2-} ions as the major ions in solution (Jones *et al*., 1998). The lake is however a productive ecosystem due to the availability of dissolved carbon dioxide, high ambient temperatures and high daily light intensities (Melack and Kilham, 1974) and harbors diverse species of microorganisms.

5.5 Conclusions

Subjecting the cyanobacterial isolates to different physicochemical conditions altered their growth. The cyanobacteria species have optimum growth at room temperature (23 ± 2 °C) with adequate light. Growth was also found to occur at 50 °C and the cyanobacteria species could

hence be described as thermotolerant. Most of the species had optimum growth rates at pH 6 but growth occurred upto pH 10 and they therefore could be considered to be alkalitolerant. Most of the isolates investigated had optimal growth at NaCl concentration of between 0.0 to 0.3 % w/v (which is less than 0.2 mol/l) hence could be classified as halotolerant.

Cyanobacteria therefore may not be distinctly classified as marine or fresh water but could be described in terms of their ability to tolerate certain conditions, such as the thermotolerant *Synechococcus*, the alkalitolerant *Microcystis* and the halotolerant *Arthrospira.* This explains why cyanobacteria are known to inhabit almost every type of environment and hence their ability to survive in the hypersaline and alkaline conditions of the soda lake.

CHAPTER SIX: EFFECT OF PHAGE INFECTION ON GROWTH PATTERNS OF PROTEOBACTERIA AND CYANOBACTERIA HOSTS

Abstract

Phages that infect photosynthetic bacteria cause lysis of their cells impacting on the population growth of these bacterial hosts and hence affecting primary productivity of aquatic ecosystems. It is important to determine the impact of such phages on the population sizes of their bacterial hosts in Lake Magadi. The media used for isolation of proteobacteria and cyanobacteria were the medium modified for halolakaliphilic bacteria and the Aiba and Ogawa medium for halophilic cyanobacteria. Phage lysate was prepared from water samples collected from Lake Magadi using phage growth medium. The plaque assay technique was used to determine host range by placing overlays of the phage lysate on colonies of bacterial isolates. Population growth of proteobacterial isolates inoculated with phage and without inoculation was estimated by measurement of optical density of liquid cultures while that of cyanobacterial isolates was estimated by enumeration of the cells using a Neubauer chamber. Plaques with a diameter of about 2 mm were formed on isolate MB5 (*Rhodobacter* sp.) and about 1.2×10^5 PFU/ml were counted in 48 hrs. The growth of isolate MB5 with phage inoculation had a significant difference ($p = 0.009$) from the one without inoculation. Inoculation with phage lysate decreased the maximum concentration of bacterial culture from 0.6 to 0.4, a decline of about 30 %. Phage infection was found to cause a significant ($p<0.001$) difference on the population of the cyanobacterial host causing a reduction of about 50 % on the cell numbers in 10 days. Physicochemical factors such as temperature of 37 ºC and pH 7 that cause a decline in the growth of the cyanobacteria host were found to reduce infectivity of the phages. Electron

microscopy on phage lysate revealed the presence of carboxysomes, cellular compartments measuring about 150 nm. Phages cause considerable reduction in numbers of their host and could be applied by environmentalists when there is need to vary primary productivity of aquatic ecosystems. Ecologists could use the data to correlate diversity and population sizes of proteobacteria and cyanobacteria species in the lake to presence of infectious cyanophages.

6.1 Introduction

Viruses in the marine environment are abundant and diverse. Surface seawater samples typically contain 10^{10} viral particles per liter, and the viral genotypes identified can be extremely diverse (Breitbart *et al.,* 2002). Bacteriophages can be found in all environments where bacteria grow such as the Sahara, hot springs, the North Sea, and polar inland waters (Lin *et al.,* 2010).

Cyanophages belong to three recognized families of double-stranded DNA viruses; Myoviridae (contractile tails), Styloviridae (long non-contractile tails) and Podoviridae (short tails). They have a complex pattern of host ranges, are widely distributed and can be readily isolated from marine and fresh waters. In marine waters, genetically diverse Myoviridae which infect *Synechococcus* spp. are the most abundant cyanophages. Styloviridae and Podoviridae are most commonly isolated from fresh waters (Suttle, 2000). Although cyanophages are related to other bacteriophages, it is likely that they evolved more than 3 billion years ago when cyanobacteria diverged from other prokaryotes (Suttle, 2000).

Since their discovery, phages have been used for various practical applications, including in human and veterinary medicine. Therapeutic applications of phages were however largely

forgotten after the widespread acceptance of antibiotics during the 1940s and 1950s. The emergence of antibiotic-resistant bacteria has rekindled interest in practical uses of phages.

Virus-caused mortality of bacteria affects levels of dissolved organic matter and inorganic nutrients contributing to biogeochemical cycling in aquatic ecosystems. It therefore has an influence on the structure and diversity of microbial communities in the ecosystem. It is important to investigate phage - mediated mortality of bacteria in Lake Magadi. This study aimed at establishing the host range of phages isolated from samples of water collected from Lake Magadi and determining their effect on the growth patterns of their hosts.

6.2 Materials and methods

6.2.1 Determination of phage host range by plaque assay technique

To ensure an adequate supply of phages, the water sample was enriched with phage growth medium at a ratio of 1:1 and incubated at 37 ^oC for 24 hrs. It was then poured into sterile tubes and placed on a slowly moving shaker for about 48 hrs. An equal amount of lysing medium was added to the growth medium during the last 6 hrs of incubation to augment the lysing properties of phage. The lysing medium was composed of 0.98 g/l NaCN (Brown, 2005). Debris and miscellaneous bacteria was removed from the enrichment culture medium by a triple centrifugation process. This was done by centrifuging at 2,500 rpm for 10 min. The supernatant culture medium was decanted into another set of tubes which was centrifuged in the same manner. A third and final decantation and centrifugation was done. Filtration of the culture medium was then done using filters with a pore size of 0.22 μm. The phage filtrate was then poured into sterile tubes, wrapped in foil and stored at -4 ^oC.

Exactly 0.1 ml of phage filtrate, of serial dilution 1:100, and 2 ml of each bacterial liquid culture was added to tubes of 2 ml soft nutrient agar (consisting of 7 g/l agar and 8 g/l nutrient broth) which was kept from solidifying by keeping the tubes in a water bath at 50 °C. The soft agar was layered over lawns of bacterial isolates MB3 and MB5 (*Idiomarina* sp. and *Rhodobacter* sp. respectively) on hard nutrient agar and incubated at 37 °C. Observation was done after every 3 hrs. When formed, the plaques (PFU/ml) were counted and images taken using a digital camera (Sony Cyber - shot model).

The cyanobacteria species used to determine cyanophage host range were isolates MCy1 and MCy2 (*Synechococcus* and *Microcystis* respectively) (Fig 6.1). The cells of the *Synechococcus* appeared to be of a darker green color and slightly longer cylindrical cells in comparison to those described in section 4.3.3 on identity of cyanobacteria and are likely to be a different species of the same genus.

To 2 ml of each cyanobacterial liquid culture was added 1 ml of phage filtrate of serial diluton 1:100. This was added to tubes of 3 ml soft nutrient agar (consisting of 7 g/l agar and 8 g/l nutrient broth) which was kept from solidifying by placing the tubes in a water bath at 50 °C (Brown, 2005). Three milliliters of the soft agar culture was layered over lawns of cyanobacterial isolates on hard nutrient agar in plates and incubated at room temperature ((23 ± 2 °C) next to a window allowing adequate sunlight for over 10 days. Plaques formed were counted and images taken using the optical microscope and digital camera.

The bacteriophage and cyanophage were isolated by cutting out agar sections containing plaques using a sterile blade. These were placed in 8 % NaCl sterile solution and incubated at 37 °C for 12 hrs. The solution was then centrifuged and filtered through a 0.22 µm filter to remove any remaining bacteria. The filtrate was preserved by addition of formalin, placed in tubes wrapped in foil, and kept at -4 °C (Brown, 2005). The phage filtrate was then sent for electron microscopy.

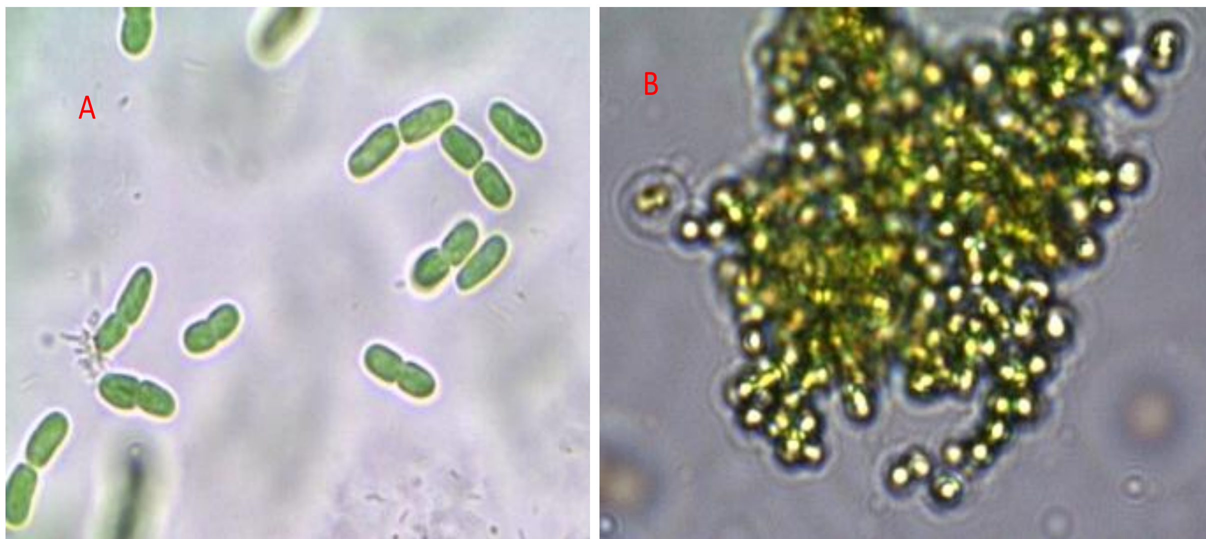


Fig 6.1: Wet mounts (x 1000) of cyanobacterial isolates used to determine cyanophage host range. (A) Cells of *Synechococcus* sp. (isolate MCy1); (B) Colonies of *Microcystis* sp. (isolate MCy2).

6.2.2 Determination of effect of phage infection on growth of their bacterial hosts

To determine the effect of bacteriophage on growth of bacterial host, about 80 ml of each bacterial broth culture, MB3 and MB5 (*Idomarina* sp. and *Rhodobacter* sp. respectively), had growth monitored by measurement of optical density using a spectrophotometer (Beckmann

Coulter Du 530 model) at wavelength of 680 nm (Brown, 2005). Measurement was done after every one hour after an initial incubation period of 12 hrs at 37 ^{o}C. On the fifth hour each bacterial culture was divided into two portions of 40 ml. Two ml phage lysate was added to the first portion while a similar amount of phage growth medium was added to the second portion to act as a control. The cultures were then incubated at 37 ^{o}C and optical density measurement resumed after every hour.

To determine the effect of cyanophage on growth of cyanobacterial host, two cyanobacterial isolates MCy1 (*Synechcoccus*) and MCy2 (*Microcystis*) cultures were each added to 19 ml Aiba and Ogawa medium and properly shaken. The number of cells of each cyanobacterial isolate was counted using a Neubauer chamber. Each of the cultures was then divided into two portions of 10 ml. Phage lysate (0.5 ml) was added to the first portion of each isolate and incubated at room temperature (23 ± 2 ^{o}C) next to a window allowing plenty of sunlight. The second 10 ml portion was a control set up with 0.5 ml phage growth medium added in place of 0.5 ml phage lysate. The number of cyanobacterial cells in each tube was counted by use of a Neubauer chamber after every two days. Analysis of variance (ANOVA) and F-test were used to test for significant differences (Payne *et al*., 2011).

6.2.3 Determination of effect of variation in temperature and pH on cyanophage infectivity

To determine the effect of change in temperature on infectivity of cyanophage isolate MCy1 (*Synechococcus*) was cultured in liquid Aiba and Ogawa media for about 10 days. One milliliter of the isolate was added to 19 ml Aiba and Ogawa medium and mixed well. The number of cells

was counted using a Neubauer chamber. The culture was then divided into two portions of 10 ml each. Phage lysate (0.5 ml) was added to the first portion while an equal volume (0.5 ml) phage growth medium was added to the second portion (control experiment). These were incubated at 37 ºC and the number of cyanobacterial cells in each tube counted after every two days. To determine the effect of pH variation on infectivity, the protocol used for temperature variation was repeated with Aiba and Ogawa medium whose pH was adjusted to pH 7 by addition of hydrochloric acid (2 Mol/l) while monitoring with universal indicator papers. The isolates were however incubated at room temperature (23 ± 2 ºC) in adequate sunlight. Counting of the cells was done at the beginning and after every

6.3 Results

6.3.1 Host range of phage

Plaque formation on proteobacterial host

Plaques of about 1 - 2 mm in diameter developed on isolate MB5 (*Rhodobacter* sp.) (Fig. 6.2) but none developed on isolate MB3 (*Idiomarina* sp.). The bacterial concentration was about 1.4 x 10^4 CFU/ml while the phage concentration was about 1.2 x 10^5 PFU/ml.

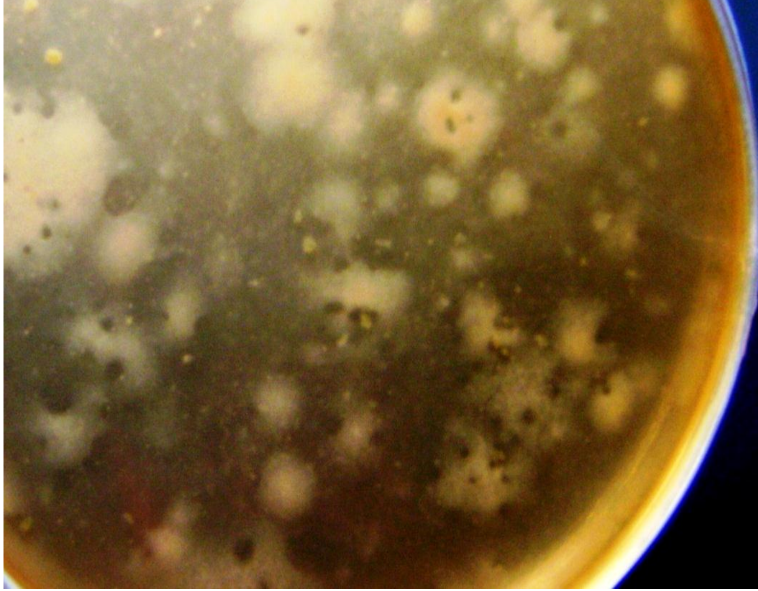


Fig 6.2: Plaques formed on proteobacterial isolate MB5 (*Rhodobacter* sp.) from Lake Magadi.

Plaque formation on cyanobacterial host

In four days small plaques of about 10 µm in diameter were observed under the microscope (x 400) on isolate MCy1 (*Synechococcus*) inoculated with phage lysate (Fig. 6.3A). After 10 days of incubation at room temperature the plaques measured about 5 mm in diameter (Fig. 6.3B). About 1.18×10^2 PFU/ml were counted which translated to 1.18×10^4 cyanophage particles/ml when the dilution factor of 1:100 was taken into account. No plaques were observed on isolate MCy2 (*Microcystis*). Transmission electron microscopy (TEM) on the phage lysate showed no identifiable phages but round encased structures (Fig. 6.3C) and polyhedral structures (Fig. 6.3D) were observed. The structures resembled phages but had varying sizes, were tailless and were identified as carboxysomes.

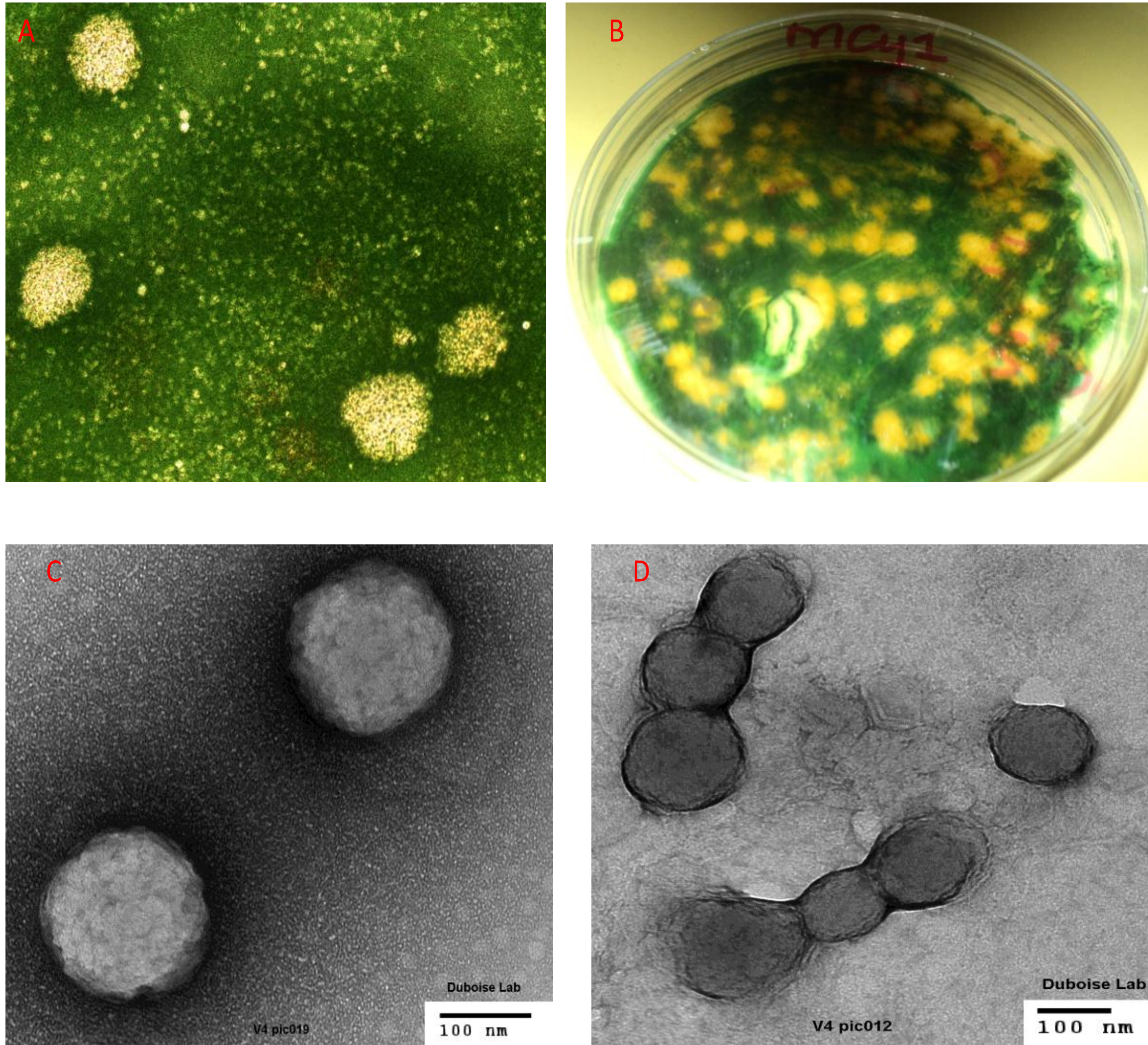


Fig. 6.3: Plaques formed on cyanobacterial isolate and carboxysomes observed on phage lysate. (A) plaques observed on cyanobacterial isolate MCy1 (*Synechococcus*) under the light microscope (x 400) in 4 days; (B) larger plaques in 10 days; (C) round encased structures, (D) polyhedral structures, observed by electron microscopy and identified as carboxysomes.

6.3.2 Effect of phage infection on growth of proteobacterial and cyanobacterial hosts

Growth of isolate MB3 (*Idiomarina* sp.) and MB5 (*Rhodobacter* sp.) followed the sigmoid

pattern of growth (Fig. 6.4). Growth of isolate MB3 inoculated with phage had little difference from the one without phage inoculation. Growth of isolate MB5 with phage infection had a significant difference ($p = 0.009$) from the one without phage infection. The maximum concentration of bacterial culture (measured in terms of optical density) without phage infection was 0.6 while with phage infection the concentration dropped to about 0.4, a decline of about 30%.

The growth of uninfected isolate MCy1 (*Synechococcus*), growing at room temperature (23 ±2 ºC), showed an increase in number of cells from about $1.5x10^7$ cells/ml to about $5.5x10^7$cells/ml in 10 days. The population of isolate MCy1 infected by phage decreased from $1.5x10^7$ cells/ml to about $0.6x10^7$ cells/ml in 10 days (Fig. 6.5). This was a decrease of about 50 % in 10 days. There was a difference of about $4.9x10^7$ cells/ml between the infected and the uninfected isolates after 10 days. Infection with phage had a significant ($p<0.001$) effect on the growth of isolate MCy1. The growth of isolate MCy2 (*Microcystis*) was not altered by inoculation with phage lysate. The difference between the two growths, with phage inoculation and without phage inoculation, was not significant ($p = 0.924$). The isolated phage did not infect the cyanobacterial isolate MCy2.

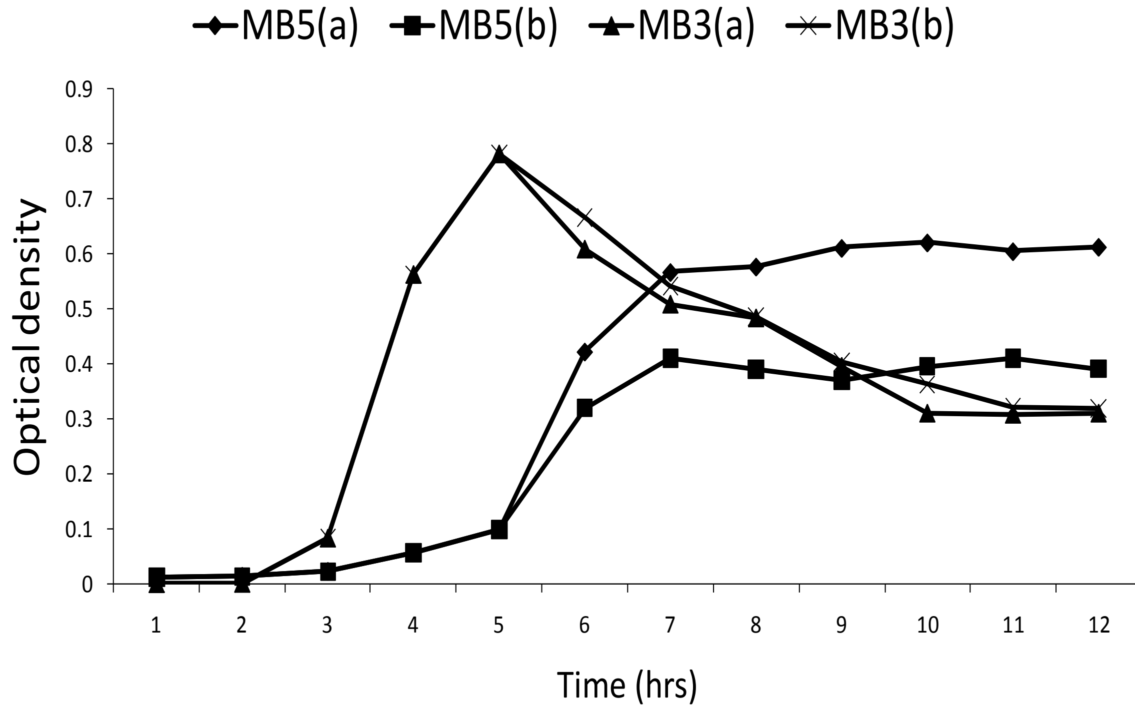


Fig. 6.4: Population growth (measured as optical density) of infected and non-infected bacteria isolated from Lake Magadi. Curve (a) growth of isolate MB3 and MB5 without phage inoculation; curve (b) growth of isolate MB3 and MB5 with phage inoculation.

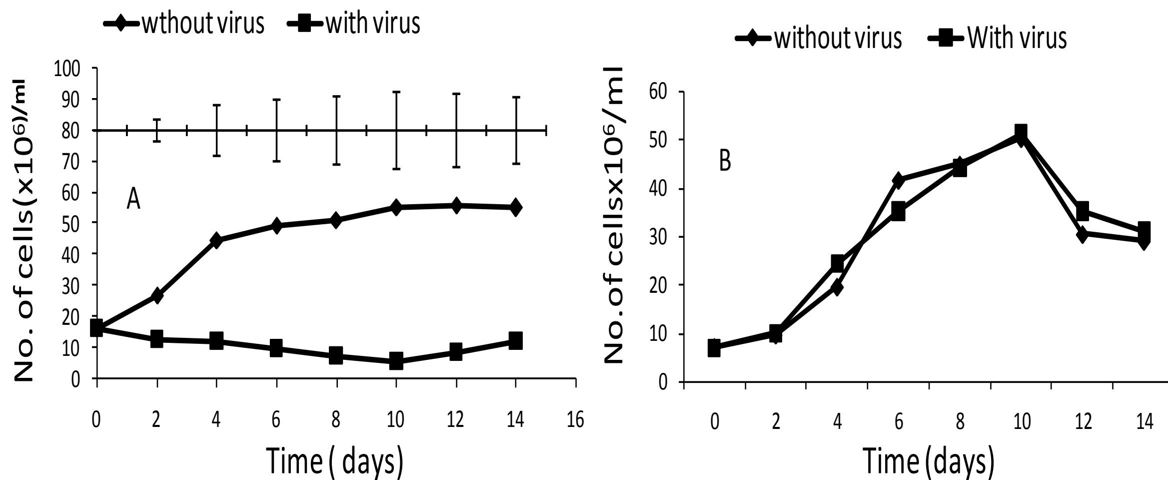
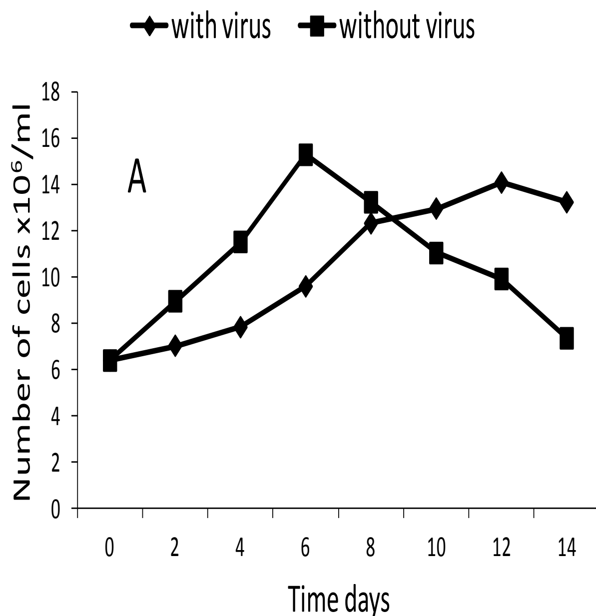


Fig. 6.5: Effect of phage infection on growth (number of cells/ml) of cyanobacerial isolates from Lake Magadi. (A) growth of isolate MCy1 with phage infection and without phage infection. Each data point represents the mean of three readings with the least significant difference bars; (B) growth of isolate MCy2 inoculated with phage lysate and without inoculation.

6.3.3 Effect of variation of temperature and pH on phage infectivity

The number of cells of isolate MCy1 (*Synechococcus*) incubated at 37 ᴼC without inoculation with phage increased from about 6.4 x10^6 cells/ml to about 1.7 x10^7 cells/ml in 6 days. When inoculated with phage lysate the number of cells increased to about 9.6 x10^6 cells/ml in 6 days and then gradually increased to a maximum of about 1.4x10^7 cells/ml (Fig. 6.6A). When isolate MCy1 was cultured at 37 ᴼC there was no significant difference ($p = 0.867$) between the growth with phage inoculation and that without phage inoculation. There was also no significant differene ($p = 0.609$) in the growth of isolate MCy1 inoculated with phage and without phage inoculation when cultured at room temperature (23 ± 2 ᴼC) and pH6 (Fig. 6.6B).

Growth at 37 °C and pH 10 **Growth at 23 ± 2 °C and pH 7**



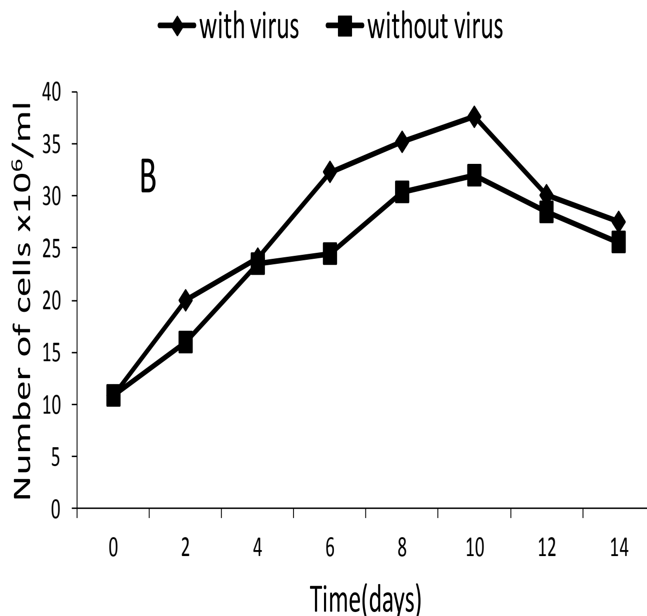


Fig. 6.6: Growth (number of cells/ml) of cyanobacterial isolate MCy1 (*Synechococcus*) inoculated with phage with variation of temperature and pH. (A) incubation at 37 °C and pH 10 with phage and without phage inoculation; (B) incubation at room temperature (23 ± 2 °C) and pH 7 with phage inoculation and without phage inoculation. Each data point represents the mean of three readings.

6.4 Discussion

The phage isolated was host specific to *Rhodobacter* sp. and failed to cross infect the *Idiomarina sp*. Reports of such bacteriophages that infect photosynthetic bacteria were made from the Caribbean, specifically causing lysis of the purple non-sulfur anoxyphototrophs, the *Rhodobacter* sp. Such bacteriophages were also isolated from Europe (Garí *et al*., 1992) and North America

(Mural and Friedman, 1974). Rojas-Dulan *et al*. (2009) reported that in order to see a plaque during the initial detection and isolation steps, it was necessary to perform two host enrichment phases in the water sampled. This suggested low concentration of viral particles and the need for their amplification in order to detect them.

The cyanophage titer in the phage lysate was about 1.18×10^4 cyanophage particles/ml as compared to concentrations of up to 10^8 cyanophages per liter detected in marine coastal waters by Scanlan and West (2002). It has been observed that most marine *Synechococcus* cyanophages are myoviruses (Mann, 2003). Cyanophages that infect specific strains of marine *Synechococcus* sp. can reach concentrations in excess of 10^6 ml^{-1} (Suttle, 2000). The cyanophage may exist in very low abundance and only become detected when the relative abundance increases. A decrease in the population growth rate of the cyanobacterial hosts may cause a reduction in the contact rate between the phages and their hosts. This would result in lessened or total lack of cell lysis. Abundance of cyanophages follows that of *Synechococcus* sp. and in surface waters abundance varies over orders of magnitude on a seasonal basis.

Different species of *Synechococcus* have different colors depending on the photosynthetic pigments they posses. These may be chlorophyll *a* (absorbing blue and red appearing green) or phycobilins such as phycocyanin (absorbing orange and red light appearing light blue color) and phycoerythrin (absorbing slightly blue-green/yellowish light appearing slightly orange-yellow). The presence of infectious cyanophages had an impact on the population size of the cyanobacteria. The population of isolate MCy1 (*Synechococcus*) decreased by about 50 % in 10 days after inoculation with phage lysate. Despite the observation that some *Synechococcus*

isolates are resistant to phages, the mechanisms of resistance have not been investigated in detail.

Several studies suggest that lysogeny may be an important type of phage-host interaction in marine environments (Paul and Kellogg, 2000) and lysogenic interactions between marine *Synechococcus* sp. and cyanophages have been documented (McDaniel *et al.,* 2002). Continual viral production could be a result of the induction of prophage from lysogenic cyanobacteria. For other well studied bacterium-phage systems, several different mechanisms of phage resistance or immunity have been described. The most common mechanism appears to be an alteration of host surface receptors which reduces or eliminates the ability of phages to attach to the host cell and establish an infection (Bohannan and Lenski, 2000).

Other mechanisms of phage resistance include the inability of a bacterium to support viral replication and the presence of restriction-modification systems in which host restriction endonucleases degrade viral DNA upon entry into the cell (Levin and Lenski, 1985). The presence of prophage in bacterial genomes can also provide immunity to infection by related phages. An alternative explanation may arise from the observation that virus particles are abundant in marine sediments, probably as a result of adsorption to suspended material in the water column that settles out and contributes to the benthic viral population (Hewson *et al.,* 2001). Indeed, infective cyanophage can be recovered from considerable depths in marine sediments which may act as phage reservoir (Suttle, 2000). Lake Magadi being a saline lake is likely to have similar patterns as the marine coastal waters.

Incubation at raised temperature (37 ^{o}C) disrupted the infectivity of the cyanophages. Wommack *et al.* (1999) suggested that viral blooms begin when host cell density reaches a critical abundance and then decline when host cell density is reduced due to viral lysis. Salinity, light, nutrient availability, depth, as well as host cyanobacterial populations, appears to influence the genetic composition of cyanophage communities (Lu *et al.,* 2001).

Theory predicts that when the host is present in low abundance and is slow-growing, temperate phages will have a great selective advantage over obligately lytic phages (Pacheco *et al.,* 1997). Temperate phages can persist within the host in the lysogenic state. Pseudolysogeny may represent one strategy by which obligately lytic *Synechococcus* phages persist when population density is low due to nutrient starvation. Temperature is a crucial factor for bacteriophage survivability (Olson *et al.,* 2004). It plays a fundamental role in attachment, penetration, multiplication, and the length of the latent period (in the case of lysogenic phages).

At lower than optimal temperatures, fewer phage genetic material penetrate into bacterial host cells; therefore, fewer of them can be involved in the multiplication phase. Higher temperatures can prolong the length of the latent stage (Tey *et al.,* 2009). Atypical environments in which bacteriophages can survive are hot springs (achieving temperatures of 40 - 90 ^{o}C). Bacteriophages isolated from such springs in California were tested at low and high temperatures (Breitbart *et al.,* 2002). It was observed that more than 75 % of the phage particles remained intact even when incubated on ice (around 0 ^{o}C). They were more sensitive when boiled at 105 ^{o}C, as only 18 - 30 % of the phage particles remained intact. Mocé-Llivina *et al.* (2003) observed that phages were more resistant to thermal treatment than bacteria.

Incubation at different pH conditions (pH 7) disrupted cyanophage infectivity. Kłak *et al.* (2010) incubated T4 bacteriophage in buffers of different pH ranging from 1.1 - 9.2 for 1 hr at 37 ^{o}C and showed that the optimum pH was 6.0 - 7.4. The titer decreased by half at pH 9.2, and no active phages were detected at pH 4.0. The suggestion was that irreversible coagulation and precipitation might be the factors limiting phage activity.

Transmission electron microscopy on the phage lysate showed presence of carboxysomes and no phages. Disintegration of phages can be caused by variation in environmental conditions such as temperature and light. Phage decay or loss of phage titer may have occurred during transportation for electron microscopy. Presence of carboxysomes is in agreement with other writers. Yeates *et al*. (2008) found carboxysomes in all cyanobacteria and many chemotrophic bacteria that fix carbon dioxide. They are bacterial micro compartments that contain enzymes involved in carbon fixation (Berger, 2003). Structurally, carboxysomes are icosahedral, typically around 80 to 120 nm in diameter. The carboxysome has an outer shell composed of a few thousand protein subunits, which encapsulates the two carbon fixing enzymes, carbonic anhydrase and ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCO) (Tanaka *et al*., 2008).

Carboxysomes are thought to concentrate carbon dioxide to overcome the inefficiency of RuBisCO, the predominant enzyme in carbon fixation and the rate limiting enzyme in the Calvin cycle. The carbon concentrating mechanism involves several types of inorganic carbon transporters in the cell membrane that increase cytosolic concentrations of bicarbonate. The intracellular bicarbonate then diffuses into the carboxysomes, where carbonic anhydrase converts

it into CO_2. RuBisCO combines CO_2 with the 5-carbon sugar ribulose-1,5- bisphosphate (RuBP) to form two molecules of 3-phosphoglycerate (3PGA). The latter leave the carboxysome for the cytoplasm where they enter the Calvin cycle (Tanaka *et al.*, 2008). A previous report that carboxysomes of *Nitrobacter agilis* contain DNA, (Westphal *et al*., 1979) together with the icosahedral shape of the particles, made it tempting to suggest that carboxysomes are evolutionarily related to bacteriophages. Subsequent studies have led to the conclusion that the DNA associated with the particles was a contaminating section of the bacterial chromosome and that carboxysomes do not contain a nucleic acid component (Holthuijzen *et al.,* 1986).

6.5 Conclusions

Lake Magadi harbors a high diversity of bacteria and it is evident that phage population is also high. Inoculation with phage lysate decreased the maximum concentration of bacterial culture (measured in terms of optical density) from 0.6 to 0.4, a decline of about 30 % in two hours. The results show that phages have an effect on the host microbe concentrations. Phages may also influence non-host microbes through a chain reaction mechanism. By lysing specific hosts that are lethal to other microorganisms, viruses can influence microbial population dynamics indirectly.

Phage infection impacts on the population of *Synechococcus* reducing it by half in 10 days. Cyanophages that infect the *Synechococcus* sp. are therefore a significant factor in determining the dynamics of *Synechococcus* populations in Lake Magadi. Variation of environmental factors (such as temperature and pH) from the optimum for growth of cyanobacteria was found to reduce infectivity of the cyanophage. These conditions cause reduction in numbers of

Synechococcus and hence reduce the contact rate between the host and the cyanophages. Prevailing extreme physicochemical conditions of the soda lake possibly affects phage-mediated cyanobacteria mortality rate. Phage infection however did not totally eradicate the cyanobacteria species. However, even with low mortality rates, cyanophages still play an important role in regulation of cyanobacterial populations. This in turn influences carbon fixation and nutrient flow in the ecosystem. There is therefore a correlation between the presence of cyanophages and the ecosystem dynamics of the soda lake. Further research needs to be done on phages inhabiting soda lakes and hence affecting microbe populations some of which like the *Rhodobacter* sp. and cyanobacteria contribute to primary production.

CHAPTER SEVEN: GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

7.1 General discussion

Species of bacteria isolated from Lake Magadi include two species of the phylum proteobacteria and seven genera of the phylum cyanobacteria. The bacterial species *Rhodobacter veldkampii* and the cyanobateria carry out anoxigenic and oxigenic photosynthesis respectively. Photosynthesis dominates activity at the surface and provides the primary energy source for life in soda lakes. Important photosynthesizers include cyanobacteria, eukaryotes such as green algae (Chlorophyta), haloalkaliphilic archaea such as *Halobacteria* and bacteria such as *Halorhodospira* (Zavarzin *et al*., 1999). Below the surface, anoxygenic photosynthesizers include purple sulfur bacteria such as *Ectothiorhodospira* and the purple non-sulfur bacteria such as *Rhodobacteraceae* (Milford et al., 2000). Soda lakes are therefore highly productive ecosystems with gross primary production (photosynthesis) rates above 10 g C m^{-2} day^{-1} (grams carbon per square meter per day), over 16 times the global average for lakes and streams (0.6 g C m^{-2} day^{-1}) (Melack and Kilham, 1974).

Physicochemical conditions affect growth of cyanobacteria. Optimum culturing conditions were 23 ± 2 °C at room temperature next to a window allowing adequate sunlight, salinity of between 0.0 and 0.3 % w/v NaCl concentration and pH 6 to 10. Rippka *et al*. (1979) suggested that *Spirulina major* as well as some other species were able to grow at pH 6.5 but at lower rates and that there was a complete absence of cyanobacteria in the environment with pH less than 4. However, slightly acidic environment is not deterrent to the growth of cyanobacteria, and in

some cases it is the preferred pH for higher growth.

Results that were in agreement with findings of Kumar-Rai and Abraham (1993) showed the effect of different salinity levels on growth of *Oscillatoria acuminata*. Growth was determined by measurement of amount of chlorophyll ‘a’ at different salinity levels. Maximum growth was observed at higher salinity (35 ppt) whereas minimum growth was observed at lower salinity (5 ppt). Thus in general, increased salinity reduces the amount of cyanobacterial growth but each species has its own optimum sodium chloride concentration. The availability of the complete sequence of the genome of strain WH8102 provided insights into the unique adaptations of this cyanobacterial *Synechococcus* group to the marine environment, including mechanisms of nutrient and metal ion transport, chemotaxis, motility, and viral interactions. This helped in understanding the factors that are ultimately important in controlling primary productivity in the oceans (Scanlan *et al*., 1993).

The infection by phage caused a decrease in numbers of about 50 % of *Synechococcus* host in 10 days. This result is in agreement with some studies that show that mortality due to cyanophage infection ranges from 0.005 % to 28 % of the *Synechococcus* community per day. This low mortality rate suggests that resistance to viral infection is common in *Synechococcus* spp. (Garza and Suttle, 1998). Viral infection accounts for a significant portion of microbial mortality and is believed to be as important as grazing by protists in keeping microbial biomass in balance (Suttle, 2005). In near shore waters the high concentrations of cyanophages and *Synechococcus* result in high encounter frequencies and selection for *Synechococcus* communities that are largely resistant to infection (Suttle, 2000).

Presence of carboxysomes was detected in phage lysate by transmission electron microscopy but no phages were observed. Suttle and Chan, (1994) reported that phages have extreme sunlight sensitivity and this may lead to very low or nonexistent titers of infective phages in surface waters. However, titers of infective cyanophages can be as high as 10^4 to 10^5 ml^{-1} in surface waters. This suggests a paradox, especially for cyanophages and algal viruses, which infect photoautotrophic hosts. A plausible explanation of this paradox is that the infectivity of sunlight-inactivated bacteriophages is restored by either host- or phage-mediated DNA repair. The phenomenon of photoreactivation of UV-inactivated bacteriophage is well known. Indeed, this phenomenon alerted researchers to the existence of specific DNA repair machinery in bacteria. Solar radiation has a major effect on cyanophage infectivity and results in the selection of cyanophage communities that are more resistant to destruction by sunlight. In contrast to surface waters, infectious cyanophages can persist in sediments for at least 100 years (Suttle, 2000).

7.2 General conclusions

i. Lake Magadi harbors genetically diverse proteobacterial species represented by the two identified species of the phylum proteobacteria. One of the species, *Idiomarina,* of the class gammaprotebacteria, was a Gram negative, organotrophic, motile rod that grew well in the medium modified for haloalkaliphiles while the *Rhodobacter* sp. of the class alphaproteobacteria, was a Gram negative, autotrophic, non motile rod with optimum growth in the Aiba and Ogawa medium for cyanobacteria.

ii. The diversity of cyanobacteria in Lake Magadi was found to be wide with seven genera having been isolated. These included unicellular *Synechococcus, Microcystis, Synechocystis, Prochlorococcus* and filamentous *Anabaena, Arthrospira* and

Oscillatoria. The species posses various photosynthetic pigments, heterocysts, carboxysomes, small sized cells usually covered by slimy sheaths. These adaptations enable them to survive in the extreme conditions of the lake.

iii. The growth of cyanobacteria was favored by relatively lower salinity (NaCl concentration of between 0.0 and 0.3% w/v, pH 6 - 10 and warm temperatures (room temperature of 23 ± 2 ^{o}C) with plenty of sunlight. They appeared tolerant to extreme conditions of temperature (50 ^{o}C), relatively high salinity (0.9% w/v) and high alkalinity (pH 10). They can be described as thermotolerant, halotolerant and alkalitolerant rather than grouping them simply as marine or freshwater.

iv. Phage infection caused a marked decline in bacterial host populations. Concentration of proteobacterial host (*Rhodobacter* sp.) culture measured as optical density decreased from 0.6 to 0.4, a decline of about 30% in 2 hrs, while cyanobacterial host (*Synechococcus*) reduced by about 50% in cell numbers in 10 days. Incubation of cyanobacteria host at pH 7 and temperature of 37 ^{o}C, conditions that were observed to cause reduction in growth of the host, led to absence or reduction of phage infectivity.

7.3 Recommendations

i. Environmentalists should come up with measures that could be applied to enhance primary productivity in the lake.

ii. It is important to determine factors that cause seasonal variation of important species such as *Arthrospira*, the source of food for flamingoes and *Anabaena,* important for nitrogen fixation.

iii. More studies focusing on temporal and spatial variations in phage abundance in Lake

Magadi should be carried out in order to fully understand the impact of phages on the ecosystem.

iv. Studies on phages in other ecosystems such as soils and biofilms could display greater complexity in terms of the impact on their hosts and on nutrient cycling and energy flow.

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