EVALUATION OF EXTREMOPHILIC BACTERIA FROM LAKES BOGORIA AND MAGADI FOR BIOCONTROL OF *FUSARIUM SOLANI* AND *RHIZOCTONIA SOLANI* IN *PHASEOLUS VULGARIS L.*

TOFICK BARASA WEKESA

MASTER OF SCIENCE

(Biotechnology)

JOMO KENYATTA UNIVERSITY

OF

AGRICULTURE AND TECHNOLOGY

2023

Evaluation of Extremophilic Bacteria from Lakes Bogoria and Magadi for Biocontrol of *Fusarium Solani* and *Rhizoctonia Solani* in *Phaseolus Vulgaris L.*

Tofick Barasa Wekesa

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Biotechnology of the Jomo Kenyatta University of Agriculture and Technology

2023

DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

Signature: ……………………………………………Date………………………….

Tofick Barasa Wekesa

This thesis has been submitted for examination with our approval as the University Supervisors.

Signature: ……………………………………………Date………………………….

Prof. Justus M. Onguso

JKUAT, Kenya

Signature: ……………………………………………Date………………………….

Dr. Vitalis W. Wekesa

Dudutech IPM Limited, Kenya.

DEDICATION

This work is dedicated to my family; My wife Bridgit Ndinda, My daughter Alena Petra Wekesa, Father Ernest Wekesa, My Mother Margret Mayabi and all my siblings. Thank you for the support you have given me throughout my research work.

ACKNOWLEDGEMENT

First, I appreciate Almighty God for life and guiding me during this journey. I appreciate the support of my family who were behind me during the research and project writing. I appreciate Jomo Kenyatta University of Agriculture and Technology administration for the opportunity to pursue my master's degree in the Institute of Biotechnology Research (IBR) during all this period.

I am grateful to Prof. Justus M. Onguso and Dr. Vitalis W. Wekesa for the guidance and supervision they gave me during the research period, financing me with stipends to ensure I complete my research work without any difficulties.

I sincerely express my gratitude to the director Institute for Biotechnology (IBR)-JKUAT Prof. Justus M. Onguso for ensuring reagents are available and smoothly access the institutions' facilities. I appreciate GEF-UNEP for financing the projects together with co-parties (KWS, DUDUTECH, UON, KIRDI and MOI) for the workshops and conferences to enrich my knowledge in research work.

Lastly, I appreciate the Institute for Biotechnology Research technicians (Mr. Richard Rotich, Dr. Grace Mungai) for their assistance during my lab. I thank my colleagues and friends (Dr. Patrick Okanya, and Dr. Eliud Wafula) for their guidance, review of my work and encouragement.

TABLE OF CONTENTS

DECLARATION ii

DEDICATION iii

ACKNOWLEDGEMENT iv

TABLE OF CONTENTS v

LIST TABLES x

LIST OF FIGURES xi

LIST OF PLATES xiv

LIST OF APPENDIX xv

LIST OF ABBREVIATIONS AND ACRONYMS xvi

ABSTRACT xviii

CHAPTER ONE 1

INTRODUCTION 1

1.1 Background of the Study 1

1.2 Statement of the Problem 4

1.3 Justification 5

1.4 Objectives 6

1.4.1 General Objective 6

1.4.2 Specific Objectives 6

1.5 Null Hypothesis 6

CHAPTER TWO 8

LITERATURE REVIEW 8

2.1 Introduction 8

2.2 Status of Beans Production in Kenya....8
2.3 Challenges of Bean Production in Kenya. 10
2.4 Description of Fusarium and *Rhizoctonia Solani* in Bean Crop 10
2.4.1 Fusarium Solani....10
2.4.2. Epidemiology of Fusarium Root Rot on Beans....11
2.4.3 Rhizoctonia Solani 12
2.4.4 Epidemiology of Rhizoctonia Root Decay on Bean....13
2.5 Management of Fusarium and Rhizoctonia on Bean Crop 14
2.6 Alternative Host of R. Solani and F. Solani....15
2.7 Global Microbial Diversity of Soda Lakes. 16
2.8 Microbial Diversity of Kenyan Soda-Lakes 17
2.9 Application of Extremophilic Bacteria in Biotechnology....18
2.10 Mode of Action of Soda Lakes as Biocontrol....19
2.11 Challenges of Soda-Lake Microbes as Biocontrol in Agricultural Environment. ...21
CHAPTER THREE 23
MATERIALS AND METHODS 23
3.1 Sampling Sites....23
3.2 Sample Collection 24
3.3 Antifungal Activity of Bacteria Isolated against R. Solani and F. Solani. 25
3.3.1 Isolation of Bacteria from Lakes Bogoria and Magadi 25
3.3.2 Preparation of Fungal the Pathogen Inoculum and Pathogenicity Test 26
3.3.3 Antifungal Activity of Bacterial Isolates against F. Solani And R. Solani 27
3.4 To Characterize Bacterial Isolates Activity against F. Solani and R. Solani....27

3.4.1 Morphological Characterization of the Bioactive Isolates 27
3.4.2 Physiochemical Characterization of Bacterial Isolates 27
3.4.2.1 Growth at Different Sodium Chloride Concentrations 27
3.4.2.2 Growth at Various Temperatures 28
3.4.2.3 Effect of pH on Growth of the Isolates 28
3.4.3 Assessment of Bioassay Activity of Bacterial Isolates. 28
3.4.3.1 Protease Activity 28
3.4.3.2 Chitinase Activity 29
3.4.3.3 Pectinase Activity 29
3.4.3.4 Hydrogen Cyanide (HCN) Production Ability 30
3.4.3.5 Phosphate Solubilization Ability 30
3.4.3.6 Indole-3-Acetic Acid (IAA) Production Ability 30
3.4.4 Molecular Characterization of Bacterial Isolates on R. Solani and F. Solani 31
3.4.4.1 DNA Extraction of the Bacterial Isolates 31
3.4.4.2 PCR Amplification of 16S Rrna Genes. 31
3.4.4.3 Phylogenetic Analysis of the Bacterial Isolates 32
3.5 The Efficacy of Bacterial Isolates against F. Solani and R. Solani on Bean. 32
3.5.1: Determination of Plant Biometric Attributes in Beans 33
3.5.1.1. Germination Rate 33
3.5.1.2. Root Mortality 33
3.5.1.3. Pre-Emergence and Post-Emergence Wilt Incidence 33
3.5.1.4. Shoot /Root Length and Biomass 34
3.5.2. Plant Defense Enzymes and Phenolic 34
3.5.2.1. Preparation of Enzyme Extracts 34
3.5.2.2 Phenylalanine Ammonia-Lyase, Polyphenol Oxidase, and Peroxidase 34
3.5.2.3 Total Phenolic Assay 35
3.6 Data Analysis 35

CHAPTER FOUR **36**

RESULTS **36**

4.1 Physiological Description of the Lakes 36

4.1.2 Bacterial Isolation 41

4.1.2.1 Pathogen Preparation and Pathogenicity Test 43

4.1.3 In Vitro Pathogenicity of F. Solani And R. Solani on Bean Plantlets. 43

4.1.4 Antibiosis of Bacterial Isolates against Selected Test Pathogen 46

4.1.5 Antifungal Activity of L. Bogoria and L. Magadi against Fusarium Solani 49

4.1.6 Comparison of Bacteria Isolates for Biocontrol of R. Solani and F. Solani 53

4.1.7 Comparison of Bacteria Isolates against R. Solani and F. Solani 54

4.1.8 Disc Diffusion Results 55

4.2 Characterization of Bioactive Bacterial Isolate against F. Solani and R. Solani. 59

4.2.1 Morphological Characterization of Bioactive Isolates. 59

4.2.2 Physiochemical Characterization of Bioactive Bacterial Isolates. 61

4.2.2.1. Growth at Different Ph. 61

4.2.2.2 Growth at Different Salinity 63

4.2.2.3 Growth at Different Temperature 66

4.2.3. Enzymatic Bioassays of Bacterial Isolates. 67

4.2.4 Molecular Characterization of Bacterial Isolates. 71

4.2.4.1 DNA Extraction. 71

4.2.4.2 PCR Amplification of 16s Rrna Genes. 72

4.2.4.3 Phylogenetic Analysis of Bacteria Isolates from L. Bogoria. 72

4.2.4.4 Phylogenetic Analysis of Bacteria Isolates from L. Magadi 76

4.3 The Efficacy of Bacterial Isolates against F. Solani and R. Solani on Bean. 78

4.3.1 Root Mortality Rate 78

4.3.2 Effect on Pre-and Post-Emergence Wilt Disease Incidences 79
4.3.3 Effect of Bacterial Isolates on the Germination Rate and Chlorophyll 80
4.3.4 Induction of Defense Enzymes 83
4.3.5 Total Phenolic Content 84
CHAPTER FIVE 86
DISCUSSION, CONCLUSION, AND RECOMMENDATIONS 86
5.1 Discussion 86
5.2. Conclusion 104
5.3 Recommendations 105
REFERENCES 107
APPENDICES 131

LIST TABLES

Table 4.1: Types of Samples Collected from Seven Points of L. Bogoria with their Description. 37

Table 4.2: Types of Samples Collected from Seven Points ff L. Magadi with their Description 38

Table 4.3: Comparative Effects of *F. Solani* and *R. Solani* on Bean Plantlets Observed 14 Days of Inoculation. 45

Table 4.4: Antifungal Activity of Lakes Bogoria and Magadi Bacterial Isolates on Mycelium Growth of *R. Solani* after 14th Days 47

Table 4.5: Antifungal Activity of Bogoria and Magadi Bacterial Isolates on Mycelium Growth of *Fusarium Solani* After 14 Days. 50

Table 4.6: *In Vitro* Effect of Bogoria and Magadi Bacterial Isolates against *Fusarium Solani* using Disc Diffusion after Seven Days of Incubation 57

Table 4.8: Enzymatic Bioassay of Bioactive Bacteria Isolates from L. Bogoria and L. Magadi against *F. Solani* and *R. Solani*. 70

Table 4.9: Blast Results of Lake Bogoria Bioactive Isolates and their Respective Closest Relatives 73

Table 4.10: Blast Results of Lake Magadi Bioactive Isolates and their Respective Closest Relatives 76

Table 4.11: Effect of Seed Bio-Priming with Bioactive Bacteria on Induction of Defense Enzymes in Common Bean Plantlets in Pathogen (*F. Solani* and *R. Solani*) Challenged Condition. 83

LIST OF FIGURES

Figure 1.1*:* Common Beans. Source: JKAUT Field 2

Figure 2.2: Status of Dry Beans Production in Kenya (2017-2019) 9

Figure 2.3*:* Symptoms of *Fusarium Solani* on the Bean. A)- Causes Adventitious and Tap Root Rot. B)-Pathogenicity Of *Fusarium Solani*. Source: (Leep 2016) 12

Figure 2.4: Symptom of *R. Solani* on Bean Plant; A)-*Rhizoctonia* Causes the Bean to Die...13

Figure 2.5: *Rhizoctonia Solani* Life Cycle. Source...14

Figure 3.6: Kenyan Map Showing Sampling Points from Lakes Bogoria and Magadi..24

Figure 3.7: Sampling Point Showing Sample Collection and Measuring of Physical Parameters..25

Figure 4.8: Venn Diagram Showing the Distribution of Isolated Bacteria from Lakes Bogoria and Magadi...42

Figure 4.9: Effect of *F. Solani* and *R. Solani* on Bean Plantlets.. 43

Figure 4.10: Venn Diagram Showing the Distribution of Bioactive Bacterial Isolates from L. Bogoria. 53

Figure 4.11: Venn Diagram Showing the Distribution of Bioactive Bacterial Isolates from L. Bogoria 55

Figure 4.12: The Growth of Bacteria at Different pH Values. 62

Figure 4.13: Annotated Hierarchical Clustergram of Assayed Bacteria Isolates At Varied pH. 63

Figure 4.14: The Growth of Bioactive Bacteria at Different Salt Concentrations. 64

Figure 4.15: Annotated Hierarchical Clustergram of Assayed Bioactive Isolates at Varying Salt Concentrations. 65

Figure 4.16: Growth of Bacterial Isolates at Different Temperatures 66

Figure 4.17: Annotated Hierarchical Clustergram of Assayed Bioactive Isolates at Varying Temperatures. 67

Figure 4.18: Annotated Hierarchical Clustergram of Assayed Bacterial Isolates at Different Enzymes. 69

Figure 4.19: Gel Showing Genomic DNA Extracted 71

Figure 4.20: A 1% Agarose Gel Showing the Size of the PCR Amplicons 72

Figure 4.21: Phylogenetic Tree of Bacterial Isolates from L. Bogoria Based on 16S Rrna Sequences. 75

Figure 4.22: Phylogenetic Tree Of Bacterial Isolates from L. Magadi Based on 16S Rrna Sequences 77

Figure 4.23: Effect of Seed Biopriming with B39, B21, and M10 on Common Beans Seedlings Root Mortality. 78

Figure 4.24: Effect of Seed Biopriming with B39, B21, And M10 on the Common Beans Seedlings Wilt Disease Incidences. 79

Figure 4.25: Effect of Seed Biopriming with B39, B21, and M10 on the Common Beans Seedlings Germination Rate and Biomass Observed Under Pathogen Pre-Inoculation Conditions..81

Figure 4.26: Hierarchical Clustergram of Assayed Seed Treatment on Shoot Length, Chlorophyll, and Root Length Of The Common Bean Plantlets................82

Figure 4.27: Effect of Seed Bio-Priming with Bioactive Bacteria on Phenolic Content in Common Beans...85

LIST OF PLATES

Plate 4.1: Modified Nutrient Agar Media Culture with different Colonies 41

Plate 4.2: PDA Plates Showing; (A) Rhizoctonia Solani and (B) Fusarium Solani 43

Plate 4.3 Antibiosis Assay of Lakes Bogoria and Magadi Bacterial Isolates against R. Solani after 7 Days of Incubation...46

Plate 4.4: Antibiosis Assay of Lakes Bogoria and Magadi Bacterial Isolates against F. Solani after 14 Days of Incubation..50

Plate 5.5: Antifungal Activity of L. Bogoria and L. Magadi Bacterial Isolates against Fusarium Using Disc Diffusion Techniques 56

Plate 6.6: Enzymatic Bioassay of Bacterial Isolates from L. Bogoria and L. Magadi against F. Solani and R. Solani. 68

LIST OF APPENDICES

Appendix I: Rstudio Scripts for Physiochemical and Enzymatic Analysis 131

Appendix II: Gram Staining of the Bioactive Bacteria 133

Appendix III: 50X TAE Electrophoresis Buffer 134

Appendix IV: Ethidium Bromide 10X 135

Appendix V: PCR Program for 16S Rrna Primers 136

Appendix VI: Chemical Used, Including Safety Data Information 137

Appendix VII: Equipment's Used 140

Appendix VIII: Molecular Biological Kits 141

Appendix IX: Antibiotics Preparation 142

Appendix X: List of Culture Media, Suppliers, Uses, Composition, and Preparation .144

Appendix XI: Green- House Assay 147

Appendix XII: Publications 148

ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
BLAST	Basic Local Alignment Search Tool
BP	Base Pair
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
EPPO	European and Mediterranean Plant Protection Organization
EU	European Union
FAO	Food and Agriculture organization
GDP	Gross Domestic Products
G+C	Guanine and Cytosine
GPS	Global Positioning system
HCN	Hydrogen Cyanide
ISR	Induction of systematic resistance
JKUAT	Jomo-Kenyatta University of Agriculture and Technology
KALRO	Kenya Agricultural and Livestock Research Organization
LB	Lysogeny Broth
NB	Nutrient Broth
NCBI	National Center for Biotechnology Information

PCR	Polymerase Chain reaction
PDA	Potato Dextrose Agar
PGPR	Plant Growth-Promoting Rhizobacteria
pH	Potential of Hydrogen
SDS	Sodium Dodecyl Sulphate
TDS	Total Dissolved solids
DNS	Dinitro salicylate
DAS	Days after sowing
SDW	Sterile distilled water
CV	Coefficient of variation
IAA	Indole-3-acetic acid
PAL	Phenylalanine ammonia-lyase
PO	Peroxidase
PPO	Polyphenol oxidase
LSD	Least Significant Difference

ABSTRACT

Common Bean (*Phaseolus vulgaris L.*) is some significant vegetable crop rich in protein, carbohydrates, and Vitamin B complex, used as a source of protein or cooked as a vegetable. It is associated with improving capillary resistance, inhibiting inflammation and act as an anticancer. Common beans face a major challenge of pests and disease hence lowering production yield. Some of the diseases are root rot triggered by *Rhizoctonia solani* and *Fusarium solani.* Application of chemical products and cultural practices are not effective in managing these diseases. Therefore, a sustainable, affordable, and effective control method needs to be devised to minimize the effect of bacterial plant diseases on the quality and quantity of bean yield. The use of bio-control agents is hypothetically self-sustaining, provides a non-target approach, spreads on its own, and is environmentally friendly. This study evaluated the biocontrol potential of extromophilic bacterial isolates from Lakes Bogoria and Magadi for against *Fusarium solani* and *Rhizoctonia solani* pathogens in beans. To explore biocontrol frontiers, a total of 110 bacteria were isolated from water, sediments, and soil of both lakes. Their antifungal properties were determined by co-culturing analyzed using SAS (ANONA) were; 17 (34.7%) isolates from L. Bogoria and 25 (41%) isolates from L. Magadi had varying mycelium inhibition rate for both *Fusarium solani* and *Rhizoctonia solani*. The characterization of the bioactive isolates revealed that 84.2% were Gram-positive and 15.8% were Gram-negative. The graphical analysis of bacterial isolates grew well at pH 7.0 and 8.5 though there was recorded growth in pH 5.0 and 10.0. In terms of temperature, the optimum temperature recorded was 30-35℃ with optimum salinity of 0-0.5M NaCl. The bioactive isolates were assayed for their ability to produce secondary metabolites whereby; most of the isolates produced phosphatase, pectinase, chitinase, protease, Indole-3-acetic acid and Hydrogen Cyanide making them potential biocontrol agents. Analysis of the partial sequence using BLASTn indicated 84.2% of the isolates were affiliated to *Bacillus* spp and 15.8% were affiliated to members of Gammaproteobacterial. Isolates B7, B11, B20, B21, B26, B29, B30, B32, B38, B39, M9, M10, M16, M47, M50 and M60 clustered with *Bacillus* at 98.71-100% similarity index. Isolates B12, B17 and B19 clustered to Gammaproteobacterial with 99.59-100% similarity index. In assessment for the selected isolates in greenhouse experiment, seed bio-priming showed significant change in terms of root mortality, germination rate, plant height, plant biomass, chlorophyll content, Phenylalanine ammonia-lyase, Polyphenol oxidase, Peroxidase and phenolic content compared to pathogen inoculated controls. In conclusion, lakes Bogoria and Magadi harbors beneficial microbes that can be used as biocontrol agents against *Fusarium solani* and *Rhizoctonia solani*.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

In Kenya, the agricultural industry is considered the most prominent and dominant, contributing up to 33% of GDP (Richardson, 2012). Vegetables are an important sector since they ensure food security and improve the livelihoods for small-scale farmers. Common beans (*Phaseolus vulgaris L.*) is an herbaceous annual plant grown for its edible dry seeds or green (Figure 1.1). Its leaf is also occasionally used as vegetable and fodder. It is a member of the legume family Fabaceae as it acquires the nitrogen through association with rhizobia. They grow well at 15℃ to 27℃ and will withstand up to a temperature of 29.5℃. The ideal growing condition is a rainfall of 350mm-550mm with the combination of low relative humidity to reduce the risk of fungal and bacterial diseases (Richardson 2012). They are warm season crops though very sensitive to temperature extremes. For instance, low temperature slows down plant growth, while high temperature accelerates it. Common beans are more adaptive during short days; they are very frost tender and need a minimum average soil temperature of 18℃ to germinate well (Rathna Priya and Manickavasagan 2020).

Figure 1.1: Common Beans. Source: JKUAT Field

Common bean is a stable food rated second after maize since, and contain nutritional value, protein content , affordability, storage stability, drought tolerance and dietary diversification (Celmeli et al. 2018) . The importance of common beans is that it does not require any industrial processing compared to other cash crops. Additionally it is a source of protein, minerals, fiber, thiamine, folate, and phytochemicals with analgesic and neuroprotective properties (Blair et al. 2013; Castro-Guerrero et al. 2016). According to Blair et al. (2013), it contains iron, zinc, and other microelements generally found to be in low concentration in cereals seed crops and root or tuber, making it a good candidate for bio-fortification. Common beans are high in dietary fiber which promote digestive health, regulating blood sugar level and aiding in weight management by providing a feeling of fullness and preventing overeating.

According to Castro-Guerrero et al. (2016), common beans are a vital cash crop for many smallholder farmers in Kenya whom they sell their bean harvests in local markets, generating income for their households and communities. This contributes to poverty alleviation and overall economic development, particularly in rural areas. Additionally,

Kenya exports common beans to various international markets, including neighboring countries and beyond. The export of beans contributes to foreign exchange earnings, supporting the country's balance of trade. The demand for Kenyan beans is significant due to their quality and taste, enhancing the country's reputation as a reliable exporter. Common beans have contributed to value addition in the form of processing beans into various products like canned beans, bean flour, and packaged beans increases their market value. This value addition provides additional economic benefits and opens up new markets for the bean industry. In terms of income, an average annual family income of USD $50.8 with a per capita share of USD $6.60 is generated specifically by common bean production.

Kenya is the largest producer of beans in Eastern and Southern Africa (Duku et al. 2020). In Kenya, it is grown in all areas. However, Eastern, Nyanza, Central, Western, and Rift valley are the major growing regions. Some of the varieties which are grown in Kenya include Mwitemania, Rose coco, and Canadian. According to KALRO, from 2010 to the present, Kenya's imports of beans have dropped, and this discrepancy is supplemented by imports from Uganda, Ethiopia, and Tanzania.

Pests and diseases contribute to the significant challenge for bean production. Some of the pests include; cutworms, bean fly, red spider mites, aphids, pod borers, white flies, and thrips (Mahmoud and Mahmoud 2016). A disease that affects beans includes block root rot, damping-off diseases, bean rust, *Fusarium* wilt, *Rhizoctonia* root rot, bacterial blight (Late, leaf, and web), and downy mildew. According to Muriungi JS et al. (2013); Sj et al. (2014), apart from pests and diseases, soil infertility and environmental stress also contribute to the low production of beans. There was a decline of 13.8% of total common bean production over the past 5 years as reported by Gossen et al., (2016) and Richardson, (2012). The decline is contributed by root and stem rot disease contributed by *Rhizoctonia* and *Fusarium* fungi and approximately 84% caused by *Pythium, Fusarium, Rhizoctonia*, which is most challenging to identify and control (Ajayi-Oyetunde and Bradley 2018; Tamiru and Muleta 2018).

Fungicides have been used over the period, but their effectiveness is compromised (Andrés et al. 2016; Gossen et al. 2016; Naseri 2014). They are linked to environmental pollution, reduces plant growth, nodulation and nitrogen fixation and leading to the expansion of resilient pathogenic strains (Ashraf et al. 2020). Therefore, biological control can be an alternative solution in managing *Fusarium* and *Rhizoctonia* fungi (Knodel et al. 2002). It calls for need to expand the biocontrol approach based on microbial antagonists to eliminate chemicals.

Soda-lakes such as Bogoria, Magadi, and Elementaita harbor extremophiles bacteria that thrive in extreme environmental conditions that would be lethal to most other forms of life. These extremophiles have adapted to survive and even flourish in habitats characterized by extremes in temperature, pH, pressure, salinity, radiation, and other environmental factors. Studying extremophiles bacteria has provided valuable insights into the limits of life on Earth and the potential for life in extreme environments on other planets. They are classified into thermophiles which typically grow between 45°C and 122°C with the habitats being hot springs, geysers and deep-sea hydrothermal vents. Secondly are the psychrophiles that grow in temperature of below 0°C and 20°C. Thirdly are the acidophiles growing at extremely acidic environments; alkaphiles growing at highly alkaline environment and halophiles growing at high salt concentration with salt flats, salt mines and salt pans as their habitats (Eunice et al. 2020). The study evaluated the potential of bacterial isolates from Lakes Bogoria and Magadi for biocontrol of both *Fusarium solani* and *Rhizoctonia solani* pathogens in beans.

1.2 Statement of the Problem

Pests and diseases cause a reduction in common bean yield, quality and increase the cost of production (Schwartz and Marcial A. 1989). Among the most devastating diseases attacking beans are *Fusarium wilt* (Zitnick-Anderson et al. 2020), caused by *Fusarium solani* (Leep 2016) and *Rhizoctonia solani* (Al-Hazmi and Al-Nadary 2015) that causes root rot and hypocotyl diseases (Ajayi-Oyetunde and Bradley 2018) and damping-off

disease (Chang et al. 2008). In general, pest causes severe losses >80% to the yield and quality of common beans worldwide (Singh and Schwartz 2010). The root rot deterioration caused by *Fusarium solani* and *Rhizoctonia solani* causes severe yield losses of up to >70% in common beans in most parts of the world, with devastating effects on the significant commercial beans cultivars in East Africa (Amongi et al. 2020).

Secondly, control of bean diseases has been a challenge since, farmers have opted to use crop rotation strategies based on climate regulation, genetic diversity, and sanitation. Farmers have also employed manipulating sowing density and time of sowing, choice of soil, use of varieties, species mixture for a specific condition, and selective weeding (Trutmann, Voss, and Fairhead 2008). Several sanitation methods, such as removing debris from the field, burning weed, and early planting, have been ineffective in controlling *Fusarium solani* and *Rhizoctonia solani*. Farmers also use synthetic chemicals in control of the weed and various pathogens which affects the fertility of the soil (Atafar et al. 2008), pH of the soil, and they destroy essential microbes in the soil, affecting the growth of most plants (Manna et al. 2005). Chemical-based strategies have also been reported to have health issues for consumers (Birnbaum 2008) and contribute to environmental degradation (Birnbaum 2008). Lastly, there is are limited chemical for managing Fusarium root rot and damping-off disease caused by *Rhizoctonia solani* (Leep 2016).

1.3 Justification

Traditional and chemical methods, has been employed in managing *Fusarium solani* and *Rhizoctonia solani*. These methods have not been effective, cause environmental degradation, and affect non-target beneficial microorganisms. Therefore, there is a need to come up with a technological invention in managing the pathogen. Additionally, it is favorable to replace chemical control approaches with less poisonous biological control methods. The attractive option is the use of bioproducts from such unique environments as soda lakes. The benefits of these biological agents are that they influence root

colonization ability and determine the population of the pathogen, are self-sustaining, spread after initial establishment, minimize the input of non-renewable capitals, and endure disease destruction in an ecologically friendly way. This will provide sustainable and environmentally sound management options and ensure farmers' increased incomes and fair prices to consumers.

The study seeks to evaluate an effective bio-product from soda lakes as an alternative method for controlling *Fusarium solani* and *Rhizoctonia solani* in beans. Soda-lakes are special in terms of their uniqueness and therefore, biocontrol from soda-lake will play a big role in agriculture. The prospective bio-product is more effective against disease, promote plant growth, and enhance yield production. Additionally, it should be environmentally friendly, sustainable, and combat disease compared to conventional methods.

1.4 Objectives

1.4.1 General Objective

Evaluation of biocontrol potential of bacterial isolates from Lakes Bogoria and Magadi against *Fusarium solani* and *Rhizoctonia solani* in *Phaseolus vulgaris L.*

1.4.2 Specific Objectives

i. To assess the antifungal activity of bacteria isolated from L. Bogoria and L. Magadi against *Rhizoctonia solani* and *Fusarium solani.*

ii. To characterize bioactive bacterial isolates against F. *solani* and R. *solani*.

iii. To determine the efficacy of the bioactive isolates against *F. solani* and *R. solani* in greenhouse.

1.5 Null Hypothesis

i. Bacterial isolates from L. Bogoria & L. Magadi have no antifungal activity against *Fusarium solani* & *Rhizoctonia solani*.

ii. There is no difference in the bioactive bacterial isolates against *F. solani* and *R. solani*.

iii. Bioactive isolates are not effective in the control *F. solani* and *R. solani* in greenhouse.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

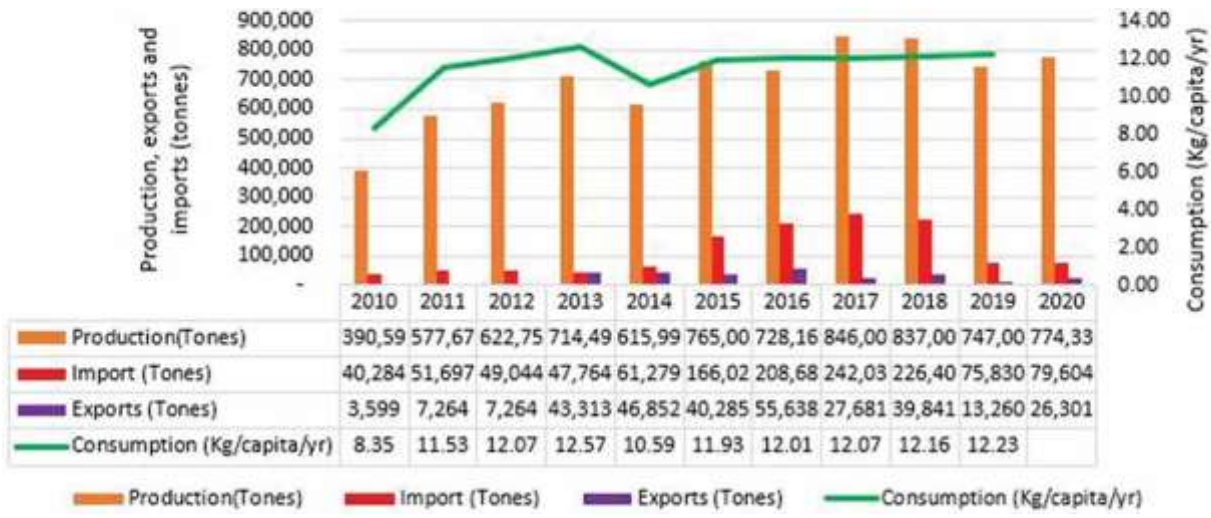
Common beans (*Phaseolus vulgaris L.*) are an essential component of the production system and a significant source of protein, especially for the poor in Eastern and Southern Africa. It is grown mainly for subsistence and contributes to approximately 40% of production, valued at USD 452 million(Duku et al. 2020). Globally, the production trend has not kept pace with the annual growth rate in population as a result of both biotic, abiotic, and socio-economic constraints (Mutari et al. 2020). Among the abiotic factors, drought is primary and common across Eastern and Southern Africa. Drought is contributed by inadequate rainfall, erratic rainfall distribution, prolonged dry spell, and delayed onset and early cessation of rains. Additionally, global climatic change has been a threat to exacerbate the drought problem in some part of country makes the production of beans unsustainable to the rapid growing population.

It is a warm-seasoned crop that does not tolerate frost or prolonged exposure to the near-freezing temperature at any growth stage. The crop requires a moderate amount of rainfall (300-600mm) though, adequate amounts are essential during and immediately after the flowering stage. Common beans are considered a short-season crop, with most varieties maturing in a range of 70-110 days from the time they emerge to physiological maturity (Babirye et al. 2023). The crop is not sensitive to soil type so long as it is fertile and well-drained.

2.2 Status of Beans Production in Kenya

In Kenya, Common beans are mainly grown in highlands and midlands. According to Duku et al. (2020), approximately 75% of the annual farming occurs in three regions; Rift-valley, Nyanza, and Eastern counties. In terms of productivity, rift-valley accounts for 33% of the national production (Wangui, Mugambi, and Mushimiyimana 2017).

Approximately 1.5 million small-scale farmers cultivate beans. This sums up to about a million hectares with a yield of 0.6 Metric tons. The consumption rate is approximated to be 600, 000MT hence resulting in a capital consumption rate of 14-66 kilograms (Wangui et al. 2017). Therefore, there is an import deficit which Ethiopia, Tanzania, and Uganda supplement. Katungi et al. (2012) reported that since 2010-2015 imports have had about a 7% consumption rate though still there is a consumption gap of approximately 20%. The production of French beans by small-scale farmers was approximately 112,666MT, which is valued at Kshs. 5.04 billion (Castro-Guerrero et al., 2016; Katungi et al., 2012; Rathna Priya & Manickavasagan, 2020). The statistics indicated a significant increase in area harvested, production, and yield from 2010-2020 (Babirye et al. 2023). This increase in output value from $4.4 billion to $5.4 billion. Worldwide, Brazil and Mexico are examples of major producing countries for national consumption. Canada, United States, Argentina, and China are exporting countries (FAO 2011).



	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020
Production(Tones)	390,59	577,67	622,75	714,49	615,99	765,00	728,16	846,00	837,00	747,00	774,33
Import (Tones)	40,284	51,697	49,044	47,764	61,279	166,02	208,68	242,03	226,40	75,830	79,604
Exports (Tones)	3,599	7,264	7,264	43,313	46,852	40,285	55,638	27,681	39,841	13,260	26,301
Consumption (Kg/capita/yr)	8.35	11.53	12.07	12.57	10.59	11.93	12.01	12.07	12.16	12.23	

Figure 2.2: Status of Dry Beans Production in Kenya (2017-2019) (Babirye et al. 2023)

In terms of production and distribution, cultivation in Kenya is widespread, but production concentrates on few counties. In Africa, Kenya is the leading producer of

common beans in terms of area. However, Kenya is the second in terms of production after Uganda (Katungi and Farrow Geagrofía 2009). This has been contributed by the relatively favorable biophysical environment in Uganda compared to Kenya (Babirye et al. 2023). Common bean is grown twice a year, with the sowing season running from March to April and from September to October. Kenya grows various types of beans, including Rose coco, Canadian Wonder, Mwezi Moja, Nyayo, and others. Farmers choose bean varieties based on factors such as adaptability to local conditions, market demand, and disease resistance. Common beans are not only consumed domestically but are also exported to various countries, particularly in East Africa, the Middle East, and Europe. Export markets play a crucial role in the income generation of farmers (Figure 2.2).

2.3 Challenges of Bean Production in Kenya.

Bean production faces various challenges that can affect the yield, quality, and overall success of the crop (Duku et al. 2020). Biotic and abiotic factors are significant factors affecting the production of beans. Some biotic factors include *Ophioma* species known as bean stem maggots, aphids, thrips, and white flies (Richardson 2012). Some of the abiotic factors such as drought, excessive rain, and poor soil fertility have affected bean production in Kenya (Jacobsen, Jensen, and Liu 2012; Mutari et al. 2020). Additionally, constraints such as inadequate capital, poor access to improved germplasm, low labor productivity, and poor marketing infrastructure have resulted in the decline of bean productivity and also low supply in the market (Birachi et al. 2011).

2.4 Description of *Fusarium* and *Rhizoctonia* solani in Bean Crop

2.4.*1 Fusarium solani*

Fusarium wilt is a significant cause of the poor production of beans. *Fusarium* spp. is characterized by its irregular light brown lesion and round (Naseri 2014). The lesion is seen along the tap root and lower hypocotyl. The physical appearance of the diseased area looks enlarged and turns brown with time (Figure 2.3). According to Toghueo et al.

(2016), young seedlings which are infected appear underdeveloped. First leaves are frequently yellow, which turns necrotic hence result in wilting. Secondary fungi infections also contribute to the dead of the seedling. The infected roots most often appear discolored reddish, which is shown to the soil's surface (Figure 2.3). There is also a sign of the proliferation of the adventurous roots, and for the severe infection, plants are always undersized, chlorotic, and defoliate early (Akrami et al. 2012).

2.4.2. Epidemiology of Fusarium Root Rot on Beans

Fusarium solani causes *Fusarium* root decay. The pathogen's characteristics include microconidia, macroconidia, and chlamydospores, infective spores of *R. solani*. It attacks plants by directly all-pervading into the root flesh through injuries and normal opening. Additionally, chlamydospores allow the pathogen to reside in the soil until it is enthused by the existence of the seedling root (Leep 2016). Therefore, it allows mycelium to penetrate the epidermis and reside in the intracellular part of the plant for a while before it extends to the longitudinal root of a plant. *F. solani* can be able to survive indefinitely on the decaying organic matter hence, makes it difficult to effectively manage its control (El-Mohamedy and Alla 2013)

In the field, the pathogen can be passed through debris from previously disposal sites for containment. This is contributing to runoff rain and soil transfer. Management of *Fusarium* has been a challenge since chemical and farming practices have been ineffective in controlling the disease(Gossen et al. 2016; Zitnick-Anderson et al. 2020).

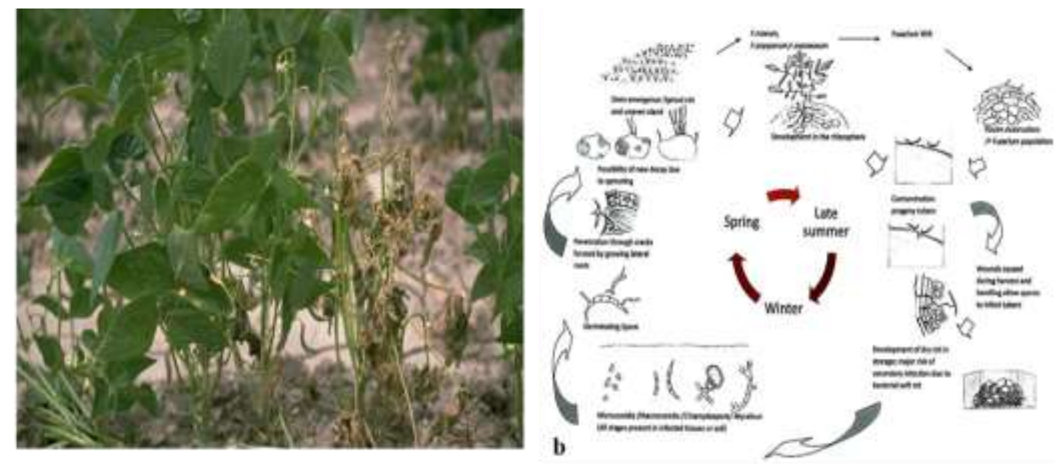


Figure 2.3*:* Symptoms of *Fusarium solani* on the Bean. a)- Causes Adventitious and Tap Root Rot. b)-Pathogenicity of *Fusarium solani*. Source: (Leep 2016)

2.4.3 *Rhizoctonia solani*

It is a soil fungus that results in seed deterioration, root decay, and shoot canker. One of the characteristics of the disease is that it causes damping-off (Mahmoudi and Naderi 2017). It attacks the stem below and above the soil surface. The young plants die soon after being infected by the *R. solani* (Figure 2.4). To the older plants, they develop reddish-brown canker spread longitudinally laterally the shoot near the soil surface. *R. solani* is a source of brick red staining of the dominant part of the inferior stem, yellowing, inhibiting and rough development, and final demise of a plant (Strausbaugh et al. 2011).

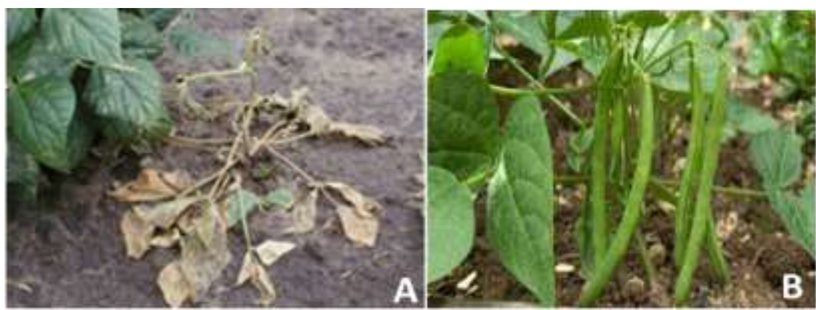


Figure 2.4: Symptom of *R. solani* on Bean Plant; A)-*Rhizoctonia* Causes the Bean to Die. B)-Health Common Bean Plant has Green Leaves. Source: (Ajayi-Oyetunde and Bradley 2018)

2.4.4 Epidemiology of *Rhizoctonia* Root Decay on Bean

Rhizoctonia solani causes *Rhizoctonia* root deterioration. The pathogen can endure hyphae in diseased crop debris for an extended period (Strausbaugh et al. 2011). The disease affects root tissues by use of propagules, sclerosis, and mycelium. It also can survive in the soil for an extended period (El-Mohamedy and Alla 2013). This is contributed by the plant host, which provides a conducive environment for survival. Therefore, it has to be transported during planting with infected seed or soil. When the pathogen meets the plant, it produces appressorium. This makes the fungal take the young radicle of the emerging seed and causes hypocotyl cuts, foliage, disfiguring, and shoot canker (Muriungi et al. 2014). According to Strausbaugh et al. (2011), the pathogen is endangered, especially from biological and biochemical deficiency by melanized hyphae which are significant for more prolonged survival (Muriungi et al. 2014). The pathogen can feast through gusting infested soil, crop wreckage, for instance, through the diseased plants' movement and during the rainy season. During moderate temperatures, the development of the disease is favored. Below is a schematic diagram showing the infectious cycle of *R. solani* (Figure 2.5).

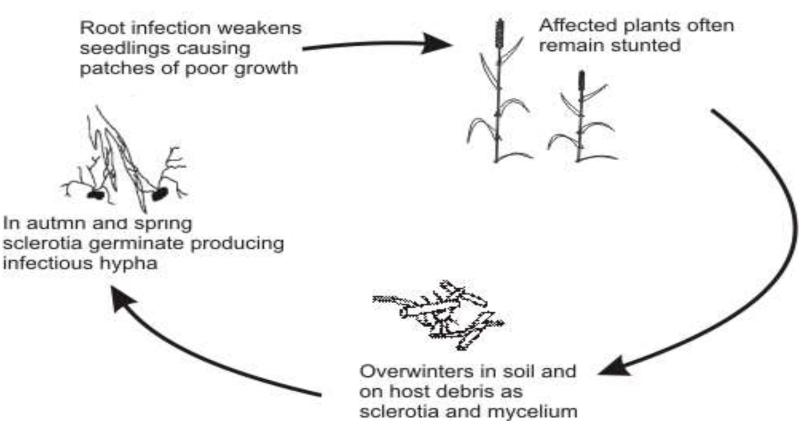


Figure 2.5:*Rhizoctonia solani* Life Cycle. Source (Ajayi-Oyetunde and Bradley 2018)

2.5 Management of *Fusarium* and *Rhizoctonia* on Bean Crop

According to Gossen et al., (2016), synthetic fungicides for seed treatment have low efficacy in disease management. Additionally, some of the chemicals used include Captan, Thiram, carboxy, metalaxyl, and difeconazole. Farmers use fungicides, for instance, methyl thiophanate, chlorothalonil, and fludioxonil which are suitable for soil application (Asad-Uz-Zaman et al. 2015; Gossen et al. 2016; Tamiru and Muleta 2018). Despite that, biocontrol agents can manage root deterioration disease caused by *R. solani* and *F. solani* (Akrami et al. 2012, 2013). The advantage of biocontrol is that it provides a season of long root rot destruction by colonizing and exciting host resistance response. Some of the microbial agents which have shown antagonist actions against root decay include; *Bacillus subtilis* (Gossen et al. 2016)

According to Ajayi-Oyetunde & Bradley (2018); Asad-Uz-Zaman et al., (2015); Gossen et al., 2016; Strausbaugh et al., (2011); Teixeira et al., (2015), managing root rot by cultural practices is focused on minimizing inoculum pressure and enhancing crop health. This is mainly attained by varied crop rotation with non-host plants.

2.6 Alternative Host of *R. solani* and *F. solani*

Weeds like pigweed (*Amaranthus spp*.), chickweed (*Stellaria media*), and lambsquarters (*Chenopodium album*) are known to harbor *R. solani*. These weeds can serve as reservoirs for the pathogen, allowing it to survive and multiply during crop off-seasons. Certain cover crops, including clover (*Trifolium spp*.) and rye (*Secale cereale*), have been identified as alternative hosts for *R. solani* (Abbas et al. 2019; Jabnoun-Khiareddine H 2018). Cover crops are commonly used to improve soil health and prevent erosion, but they can inadvertently facilitate the survival and spread of the fungus(Strausbaugh et al. 2011). Ornamental plants like geraniums (*Pelargonium spp*.) and poinsettias (*Euphorbia pulcherrima*) can host *R. solani*, acting as potential reservoirs in greenhouses or nurseries. The fungus can persist in the soil and plant debris associated with these ornamentals. Crop residues left in the field after harvest, particularly those of susceptible crops like maize and soybeans, can harbor *R. solani*. The fungus can survive on these residues, infecting subsequent crops and perpetuating the disease cycle (Ajayi-Oyetunde and Bradley 2018).

Fusarium solani is another widely distributed and highly versatile plant pathogenic fungus that causes a variety of diseases in plants. Similar to *R. solani*, *F. solani* also has a broad host range and can infect numerous plant species. Some of its alternative hosts include (Stefańczyk and Sobkowiak 2018). Various leguminous crops, such as beans, lentils, and peas, can serve as alternative hosts for *F. solani*. The fungus can cause root rot and vascular wilt diseases in these crops, impacting their growth and yield. Solanaceous crops like tomatoes, potatoes, and eggplants are susceptible to *F. solani* infection (Aydi et al. 2016). The fungus can cause damping-off, root rot, and stem canker, leading to significant losses in yield and quality. Cucurbitaceous crops like cucumbers, pumpkins, and squash are also known to be alternative hosts of *F. solani* (Won et al. 2018). The fungus can infect the roots, causing root rot and wilting of the plants. *F. solani* is capable of infecting and causing disease in a variety of woody plants,

including trees and shrubs. The fungus can lead to root rot, stem cankers, and dieback in these plants (Jangir et al. 2018).

2.7 Global Microbial Diversity of Soda Lakes.

Soda lakes are predominantly found in the arid, semi-arid and desert regions around the world (Samylina et al., 2014). Soda lakes from around the world that have been studied include; Mono Lake (Humayoun et al., 2003; Scholten et al., 2005) soda lakes in the Kenyan-Tanzanian Rift Valley (Rees et al., 2004), soda lakes in Mongolia (Sorokin et al., 2004) and Inner Mongolia in China (Ma et al., 2004), athalassohaline lakes of the Atacama desert, Chile (Demergasso et al., 2004), saline, meromictic lake Kaiike in Japan (Koizumi et al., 2004), saline Qinghai Lake, China (Dong et al., 2006) and athalassohaline Lake Chaka, China (Jiang et al., 2006). Despite these studies, the knowledge of microbial populations in hypersaline/alkaline environments are still limited when compared with microbial communities in marine or fresh water bodies.

The knowledge about molecular mechanisms of extremophilic microbes stemmed mainly from studies by Horikoshi (1999) on hot springs in Yellowstone National Park (Wyoming, U.S.A) that revealed large bacterial communities in the hot spring that had twelve novel division-level lineages. These studies revealed that members belonging to the bacterial domain appeared to outgrow the Archaea found the hydrothermal environment (Keller & Zengler, 2003; Simasi, 2009). Novel obligately anaerobic, alkalithermophilic, chemo-organotropic bacterium was identified from an alkaline hot spring located on Paoha Island in Mono Lake, California, USA. This bacterium is rod-shaped; it reduces Fe (III) and Se (IV) in presence of organic matter. The strain was identified as Anaerobranca californiensis sp. Nov on the basis of physiological properties, 16SrRNA gene sequence and DNA–DNA hybridization data (Vladimir et al., 2004).

Subsequent studies of microbial diversity in the hot springs of Yellowstone National Park in (Wyoming) U.S.A, have shown that although all the hot springs were in close

geographical proximity, they had similar temperatures (between 85°C and 95°C) and pH value (7.8–8.9), but differed remarkably in regard to their overall microbial diversity. This finding indicated that, in the common complexity of microbial diversity in soil, geochemical variations affect microbial biodiversity and that only studies that incorporate measurements of geochemical parameters will allow the understanding and prediction of biodiversity (Simasi, 2009).

Studies by Yanhe in 2004 on Baer Soda Lake located in the Inner Mongolia region of China, indicated that the 16S rDNA phylogenetic analysis of bacterial diversity in the alkaline Lake could be isolated and characterized using both culture dependent and molecular methods. Fifty-three alkalithermophilic bacteria were isolated from the sediment samples, 20 of the isolates were subjected to 16S rRNA gene sequence analysis. The results showed some of the clones were related to extremophilic bacteria from soda lakes such as Alkalispirillum, Thioalcalovibrio denitrificans, and Halomonas campisalis, while others were related to known species with more than 97 % similarity from environments that are not alkaline. These isolates were affiliated to the genera *Bacillus, Amphibacillus, Gracilibacillus, Alkalibacterium, Salinicoccus, Exiguobactrium, Halomonas, Pseudomonas, Marinospirillum,* and *Cyclobacterium*. Out of the 20 bacterial isolates, 4 were Gram-negative while the rest of them were Gram-positive isolates (Borsodi et al., 2008).

2.8 Microbial Diversity of Kenyan Soda-Lakes

Kenyan soda lakes range from approximately 5-30% (w/v) of the salinity and pH of 9-11.5. Lake Magadi is among the most stable, highly alkaline environments on earth, with a pH greater than 10.5 (Antony et al. 2012). Soda lakes in Kenya have been shown to have major trophic groups of microbes. Cyanobacteria are less in saline-alkaline lakes, *spirulina platensi*s, and cyanopsia. Unicellular species, for instance, *Chorococcus spp*, have been found as the dominant primary producers.

Lake Bogoria, which is mainly characterized by hot springs and therefore, has hyperthermophilic and haloalkalithermophilic microorganisms. The bacteria from this lake can grow at higher temperatures of >80°C. They can also adapt to high pH, making them more useful for industrial purposes such as enzymes. Some of the microbes identified from such an environment included the genera of Nitzchia and Navicular, which are predominant in these ecosystems. Alkaliphic anoxygenic phototrophic bacteria belong to Ectothiorhodospira and Halorhodospira genera. These genera can form visible blooms in the soda lakes and provide substantial contributions to the primary production. The genera can oxidize sulphide to sulphate and deposit extracellular elemental Sulphur (Eunice et al. 2020; Kambura et al. 2013; Nyakeri, Mwirichia, and Boga 2018).

The lakes also contain anaerobic groups such as Desulfonatronovibrio and Desulfonatronu, which oxidize Sulphur, menthane (*Methylobacter alcaphilus*), ammonia (*Methylomicrobium spp*). The studies on Lake Bogoria reveal diverse populations of aerobic sulphur-oxidising bacteria of Thioalkamicrobium and Thioalkolivibrio. Several strains of *Bacillus, alcaligenes faecalis, stenotrophomonas, Rhodobacter spp*, and *pseudomonas* were identified from Kenya soda lakes (Kiplimo et al. 2019; Mwirichia et al. 2011; Mwirichia, A. Muigai, et al. 2010). Therefore, the study indicates that soda lakes harbor diverse microorganisms which might have important use from industrial, medical, agricultural, and plant pathology. The study of the soda lakes has attracted various scientists to exploit microorganisms in terms of their composition and use.

2.9 Application of Extremophilic Bacteria in Biotechnology

The microbial community in soda lakes has attracted attention as a possible source of novel microorganisms which can be used as enzymes and metabolites in biotechnology (Scoon 2018). Most microbes are used as a source of enzymes because they are cheap to produce, predictable and controllable. Thermoalkaphilic bacteria are believed to have biotechnological potential as the source of alkali-stable enzymes (Kambura et al. 2013). Proteases from extremophiles are also applied in the manufacture of leather, xylanases

for use in the pulp paper industry, and cyclodextrin glucosyltransferase for cyclodextrin manufacture from starch, frequently used in foodstuffs, chemicals, cosmetics, and pharmaceuticals (Mulango et al. 2020). Glycosyltransferases and hydrolases from extremophiles are essential because they can perform reactions at high temperatures and with high contents of organic solvents. Subsequently, they have advantages over 'conventional' enzymes (Antony et al. 2012).

Detergent enzymes account for approximately 60 % of total worldwide enzyme production (Sorokin et al. 2014). They usually have a pH range of 8 and 10. The main reason for selecting enzymes from alkaliphiles is their long-term stability in detergent products, energy cost saving by lowering the washing temperatures, quicker and more reliable product, reduced effluent problems during the process, and stability in the presence of detergent additives such as bleach activators, softeners, bleaches, and perfumes (Mwirichia et al. 2010).

2.10 Mode of Action of Soda Lakes as Biocontrol

Soda lakes, with their extreme alkaline conditions and unique microbial communities, have shown promise as potential sources of biocontrol agents for managing plant pathogens and promoting sustainable agricultural practices. The mechanisms of action for utilizing soda lake microorganisms in biocontrol are multifaceted and include both direct and indirect interactions with pathogens and the plant ecosystem. Here are some key mechanisms of action (Yehia et al. 2023)

Antagonism and Antimicrobial Compounds: Microorganisms from soda lakes can produce antimicrobial compounds that inhibit the growth and development of plant pathogens. These compounds may include antibiotics, secondary metabolites, volatile organic compounds, and enzymes that have antimicrobial properties (Jabnoun-Khiareddine H 2018). The antagonistic activity of these compounds directly suppresses the growth of harmful pathogens. Soda lake microorganisms can outcompete plant pathogens for essential nutrients and space. By utilizing available nutrients more

efficiently, they limit the resources available for the growth of pathogens, reducing their population and impact on plants (Tariq et al. 2020).

Some microorganisms from soda lakes are capable of forming biofilms on plant surfaces. These biofilms act as physical barriers that prevent the attachment and colonization of pathogenic microorganisms. Additionally, biofilms can produce antimicrobial substances that inhibit the growth of pathogens (Yehia et al. 2023). Certain microorganisms from soda lakes can induce systemic resistance in plants. They trigger the plant's defense mechanisms, resulting in a heightened immune response against potential pathogens (Rais et al. 2017). This systemic resistance can provide long-lasting protection to plants against a range of diseases. Microorganisms from soda lakes may possess the ability to detoxify harmful substances present in the soil or plant environment (Cawoy et al. 2014). They can degrade or transform toxic compounds into non-toxic forms, reducing the detrimental effects of these substances on plant health (Won et al. 2018; Wu et al. 2021).

Some microorganisms from soda lakes are plant growth-promoting rhizobacteria (PGPR) that enhance plant growth and vigor by improving nutrient uptake, increasing root development, and producing growth-promoting substances like auxins and cytokinins (Sharma et al. 2019). Healthy plants are more resilient to pathogen attacks. Soda lake microorganisms can modify the soil or rhizosphere environment to make it less favorable for pathogen survival and growth (Panpatte et al. 2016). This alteration may include changes in pH, nutrient availability, or the release of substances toxic to pathogens (Baazeem et al. 2021). Soda lake microorganisms can contribute to stabilizing the local environment by reducing the impact of soil-borne pathogens, thereby enhancing soil health and overall ecosystem stability (Jiao et al. 2021).

2.11 Challenges in the Use of Soda-Lake Microbes as Biocontrol in Agricultural Environment.

While the use of microbes from soda lakes as biocontrol agents holds promise for sustainable agricultural practices, several challenges need to be addressed to realize their full potential effectively. Here are some key challenges associated with utilizing soda lake microbes as biocontrol agents in the agricultural environment(Panpatte et al. 2016).

Microbes from soda lakes have evolved to thrive in extreme alkaline environments. Adapting them to more neutral or slightly acidic agricultural soils can be challenging (Duckworth et al. 2000). Ensuring that they can effectively establish and function in these different soil conditions is crucial for their efficacy as biocontrol agents (Tamiru and Muleta 2018). The survival and persistence of soda lake microbes in the agricultural soil environment, which may have different nutrient availability, microbial communities, and stressors, is a significant challenge. Enhancing their survival rates and long-term effectiveness after application is essential for successful biocontrol. Integrating soda lake microbes into existing agricultural practices, such as crop rotations, irrigation methods, and the use of fertilizers and pesticides, requires careful consideration (Szilagyi-Zecchin, Mógor, and Figueiredo 2016). Ensuring that these biocontrol agents do not interfere with conventional agricultural approaches is crucial for widespread adoption (Antony et al. 2012).

Achieving a high level of specificity against target pathogens while maintaining efficacy is a significant challenge. Ensuring that the biocontrol agents effectively target and suppress harmful pathogens without affecting beneficial microbes or non-target organisms is essential for successful biocontrol. Scaling up the production of biocontrol agents derived from soda lake microbes to meet agricultural demands is a logistical and economic challenge (Scoon 2018). Developing cost-effective and efficient production processes to obtain sufficient quantities for widespread use is critical. Meeting regulatory requirements for the safe use of biocontrol agents in agriculture is a complex process. Conducting thorough safety assessments and obtaining necessary approvals

from regulatory bodies can be time-consuming and resource-intensive (Jangir et al. 2018).

The interactions between soda lake microbes and the diverse soil microbial communities in agricultural environments are not fully understood. Understanding these interactions is crucial for predicting the impact and success of biocontrol agents. Public perception and acceptance of using extremophile microbes from soda lakes in agriculture may present a challenge (Elmahdi et al. 2015). Public education and awareness campaigns regarding the safety and benefits of such biocontrol agents are essential to gain public acceptance (Maina et al. 2020). Assessing the cost-effectiveness and economic viability of utilizing soda lake microbes as biocontrol agents compared to traditional control measures is necessary. Demonstrating the economic benefits and long-term sustainability of these biocontrol strategies is crucial for adoption by farmers (Syed Ab Rahman et al. 2018).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Sampling Sites

Lake Bogoria is in the Kenyan Rift-valley (0" 20'N and 36" 15'E). Due to salinity and alkalinity, its pH is around 9.0. The lake is described as a hot spring with a stream temperature range from 76-90℃. It is shallow, and it is approximately 34 km long and 3.5 Km wide. Therefore, it has a drainage sink of 700km2 with surface advancement of 990m.

Lake Magadi is a hypersaline lake located in the southern part of Kenyan Rift-valley (2° S and 36° E) close to the Tanzania border. It has an elevation of 600 m above marine level. It covers an area of 90 km^2, thus referred to as the smaller Rift-valley Lake. Liotta Hills and Mau escarpment play a significant role in shielding the valley floor from rainfall; hence it results in approximately 500mm of rainfall for the two rainy reasons. The lake's location is underneath the surface, and therefore, superficial water is typically only found around the boundaries of the crystalline deposit where updraft springs forage the lake (Kiplimo et al. 2019). No permanent river enters the lake basin. But there are solute supplies of alkaline springs with a temperature of 70℃. The location of the springs is around the border of the lake (Figure 3.6).

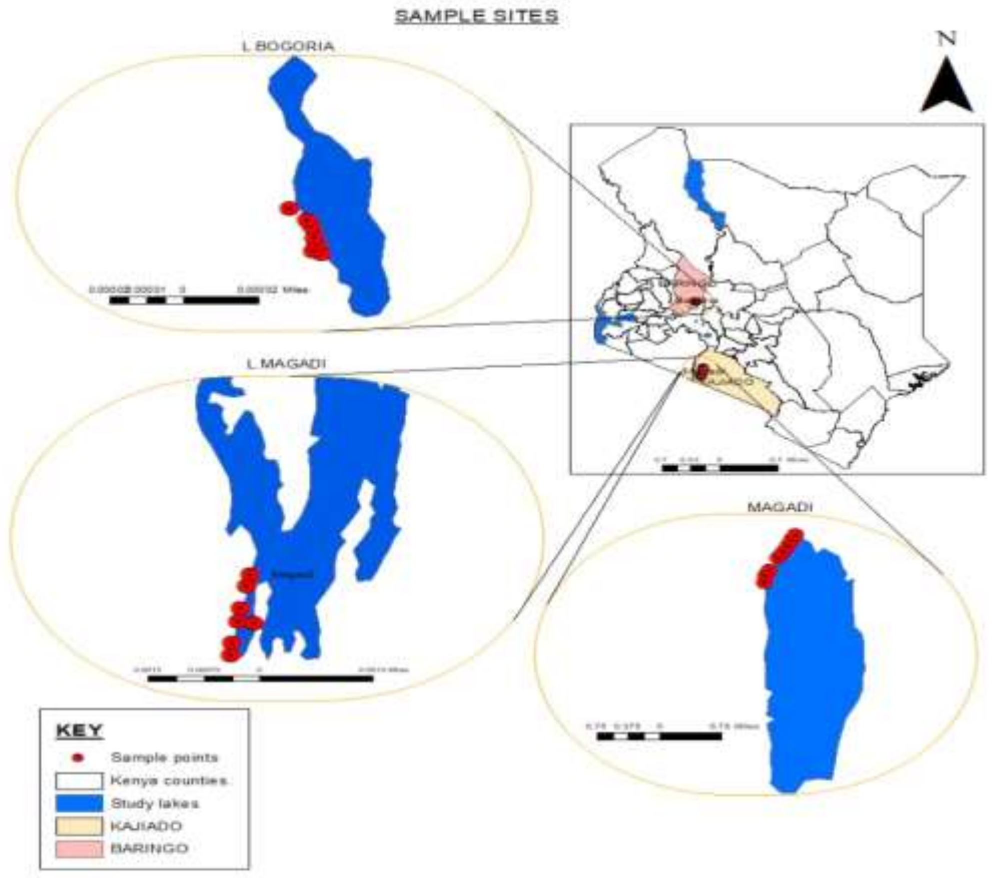


Figure 3.6: Kenyan Map Showing Sampling Points from Lakes Bogoria and Magadi.

3.2 Sample Collection

The purposive sampling method was used where the different stations of watering point, sediment, and soil labeled A, B, and C respectively of thermal spring were sampled. A total of 100 ml water, 50 g sediment, and soil were collected in triplicate and stored in sterile tubes. A total of sixteen sampling points from L. Magadi and seven points from L. Bogoria with a range of 100 m apart was considered for sample collection (Figure 3.7). The physiochemical properties such as temperature, pH, Salt, TDS (Total Dissolved Solids), and Conductivity were recorded using ISOLAB S/n: 0005596. The

collected samples were put in a sterile bottle, labeled, and preserved in a cool box (4°C). They were transported to the Institute of Biotechnology Research Laboratory in Jomo Kenyatta University of Agriculture and Technology (JKUAT, Nairobi, Kenya), for further analysis.

Figure 3.7: Sampling Point Showing Sample Collection and Measuring of Physical Parameters. a) Hot Spring Physical Parameters; b) Offshore of the Lake Soil Sampling.

3.3 Assessment of the Antifungal Activity of Bacteria Isolated from L. Bogoria and L. Magadi against *Rhizoctonia Solani* and *Fusarium Solani.*

3.3.1 Isolation of Bacteria from Lakes Bogoria and Magadi

The isolation of bacteria strains from water, soil and sediment was carried out using methods described by Thomas et al., (2015) on modified Nutrient Agar-Himedia [10.0 g Peptone, 10.0 g Meat extract B#, 5.0 g NaCl and 12.0 g Agar] per liter supplemented with cycloheximide (0.01 mg) (inhibit the growth of fungus), and 35 g/L (w/v) of salt. A 1 g of soil and sediment were weighed and then homogenized in a sterile test tube containing 9 mL of sterile physiological saline (0.85% NaCl). The resulting soil and sediment suspensions were then vigorously vortexed at 150 rpm for 1 min. A five-fold serial dilution of soil and sediment suspension with physiological saline (0.85% NaCl) was done in 1 mL to 9 mL. The dilutions were as follows 10^{0}, 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4}.

An aliquot of 30 µL from dilutions 10^{-3} and 10^{-4} was cultured according to Hartman, (2011) on modified nutrient agar-Himedia. The plates were incubated at 39.5°C for 24 h, followed by a subculture of the bacterial colonies using an isolation medium (modified nutrient agar) until pure colonies were obtained. The isolates were cryopreserved at -86°C in an isolation medium (Nutrient Agar) supplemented with 20% glycerol for further analysis. The pure bacterial isolates were characterized using standard microbiological techniques as described by Mullane (2010).

3.3.2 Preparation of Fungal the Pathogen Inoculum and Pathogenicity Test

Both *Fusarium solani* and *Rhizoctonia solani* were obtained from Dudutech Laboratory (Naivasha). Isolates were sub-cultured on Potato dextrose agar-Himedia (PDA) [200.0g Potatoes, 20.0 g Dextrose and 15.0 g Agar] per litre, incubated at 30°C for 7 days and preserved at -80°C for future reference and use.

Soil preparation for pathogenicity was done following the method as described by Asaka & Shoda (1996) was used. The Tantalization technique was used for autoclaving the soil for 60 mins at 121°C. Sterilized soil (150 g) was packed in plastic containers of maximum water holding capacity by adding sterile distilled water (SDW). Both spore solution of *F. solani* and mycelium mat of *R. solani* were inoculated into the soil at a ratio of 5.0 ml of spore/mycelium to one pot five days before planting the germinated bean seeds. SDW was used as control. Plant tests followed the procedure reported by Asaka & Shoda (1996). The seeds were surface sterilized with 70% ethanol for 30 secs and 5% NaOCl for 5 min and rinsed three times with SDW. After rinsing with SDW, the seeds were germinated on a 2% (w/v) agar plate at 28℃ for 3-5 days. Each pot was sown with two pre-germinated seeds and placed in a growth chamber at 28°C with 80% relative humidity under 8 hours of light (about 8,000 lux calculated using lx = W × (l m/W) / m2). After two weeks, the percentage of diseased seedlings (disease severity) was evaluated using a scale from 1-7, as described by Godoy et al. (1990). Furthermore, plant height, length of the root, root fresh weight, shoot length and shoot fresh weight were determined.

3.3.3 Assessment of the Bacterial Isolates for Anti-Fungal Activity against *F. Solani* and *R. Solani*

Bacterial were evaluated for antifungal activity using the co-culture method was used on PDA media-Himedia as described by Qadri et al., (2013). Bacterial isolates were perpendicularly streaked across the center of the petri-dish. Two agar plugs (8 mm) were cut from an active 5 day-old growing pathogen (*F. solani* and *R. solani*). The plugs were placed at the end of each side of the plates. The control of pathogen only was used by placing active growing pathogen at the center of the plate. The cultures were incubated at 30℃. The colony diameter of the pathogen was determined by use of Aydi et al. (2016) formula;

%I. R= [(C2-C1)/C2] *100

Where I. R= Inhibition Rate, C2= Colony diameter of the pathogen in control, and C1= Colony diameter of the pathogen co-cultured with bacteria.

3.4 To Characterize Bacterial Isolates Activity against *F. Solani* and *R. Solani*

3.4.1 Morphological characterization of the bioactive isolates

Standard microbiological criteria were used to describe morphologies of bacterial colonies with respect to pigmentation, shape, size, color, margin, texture, opacity, consistency, and pigmentation as described by Mullane, (2010). Gram staining as a preliminary identification characteristic of each isolate was done following procedure described by Beveridge, (2001) and Tripathi & Sapra, (2020) using a light microscope (MD827S30L, USA).

3.4.2 Physiochemical Characterization of Bacterial Isolates

3.4.2.1 Growth at Different Sodium Chloride Concentrations

Bioactive isolates were cultured on Luria-Bertani (LB) Himedia [10.0 g Tryptone, 5.0 g Yeast extract, 10.0 g NaCl] per litre supplemented with different concentrations of

sodium (0.0 M, 0.5 M, 1.0 M, 1.5 M, and 2.0 M NaOH), according to the manufacturer. The optical density (O.D. 600 nm) of the bioactive isolates were recorded after 48 h at 30°C in a shaking incubator at a speed of 120 rpm/min using VERSA MAX spectrophotometer equipped with SoftMax Pro version 6.4. An uninoculated L.B was used as a control.

3.4.2.2 Growth at Various Temperatures

Bioactive isolates were cultured on L.B. broth at varying temperatures of 20, 25, 30, 35, 40, 45, 50 and 60°C. They were incubated in a shaking incubator of 120 rpm at varying temperatures. The growth of the isolates was determined by measuring optical density at 600 nm after 48 h. Blank absorption was obtained using LB Himedia without bacteria isolates.

3.4.2.3 Effect of pH on Growth of the Isolates

The LB Himedia was prepared by adjusting its pH to 5, 7, 8.5, and 10 using 1 M HCl and 1 M NaOH. The media was autoclaved and dispensed on sterile tubes. Bioactive bacterial isolates were cultured on each tube and incubated at 30°C for 48 hours. Optical density (600nm) was measured using spectrophotometer. Blank absorption was obtained using LB Himedia without bacteria isolates as control.

3.4.3 Assessment of Bioassay Activity of Bacterial Isolates.

3.4.3.1 Protease Activity

The proteolytic activity of the bacterial strains was assessed following the protocol described by Saran et al., (2007) on skim milk agar-Himedia (28.0 g S.M. powder, 5.0 g Tryptone, 2.5 g yeast extract, 1.0 g Dextrose or glucose, and 15.0 g Agar) per liter (3%) (v/v) medium. The bacteria strains were cultured overnight, and 5 µL of the bacterial suspension was soaked on a filter-paper disc, allowed to dry, and placed on a Petri-dish containing skim milk agar. Sterilized water was used as a control. The experiment was performed in triplicate and incubated at 30°C for 24 hours. Proteolytic activity was

detected by forming clear zones or a halo around the bacterial spots, and the inhibition zone diameter was measured and recorded.

3.4.3.2 Chitinase Activity

The bacterial strains were screened for chitin hydrolysis by spotting on the center of 1% CCA (1% Colloidal Chitin, 0.2 g NaNO3, 0.1 g K2HPO4, 0.1 g MgSO4, 0.1 g CaCO3, 0.001 g FeSO4_7H2O, 0.05 g KCL) media at pH 7.0 (Ebrahim, Usha, and Singh 2011; Teish et al. 2003). Sterilized water was used as a control. The plates were conducted in triplicates and incubated at 30°C for 72 hours. Chitinase activity was detected by forming a clear zone around the bacterial spots.

3.4.3.3 Pectinase Activity

Pectinase enzyme assay was based on the determination of reducing sugars produced as a result of enzymatic hydrolysis of pectin by the dinitrosalicylic acid reagent (DNS) method (Miller 1959). A total of 1.5 mL of freshly grown culture was taken for enzyme assay and centrifuged at 10,000 rpm for 5 min. The enzyme's source was the supernatant (100 µL) from the culture broth. In addition, the substrate was prepared by mixing 0.5% (w/v) citrus pectin in 0.1 M of pH 7.5 phosphate buffer.

From the prepared substrate, 900 µL was added to three clean labeled test tubes; one for the enzyme, one for the enzyme blank, and one for the reagent blank. Then, 100 µL of the crude enzyme was added to the test tube labeled as an enzyme, and 100 µL of distilled water was added to the test tube labeled as regent blank while the test tube labeled as enzyme blank remained as it was. Then, the test tubes were incubated at 50°C for 10 min in the water bath. After incubation, 2000 µL of dinitrosalicylic acid reagent (DNS) was added to all test tubes to stop the reaction. Meanwhile, in a test tube labeled enzyme blank, 100 µL of the crude enzyme was added after the DNS. Then, all the test tubes were placed in a boiling water bath (92°C) for 10 min. Finally, the tubes were cooled, and optical density (O.D.) was measured using a spectrophotometer at 540 nm. Enzyme activity was measured against enzyme blank and reagent blank. The enzyme

unit was defined as the amount of enzyme that catalyzes µmol of galacturonic acid per minute (µmol min^{-1}) under the assay conditions.

Relative activity was calculated as the percentage enzyme activity of the sample against the maximum enzyme activity obtained:

Relative Activity= (Activity of sample (U) *100)/Maximum enzyme activity (U)

3.4.3.4 Hydrogen Cyanide (HCN) Production Ability

Hydrogen cyanide (HCN) was detected qualitatively by use of Rijavec & Lapanje, (2016) method. Bioactive bacterial isolates were inoculated on petri-dish containing nutrient agar supplemented with glycine (4.4 g/L) (w/v). Sterile discs (9 cm diameter) were soaked in picric alkaline solution (2% Sodium carbonate and 0.5% picric acid) and placed on the lid of each petri-dish. Un-inoculated control plates were used for comparison. Plates were sealed with parafilm and incubated at 25°C for 4 days. Color change from yellow to light-reddish brown indicates a positive HCN production (Rijavec and Lapanje 2016).

3.4.3.5 Phosphate Solubilization Ability

Phosphate solubilization was done qualitatively, according to Katznelson & Bose (1959). Bacterial colonies were cultured on Pikovskaya medium. Un-inoculated plates were used as control. The culture was incubated at 30°C for 7days. A clear zone formed around colonies due to the utilization of tricalcium phosphate present in the medium was measured (Tamrela et al. 2021).

3.4.3.6 Indole-3-Acetic Acid (IAA) Production Ability

The colorimetric method described by Gang et al. (2019) was used to detect the ability of bioactive bacterial strain to produce indole-3-acetic acid. Bacterial isolates were inoculated into 20 mL of Luria-Bertani Broth (LB) supplied by L-tryptophan (2.0 µg/mL) (w/v). Additionally, 2 mL of Salkowski's reagent and 3drops of orthophosphoric acid were added to 1 mL of the culture supernatant. Un-inoculated growth mediums

were used as a negative control. The red color indicates positive IAA production (Etesami, Alikhani, and Hosseini 2015).

3.4.4 Molecular Characterization of Bacterial Isolates against *R. Solani* and *F. Solani*

3.4.4.1 DNA Extraction of the Bacterial Isolates

Genomic DNA extraction was done using a bacterial DNA isolation kit (Norgen Biotek Corp., Thorold, ON, Canada) as per the manufacturer recommendation. A spectrophotometer was used to measure the DNA concentration and purity at 230/260nm. The fragments were separated by electrophoresis on 0.8% agarose gel stained with ethidium bromide (Sigma-Aldrich, St. Louis, Missouri, United States) gel for 1 h. The DNA template was then stored at -20℃ for further analysis.

3.4.4.2 PCR Amplification of 16S rRNA Genes.

To amplify the 16S rRNA genes, genomic DNA from each bacterial isolate was used as a template. A pair of 27F Forward (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R Reverse (5'-CGGCTACCTTGTTACGACTT-3') bacterial primers were used to amplify the 16S rRNA gene in response to the *Escherichia coli* gene sequence. The amplification was performed using Peqlab Primus 96 PCR equipment. It was amplified in a 40 µL mixture comprising 20 µL of Master mix, 18.2 µL of PCR water, 0.4 µL of 27F forward primer, 0.4 µL of 1492R reverse primer, and 1 µL of template DNA (750 ng/L) DNA.

The following temperature cycling profiles were applied for the reaction mixtures: A 10 min enzyme activation at 96℃ for a single cycle, which was followed by 35 cycles of 45 s of denaturation at 95℃, 45 s of primer annealing at 53℃, 1 min of the chain of elongation at 72℃, and 10 min of the chain of final extension at 72℃ (Torome* et al. 2015). The presence and size of PCR amplicon were verified on 1.2% agarose gel and visualized under U.V. light (Torome* et al. 2015). PCR amplicons were purified using the QIAquick PCR amplification kit protocol (Qiagen) according to the manufacturer's instructions. The PCR amplicon was sent to Macrogen for sanger sequencing.

3.4.4.3 Phylogenetic Analysis of the Bacterial Isolates

The 16S rDNA of the nineteen selected isolates were compared in the primary database using the Basic Local Alignment Search Tool (BLAST) in the National Center for Biotechnology Information (NCBI) site. Alignment was done utilizing CLUSTAL W 2.0 programming. The 16S rDNA gene sequence with the highest percentage similarity from the BLAST results was selected and retrieved. Both pairwise and multiple sequence alignment was done using Mega 7 (Tamura et al. 2007). Therefore, the phylogenetic data were analyzed using the neighbor-joining method and maximum composite Likelihood method using Mega 7 (Tamura et al. 2007) and Bootstrap analysis using Mega 7.

3.5 To Determine the Efficacy of Bacterial Isolates against *Fusarium Solani* and *Rhizoctonia Solani* on Bean Seedlings.

The experiment was carried out in January 2021 at Jomo Kenyatta University of Agriculture and Technology. Isolate B21, B39, and M10, which produced the highest percentage inhibition rate against *F. solani* and *R. solani*, respectively, were selected to investigate their ability to reduce the incidence of root rot and wilt in common beans. The antagonistic effects of B21, B39, and M10 against *R. solani* and *F. solani* was studied under greenhouse condition by pot culture method as described by (Baazeem et al. 2021). Seeds were surface sterilized by soaking with 70% ethanol for 30 secs and 5% NaOCl for 5 min and rinsed three times with sterile distilled water. Soil preparation (manure: soil: sand in a ratio of 1:3:2 (v/v)) as the plant growth medium was carried out by steam sterilization and left to cool before being placed in sterilized pots. The pathogen inoculums containing 2×10^8 spores ml^{-1} were incorporated into the potting medium at 150 ml kg^{-1} of soil and incubated for 5–7 days to achieve proper spore germination and establishment of pathogen mycelium (Pandey and Dubey 1994). Surface-sterilized seeds were sown in each pot (5 seeds pot^{-1}), and daily observations were made on germination and wilt incidence. Each treatment had 5 replications. Treatment details of the pot bioassay under greenhouse conditions included P_1 absolute

(Uninoculated) control; P_2 TRICHOTECH® WP control; P_3-*F. solani* only; P_4- *R. solani* only; P_5-B39+*F. solani*; P_6-B21+ *F. solani*; P_7- M10+ *R. solani* and P_8- B21+*R. solani*. For P_5 - P_8 treatments, surface-sterilized seeds were inoculated with 24 h grown cultures (OD600 = 1, consisted of corresponding to 10^8 cells ml^{-1}) of B39, B21, and M10. The absolute control (P_1), the potting medium was without either bacterial or pre-fungal inoculation. Seeds treated with TRICHOTECH® WP (Concentration of 4.0 x 10^9 CFU per gram in an inert carrier.) were used as the recommended bioproduct treatment (P_2). The assay for plant defense enzyme was performed at the end of 4 weeks of seed sowing. After 35 days after sowing (DAS), plant samples were cleanly uprooted from the pots and immediately taken to the laboratory for different biometric measurements. The pot bioassay experiment was repeated twice with 5 replications each time.

3.5.1: Determination of Plant Biometric Attributes in Beans

3.5.1.1. Germination Rate

The rate of germination was observed by counting the number of the seeds germinated against the total number of seeds planted in all treatments after 7 days of sowing (DAS)

3.5.1.2. Root Mortality

Pathogen-induced mortality in beans roots in the presence and absence of seed inoculated biocontrol agents was estimated. Mortality caused in seedlings' roots due to soil inoculation of the wilt pathogen *F. solani* and *R. solani* were estimated using a modified method described by Dukare & Paul, (2021). Root mortality was expressed as the percentage of dead roots dry weight (D.W) of the total dry weight of roots.

3.5.1.3. Pre-Emergence and Post-Emergence Wilt Incidence

The Pre- and post-emergence wilt disease severity indices were calculated by counting the number of germinating seeds and surviving seedlings (those seedlings which were not showing any symptoms of wilt disease such as brownish lesions/premature drooping of leaves/partial or complete wilting of part or whole seedlings) among those germinated

as described by (Gossen et al. 2016). A percentage of disease incidences was calculated based on visible wilt symptoms observed on the plant after 15 days up to 35 DAS.

3.5.1.4. Shoot /Root Length and Biomass

The effects of seed inoculation with bacteria on plant elongation parameters were studied as shoot and root length. Additionally, plant biomass such as fresh shoot weight and fresh root weight were studied. After 35 DAS, fresh shoot/roots length (cm), shoot fresh weight, and fresh root weight (g) were measured using weighing machine and ruler. A total of five plants from each treatment was considered.

3.5.2. Plant Defense Enzymes and Phenolic

3.5.2.1. Preparation of Enzyme Extracts

From each treatment, 1 g of 4-week-old leaf samples were homogenized in 1.5 ml of 50 mM Tris HCl buffer (pH 7.5) at 4°C in liquid nitrogen and centrifuged at 18,000 rpm for 20 min at 4°C. The resulting supernatant was collected in sterilized 2 ml Eppendorf tubes and stored in a deep freezer (− 20°C) for further use as crude enzyme extract. This enzyme extract was used for the assay of phenylalanine ammonia-lyase (PAL), polyphenol oxidase (PPO), and peroxidase (PO).

3.5.2.2 Phenylalanine Ammonia-lyase (PAL), Polyphenol Oxidase (PPO), and Peroxidase (PO) Assays

Phenylalanine ammonia-lyase (PAL), activity was estimated based on the production of trans-cinnamic acid (Sundaramoorthy et al. 2012). Enzyme activity was expressed as μg cinnamic acid $hr^{-1}g^{-1}$ fresh plant weight. PPO activity was assayed by measuring the change in the color intensity of catechol oxidation products (Mayer et al. 1965). PPO enzyme activity was expressed as a change in absorbance at 495 nm per $min^{-1}g^{-1}$ of fresh plant weight. For PO assay, 0.5 ml crude enzyme extract was taken in a cuvette, and subsequently, 0.5 ml of 1% guaiacol solution and 1.5 ml 50 mM Tris buffer (pH 7.5) was added. Then, the reaction was initiated by adding 0.5 ml of 1% H_2O_2, and change in absorbance at 470 nm was recorded at an interval of 30 secs for 3 min. The unit of

peroxidase enzyme activity was expressed as the change in absorbance $min^{-1}g^{-1}$ of fresh weight (Hammerschmidt, Nuckles, and Kuć 1982).

3.5.2.3 Total Phenolic Assay

The total phenolic content in fresh leaf and root tissue was analyzed using the Folin-Ciocalteu colorimetric method (Zieslin, Biochemistry, and 1993 1993). The OD of the developed blue color was measured at 725 nm. The phenolic content in plant tissue was expressed as μg $catechol^{-1}g^{-1}$ fresh plant weight.

3.6 Data Analysis

Antifungal activity enzymatic assay and plant biomass were subjected to a one-way analysis of variance (ANOVA) using SAS version 8.0 software. For physiochemical data, RStudio version-2022.12.0-353 was used for animated heatmaps. For greenhouse data, GraphPad-Prism version 6.0 was used to present the data in graph version.

CHAPTER FOUR

RESULTS

4.1 Physiological Description of the Lakes

From sampling points, water, sediment, and soil samples were collected from seven points of L. Bogoria and sixteen points of L. Magadi (**Error! Reference source not found.**;

Table **4.2**). Different physical parameters during sampling from both lakes, such as pH, Temperature, conductivity, salt, and Total Dissolved Solids (TDS), were determined at every sampling point (**Error! Reference source not found.**). The elevation of the two lakes varied in that L. Bogoria is a highland lake with an elevation range of 1004M-1010M while L. Magadi is a lowland lake with an elevation range of 605 M-618 M (**Error! Reference source not found.**; Table 4.2). From the physical parameters measured during sampling, L. Magadi recorded the highest pH (8.7-10.6), conductivity (433 µS/cm-49.6 mS/cm), salt concentration (12.2 ppt-46.7 ppt) and TDS (19.0 ppt-31.2 ppt) compared to L. Bogoria. Additionally, L. Bogoria recorded the highest temperature (32.0°C-90.0°C) compared to L. Magadi.

Table 4.1: Types of Samples Collected from Seven Points of L. Bogoria with their Description.

Sampling point	Location & elevation	Sample Type	pH	Temp	Conductivity	Salt	TDS
BP01	N10°43.692 E058°25.621 N. A	Water & Sediment	9.6	69.0	6.0 mS/cm	2.9 ppt	28.6 ppt
BP02	N00°13.730 E036°05.572 1009M	Water, Sediment &Soil	9.2	45.0	25.6 mS/cm	12.1 ppt	17.0 ppt
BP03	N00°13.711 E036°05.575 1010M	Soil	9.6	75.0	100.0 μS/cm	N. A	N. A
BP04	N00°13.716 E036°05.577 1008M	Water, Sediment &Soil	8.8	70.0	6.1 mS/cm	2.9 ppt	3.9 ppt
BP05	N00°13.724 E036°05.584 1007M	Water, Sediment &Soil	8.7	68.0	5.9 mS/cm	3.1 ppt	4.1 ppt
BP06	N00°13.673	Water, Sediment	8.9	90.0	N. A	2.9	3.8

Sampling point	Location & elevation	Sample Type	pH	Temp	Conductivity	Salt	TDS
	E036°05.603 1007M	&Soil				ppt	ppt
BP07	N00°14.917 E036°05.085 1004M	Water, Sediment &Soil	8.2	32.0	26.1 mS/cm	13.0 ppt	17.2 ppt

Table 4.2: Types of Samples Collected from Seven Points of L. Magadi with their Description

Sampling Point	Location & Elevation	Sample Type	pH	Temp	Conductivity	Salt	TDS
MP01	S02°00.069 E036°13.925 606M	Soil	9.51	67.5	433 µS/cm	21.2 ppt	28.6 ppt
MP02	S02°00.037 E036°13.724 605M	Soil	10.3	55.0	21.4 mS/cm	23.1 ppt	27.6 ppt
MP03	S02°00.024 E036°13.725	Soil	9.2	30.1	12.4 mS/cm	20.1 ppt	29.2 ppt

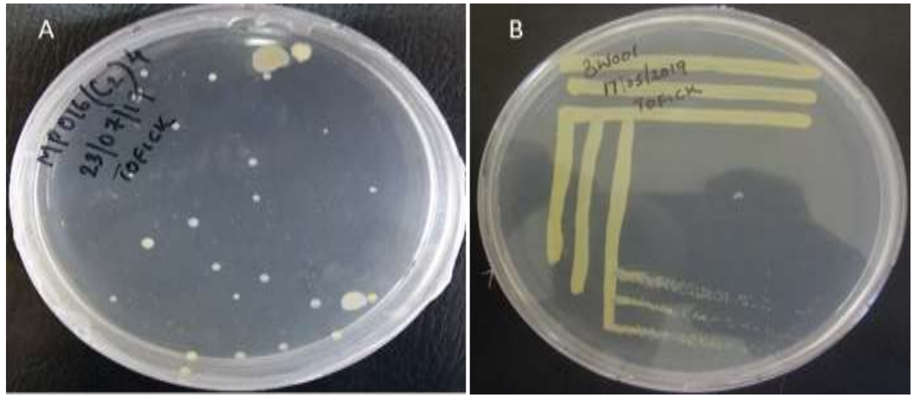
Sampling Point	Location & Elevation	Sample Type	pH	Temp	Conductivity	Salt	TDS
	605M						
MP04	S01°59.975 E036°13.748 608M	Soil	10.4	27.8	20.3 mS/cm	12.2 ppt	23.7 ppt
MP05	S01°59.925 E036°13.763 607M	Soil & sediment	9.2	23.4	46.8 mS/cm	24.1 ppt	21.9 ppt
MP06	S02°00.091 E036°13.714 609M	Soil & sediment	9.1	25.7	34.1 mS/cm	19.4 ppt	19.0 ppt
MP07	S02°00.144 E036°13.704 607M	Soil	10.6	28.9	23.7 mS/cm	22.2 ppt	29.3 ppt
MP08S	S01°42.985 E036°16.277 618M	Water, Soil & sediment	9.7	30.8	30.5 mS/cm	15.5 ppt	20.3 ppt
MP09S	S01°43.128	Water, Soil &	9.4	28.6	46.4 mS/cm	21.2 ppt	30.2 ppt

Sampling Point	Location & Elevation	Sample Type	pH	Temp	Conductivity	Salt	TDS
	E036°16.263 618M	sediment					
MP010S	S01°43.139 E036°16.265 616M	Water, Soil & sediment	9.1	38.7	40.5 mS/cm	20.1 ppt	26.1 ppt
MP011S	S01°43.145 E036°16.266 615M	Water, Soil & sediment	8.9	66.8	43.0 mS/cm	21.4 ppt	28.4 ppt
MP012S	S01°43.198 E036°16.253 614M	Water, Soil & sediment	8.9	74.3	45.3 mS/cm	23.1 ppt	29.5 ppt
MP013S	S01°43.248 E036°16.235 617M	Water, Soil & sediment	9.2	32.4	36.3 mS/cm	18.5 ppt	24.4 ppt
MP014S	S01°43.311 E036°16.228 612M	Water, Soil & sediment	9.2	77.8	46.5 mS/cm	46.7 ppt	30.8 ppt

Sampling Point	Location & Elevation	Sample Type	pH	Temp	Conductivity	Salt	TDS
MP015S	S01°43.304 E036°16.279 613M	Water, Soil & sediment	8.7	69.1	43.6 mS/cm	22.0 ppt	29.1 ppt
MP016S	S01°43.359 E036°16.284 615M	Water, Soil & sediment	9.2	28.6	49.6 mS/cm	23.5 ppt	31.2 ppt

4.1.2 Bacterial Isolation

Inoculum samples cultured on modified Nutrient Agar (N.A) media and incubated at 39.5°C showed different colonies (morphologically) after 48hours (Plates 4.1). The colonies were purified on the same isolation media and observed 24 hours (Plates 4.1).



Plates 4.1: A) Modified Nutrient Agar Media Culture with Different Colonies; B) Nutrient Agar Media Culture with Pure Bacteria Isolates.

A total of forty-nine (49) bacterial isolates were obtained from Lake Bogoria. A total of twenty-two isolates (45.0%) were from the soil, seven (14.0%) from sediment, and four (8.0%) from water. Additionally, three isolates (6.0%) were obtained from both water and soil, eight isolates (16.0%) from both water and sediment, two isolates (4.0%) from sediment and soil, and three isolates (6.0%) from all samples type (Water, sediment, and soil) (Figure 4.8).

A total of 61 bacterial isolates were obtained from Lake Magadi. Twenty-five (41.0%) were obtained from soil, eight from sediment (13.0%) and three from water (5.0%). Additionally, ten isolates (16.0%) were obtained from sediment and soil, seven isolates (11.0%) from sediment and water, three isolates (5.0%) from water and soil, and five isolates (8.0%) from all the sample types (Water, sediment, and soil) (Figure 4.8).

A comparison between the two lakes showed that, the soil sample had the highest microbial composition (41.0-45.0%), and the water sample had the lowest (5-8.0%). There were isolates obtained from both water and sediment samples (11.0-16.0%). Lastly, other isolates were obtained from all the sample types.

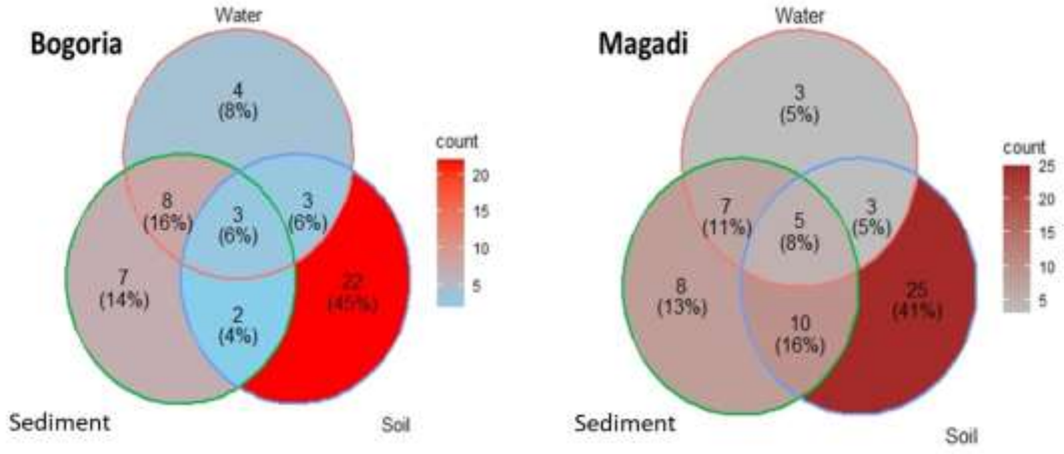
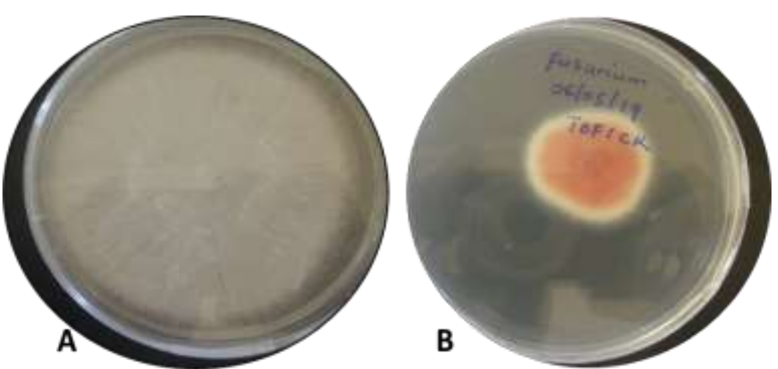


Figure 4.8: Venn Diagram Showing the Distribution of Isolated Bacteria from Lakes Bogoria and Magadi. The Shared OTUS within Various Sample Types. OUT- Operational Taxonomic Unit

4.1.2.1 Pathogen Preparation and Pathogenicity Test

Inoculated plates of *Fusarium solani* and *Rhizoctonia solani* on PDA at 28°C were observed after 5 days of incubation (Plate 4.2)



Plates 4.2: PDA plates showing; (A) Rhizoctonia solani and (B) Fusarium solani.

4.1.3 In vitro Pathogenicity of *F. solani* and *R. solani* on Common Bean Plantlets.

In pathogenicity test, the plantlets showed narrow root and red-brown lesion on the hypocotyl for *F. solani* and *R. solani* compared to control. Lesions extend down to the central taproot, which caused the wilting and death of the plantlet for both pathogens tested. However, there was no incidence of wilting observed in control despite the reported browning of the adventurous root.

Figure 4.9: Effect of *F. Solani* and *R. Solani* on Bean Plantlets. The Experiment was Carried Out in A Growth Chamber for 14 Days.

A) Growth Chamber, B) Negative Control (No Pathogen-Infected), C) Infected with *F. Solani* and D) Infected with *R. Solani.*

The severity was recorded after 14 days of incubation in the growth chamber, whereby control had the zero disease index (0.00%) compared to *F. solani* (43.75%) and *R. solani* (37.50%). For germination rate, control had the highest rate (94.00%) compared to *F. solani* (75.00%) and *R. solani* (63.00%). A comparison between *Fusarium* root rot and *Rhizoctonia* root rot showed that, *F. solani* had the highest severity (43.75%) compared to *R. solani* (37.50%) (Table 4.3).

Table 4.3: Comparative Effects of *F. Solani* and *R. Solani* on Bean Plantlets Observed 14 Days of Inoculation.

Treatments	Germination rate (%)	Plant Height (cm)	Severity (dead) (%)	Root Length (cm)	Roots fresh weight(g)	Shoot fresh weight (g)
Control	94.00%	34.28±1.98[a]	0.00%	10.08±1.89	0.33±0.13[a]	1.85±0.43
F. solani	75.00%	18.88±4.80[b]	43.75%	3.80±0.55[b]	0.26±0.06[a]	0.91±0.33
R. solani	63.00%	18.28±2.75[b]	37.50%	4.28±0.57[b]	0.24±0.07[a]	0.96±0.29
F-Value	0.00	31.82	0.00	43.73	76.46	64.49
LSD	0.00	7.16	0.00	8.72	0.28	2.19
P>	0.00	10.44	0.00	3.65	0.29	1.10
CV	0.00	0.009	0.00	0.005	0.761	0.155

*Means in a column followed by the same letter do not significantly differ according to the Fisher's LSD test (P<0.05).

CV- Co-effience of variance, F-Value-F test; LSD- Least Significant Difference

Plant height differed significantly (P<0.05) between treatments tested with the control plant having the highest length (34.28±1.98 cm), compared to *F. solani* (18.88±4.80 cm) and *R. solani* (18.28±2.75 cm). However, there was a no significant difference between *F. solani* and *R. solani* in plant height (Table 4.3).

Beans root length indicates that control differed significantly from the treatments tested. Control had the highest root length (10.08±1.89 cm) compared to *F. solani* (3.80±0.55 cm), and *R. solani* (4.28±0.57 cm). Root fresh weight was recorded after 14 days of inoculation. The root fresh weight did not differ significantly ($P<0.05$) between the treatments (Table 4.3). However, control had highest weight (0.33±0.13 g) *F. solani* (0.26±0.06 g) and *R. solani* (0.24±0.07 g). In fresh shoot weight, it is indicated that control does not differ significantly among the treatments tested. The control plant however, had the highest weight (1.85±0.43 g) compared to *F. solani* (0.91±0.33 g), and *R. solani* (0.96±0.29 g) treatments.

4.1.4 Antibiosis of Bacterial Isolates against Selected Test Pathogen

Analysis of bacterial antibiosis assays by use of disc diffusion technique and co-culturing technique against *F. solani* and *R. solani* revealed that some isolates from L. Bogoria and L. Magadi exhibited positive inhibition of mycelium growth and clear zone of inhibition of the selected test pathogen (Plate 4.3)

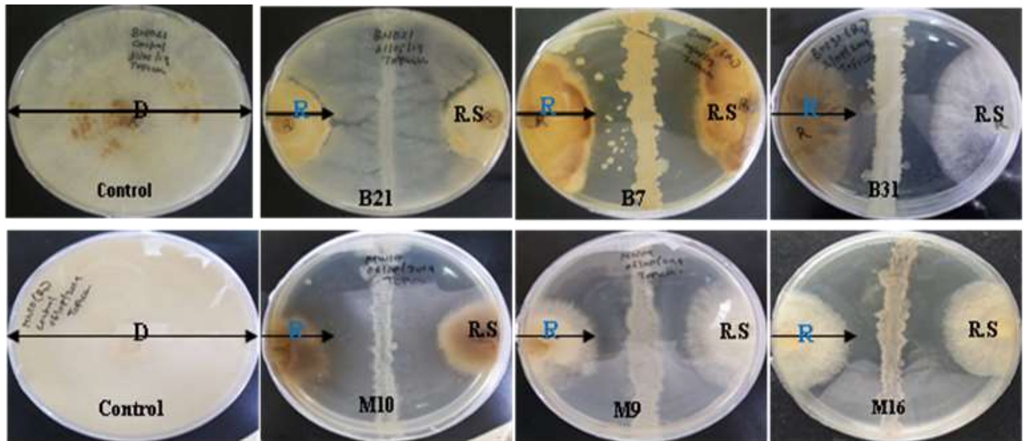


Plate 4.3: Antibiosis Assay of Lakes Bogoria and Magadi Bacterial Isolates against Rhizoctonia Solani after 7 Days of Incubation at 28°C±2.0 using the Co-Culturing Technique.

(D) Diameter of pathogen mycelium in un-inoculated control, **(R)** Radius of pathogen mycelium co-cultured with bacterial isolate, **(R.S)** *R. solani* (Pathogen), **(B)** Bogoria isolate, and **(M)** Magadi isolate.

Table 4.4: Antifungal Activity of Lakes Bogoria & Magadi Bacterial Isolates on Mycelium Growth 0f *R. Solani* after 14th Days

Site	Isolate code	Mycelium length (cm)	% Inhibition rate
	Control	8.4 ± 0.00^{a}	0.00
	B35	7.77 ± 0.33^{b}	7.54
	B4	7.13 ± 0.09^{c}	15.08
Lake Bogoria	B23	6.83 ± 0.15^{d}	18.65
	B39	6.63 ± 0.12^{d}	21.43
	B38	6.40 ± 0.21^{e}	23.81
	B30	6.37 ± 0.23^{e}	24.21
	B31	6.00 ± 0.12^{f}	28.57
	B20	5.90 ± 0.15^{f}	30.95
	B07	5.50 ± 0.10^{g}	34.52
	B21	5.20 ± 0.10^{h}	38.10
CV		1.71	
F-Value		124.72	
LSD		0.22	

Site	Isolate code	Mycelium length (cm)	% Inhibition rate
P>		0.0001	
	Control	8.40±0.00^{a}	0.00
	M24	7.90±0.26^{b}	5.95
	M17	7.43±0.97^{b}	11.55
	M33	6.90±0.06^{c}	17.86
	M16	6.83±0.78^{c}	18.69
	M43	6.80±0.17^{c}	19.05
	M21	6.73±0.20^{c}	19.84
Lake Magadi	M32	6.63±0.17^{cd}	21.03
	M50	6.60±0.15^{cd}	21.43
	M37	6.50±0.26^{cde}	22.62
	M52	6.50±0.17^{cde}	22.62
	M11	6.47±0.26^{cde}	23.02
	M58	6.23±0.12^{de}	25.79
	M60	6.10±0.15^{e}	27.38
	M47	5.30±0.06^{f}	36.90
	M09	5.03±0.14^{fg}	40.08
	M10	4.80±0.06^{g}	42.86

Site	Isolate code	Mycelium length (cm)	% Inhibition rate
CV		3.92	
F-Value		28.93	
LSD		0.50	
P>		0.0001	

*Means in a column followed by the same letter do not significantly differ according to the Fisher's LSD test ($P<0.05$)

CV- Coeffience of variance, F-Value-F test; LSD- Least Significant Difference

The diameter of the *R. solani* colony noted after 14 days of incubation at 30°C varied significantly *(P<0.05)* depending on the antagonistic bacteria tested. Ten (10) out of forty-nine (49) bacteria isolated from L. Bogoria and sixteen (16) out of sixty-one (61) bacteria isolated from L. Magadi reduced *R. solani* radial growth using co-culturing techniques. Isolate B21 (38.10%) isolated from L. Bogoria had the highest percentage inhibition rate, whereas isolate B35 (7.54%) had the lowest percentage inhibition rate (

Table 4.4).

4.1.5 Antifungal Activity of L. Bogoria and L. Magadi against *Fusarium solani*

The antifungal activity of isolates from lakes Bogoria and Magadi revealed varied mycelium length of *Fusarium solani*. Isolate B21 had the lowest mycelium length compared to B23, B7 and control. Additionally, isolates M16, M17 and M18 had varied mycelium length of F. solani compared to control

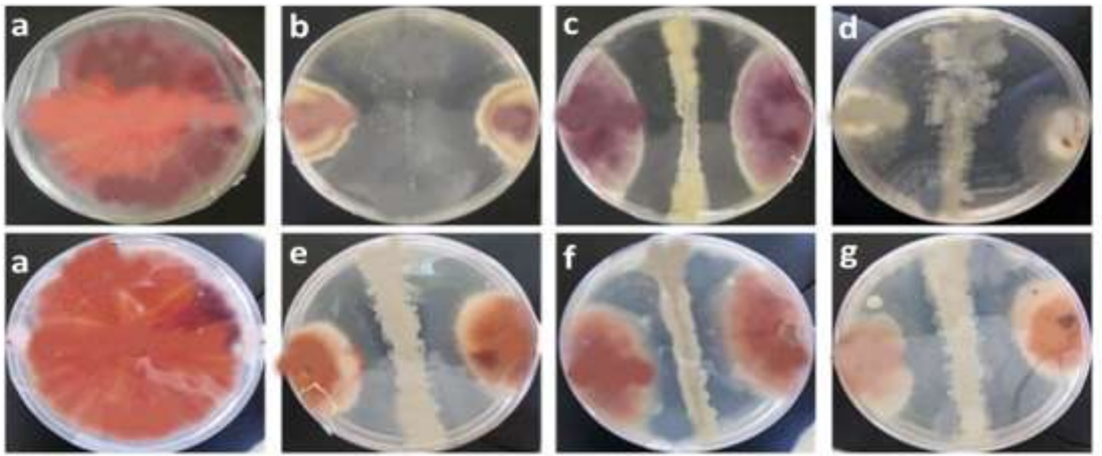


Plate 4.4: Antibiosis Assay of Lakes Bogoria and Magadi Bacterial Isolates against Fusarium Solani after 14 Days of Incubation at 30°C±2.0 using the Co-Culturing Technique. A) Control B) Isolate B21, C) Isolate B23, D) Isolate B7, E) Isolate M16, F) Isolate M08 and G) Isolate M17.

In Table 4.5, ANOVA analysis revealed significant *($P<0.05$)* variation in the *F. solani* mycelium diameter upon bacterial treatment tested. Thirteen (13) out of forty-nine (49) bacteria isolated from L. Bogoria and twelve (12) out of sixty-one (61) bacterial isolated from L. Magadi had antagonistic effects against *F. solani*. Isolate B39 (59.13%) had the highest inhibition rate, while B27 (14.68%) was the least (Table 4.5). For L. Magadi, M16 (30.56%) had the highest percentage inhibition rate, while M45 (9.92%) had the lowest.

Table 4.5: Antifungal Activity of Bogoria and Magadi Bacterial Isolates on Mycelium Growth of *Fusarium Solani* after 14 Days.

Lakes	Isolate code	Mycelium length (cm)	% Inhibition rate
Bogoria	Control	$8.4±0.00^{a}$	0.00
	B27	$7.17±0.09^{b}$	14.68

Lakes	**Isolate code**	**Mycelium length (cm)**	**% Inhibition rate**
	B15	6.73±0.09[c]	19.84
	B31	6.27±0.14[d]	25.40
	B19	6.03±0.14[de]	28.17
	B23	6.00±0.55[de]	28.57
	B38	6.00±0.06[de]	28.57
	B32	5.87±0.26[ef]	30.16
	B30	5.60±0.11[f]	33.33
	B29	5.23±0.23[g]	37.70
	B7	5.20±0.23[g]	38.10
	B26	4.67±0.20[h]	44.44
	B21	4.17±0.09[i]	50.40
	B39	3.43±0.17[j]	59.13
CV		2.54	
F-Value		145.59	
LSD		0.31	
P>		0.0001	
Magadi	Control	8.4±0.00[a]	0.00
	M45	7.57±0.24[b]	9.92

Lakes	Isolate code	Mycelium length (cm)	% Inhibition rate
	M6	7.27 ± 0.15^{c}	13.49
	M25	6.80 ± 0.06^{d}	19.05
	M50	6.80 ± 0.06^{d}	19.05
	M60	6.77 ± 0.09^{d}	19.44
	M20	6.47 ± 0.29^{e}	23.02
	M18	6.40 ± 0.21^{ef}	23.81
	M5	6.20 ± 0.23^{fg}	26.19
	M17	6.03 ± 0.30^{gh}	28.17
	M08	6.00 ± 0.36^{gh}	28.57
	M10	5.97 ± 0.03^{gh}	28.97
	M16	5.83 ± 0.09^{h}	30.56
CV		1.94	
F-Value		76.88	
LSD		0.25	
P>		0.0001	

*Means in a column followed by the same letter do not significantly differ according to the Fisher's LSD test (P<0.05)

CV- Coeffience of variance, F-Value-F test; LSD- Least Significant Difference

4.1.6 Comparison of Bacteria Isolates for Biocontrol of *Rhizoctonia solani* and *Fusarium solani* from L. Bogoria

From the forty-nine (49) isolated bacteria from L. Bogoria, seventeen (17) bacterial isolates inhibited the mycelium growth of both *Rhizoctonia solani* and *Fusarium solani*. Four inhibited *R. solani*, and six (6) isolates were able to inhibit *Fusarium solani*. Additionally, seven (7) isolates were able to inhibit both *R. solani* and *F. solani*. In terms of sample types, for *Fusarium*, most of the bioactive isolates were obtained from soil (62%) compared to water (0%) and sediment (8%). For *Rhizoctonia*, most of the bioactive bacteria isolates were obtained from soil samples (73%) in comparison to water (0%) and sediment (0%). Additionally, there were bacterial isolates that were obtained from water and sediment (18%) and water and soil (9%) (Figure 4.1010).

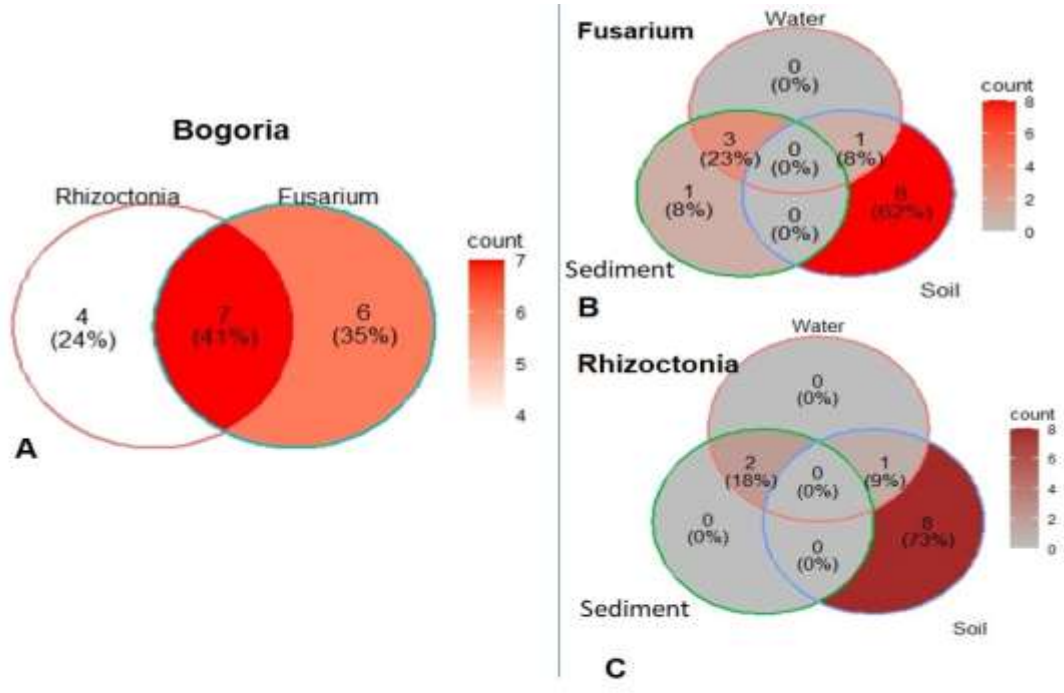


Figure 4.10: Venn Diagram Showing the Distribution of Bioactive Bacterial Isolates from L. Bogoria and Shared OTUS within Various Sample Types. OUT-Operational Taxonomic Unit.

(A) Distribution of bacteria isolates with antagonistic potential against *R. solani* and *F. solani*; (B) distribution of bacteria isolates against *R. solani*; (C) Distribution of bioactive isolates against *F. solani*.

4.1.7 Comparison of Bacteria Isolates for Biocontrol of *Rhizoctonia solani* and *Fusarium solani* from L. Magadi

From the sixty-one (61) isolated bacteria from L. Magadi, twenty-five (25) bacterial isolates could inhibit the mycelium growth of both *R. solani* and *F. solani*. Twelve (12) isolates could inhibit *R. solani*, and ten (10) isolates could inhibit *F. solani* (Figure 4.11). Additionally, three (3) isolates inhibited both *R. solani* and *F. solani*. In terms of sample types, most of the bioactive isolates were obtained from soil (54%) compared to water (0%) and sediment (0%). For *Rhizoctonia*, most of the bioactive bacteria isolates were obtained from soil samples (38%) in comparison to water (0%) and sediment (12%). Additionally, there were bioactive bacterial isolates which were obtained from water and sediment (6%); water and soil (6%); sediment and soil (19%) and water, soil, and sediment (19%) (Figure 4.11).

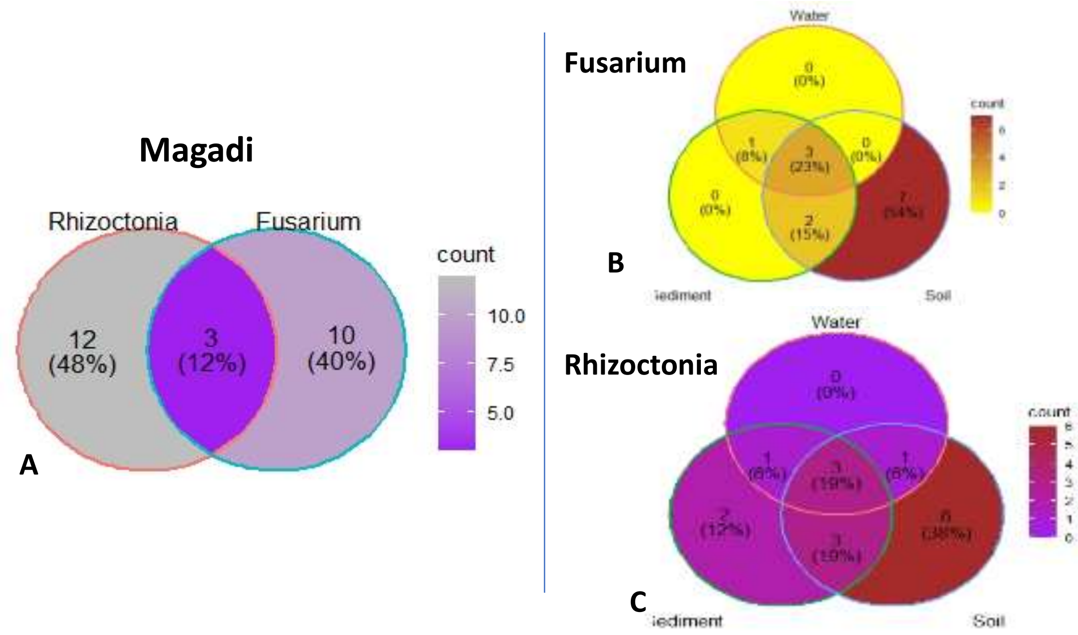


Figure 4.11: Venn Diagram Showing the Distribution of Bioactive Bacterial Isolates from L. Bogoria and Shared OTUS within Various Sample Types. OUT-Operational Taxonomic Unit

(A) Distribution of bacteria isolates with antagonistic potential against *R. solani* and *F. solani*; (B) distribution of bacteria isolates against *R. solani*; (C) Distribution of bioactive isolates against *F. solani.*

4.1.8 Disc Diffusion Results

The antifungal activity of isolates from lakes Bogoria and Magadi revealed varied mycelium length of *Fusarium solani* (Plate 4.5). The zone of inhibition varied across the isolates with Isolate MW60 had highest zone of inhibition compared to BW30 and MW16 (Plate 4.5). Additionally, isolates Mw16 and BW30 showed lowest zone of inhibition (Plate 4.5).

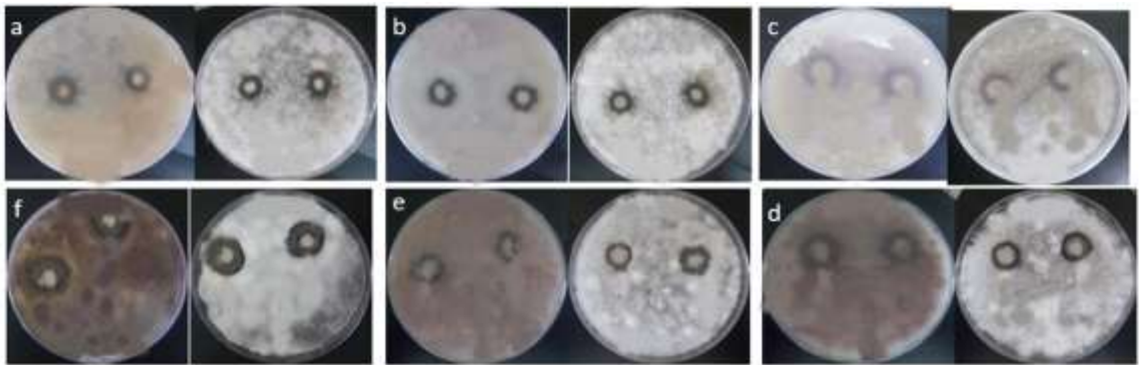


Plate 5.5: Antifungal Activity of L. Bogoria and L. Magadi Bacterial Isolates against Fusarium using Disc Diffusion Techniques (A) BW11, (B) BW17, (C) B30, (D) MW16, (E) BW21 And (F) MW60.

Table 4.6: *In Vitro* Effect of Bogoria & Magadi Bacterial Isolates against *Fusarium Solani* using Disc Diffusion after Seven Days of Incubation.

Lakes	Isolate code	Zone of Inhibition (cm)
Bogoria	Control	0.00 ± 0.00^{g}
	B21	1.93 ± 0.07^{a}
	B7	1.83 ± 0.09^{ab}
	B38	1.77 ± 0.15^{bc}
	B11	1.70 ± 0.06^{cd}
	B29	1.67 ± 0.07^{cd}
	B30	1.60 ± 0.06^{de}
	B17	1.53 ± 0.12^{e}
	B12	1.13 ± 0.09^{f}
	B19	1.07 ± 0.03^{f}
CV		22.40
F-Value		288.60
LSD		0.10
P>		0.05
Magadi	Control	0.00 ± 0.00^{d}
	M60	2.07 ± 0.26^{a}

Lakes	Isolate code	Zone of Inhibition (cm)
	M60	1.93±0.39[a]
	M16	1.43±0.13[b]
	M23	1.00±0.17[c]
	M21	0.96±0.14[c]
CV		46.77
F-Value		175.29
LSD		0.09
P>		0.0001

*Means in a column followed by the same letter do not significantly differ according to the Fisher's LSD test (P<0.001)

CV- Coeffience of variance, F-Value-F test; LSD- Least Significant Difference

ANOVA analysis from Table 4.6 revealed significant *(P<0.05)* variation in the zone of inhibition upon the bacterial treatment tested. L. Bogoria had nine isolates while L. Magadi had five isolates that inhibited the growth of *F. solani* by use of disc-diffusion. Isolate M60 (2.07±0.26 cm) had the overall highest diameter of the zone of inhibition, while M21 (0.96±0.14 cm) had the lowest inhibition diameter. Therefore, these results indicate that the positive bacterial isolates produce diffusible bioactive substances responsible for the clear zone of inhibition (Table 4.6).

4.2 Characterization of Bioactive Bacterial Isolate against *F. solani* and *R. solani*.

4.2.1 Morphological Characterization of Bioactive Isolates.

Morphological characterization was done using microscopic techniques whereby colony and cell morphology were determined. Some of the colony morphology characteristics included; circular, irregular, and penctiform; elevation whereby 63.15% of the isolates had raised elevation, and 36.85% were flat (Table 4.7) Margin differences were also observed, ranging from circular, irregular, wavy, lobate, smooth, and filamentous. In terms of size, 36.84 % were small, 57.89% were medium, and 5.26% were large. The color of the isolates ranged from white, cream, cream-white, teel, blue, and cream-yellow.

Additionally, the surface of the isolates ranged from smooth, dull, and rough. The opacity of the colonies showed that 89.47% were opaque, and 10.53% were transparent. The cell morphology of the pure isolates ranged from rods to cocci. The Gram staining technique showed that 31.58% were Gram-positive and 62.42% were gram-negative (Table 4.7).

Table 4.7: Morphological Characteristics of Bioactive Bacterial Isolates

Isolates	Form	Elevation	Margin	Size	Surface	Shape	G-stain
B7	Circular	Raised	Circular	Medium	Smooth	Rod	Positive
B11	Circular	Raised	Irregular	Small	Dull	Rod	Positive
B12	Circular	Flat	Circular	Small	Smooth	Rod	Negative
B17	Circular	Raised	Circular	Small	Smooth	Rod	Negative
B19	Irregular	Raised	Circular	Small	Smooth	Rod	Negative
B20	Penctiform	Raised	Wavy	Medium	Smooth	Rod	Negative
B21	Irregular	Flat	Lobate	Medium	Rough	Rod	Positive
B26	Circular	Raised	Circular	Small	Smooth	Rod	Positive
B29	Irregular	Raised	Irregular	Medium	Smooth	Rod	Positive
B30	Irregular	Raised	Circular	Medium	Smooth	Rod	Positive
B32	Irregular	Flat	Filamentous	Large	Dull	Rod	Positive
B38	Circular	Flat	Smooth	Medium	Smooth	Rod	Positive
B39	Circular	Raised	Smooth	Medium	Smooth	Rod	Positive

Isolates	Form	Elevation	Margin	Size	Surface	Shape	G-stain
M9	Circular	Raised	Smooth	Medium	Smooth	Rod	Positive
M10	Irregular	Flat	Circular	Medium	Rough	Rod	Positive
M16	Circular	Flat	Irregular	Medium	Dull	Rod	Positive
M47	Circular	Raised	Smooth	Small	Smooth	Rod	Positive
M50	Penctiform	Raised	Circular	Small	Rough	Rod	Positive
M60	Circular	Flat	Circular	Medium	Smooth	Rod	Positive

4.2.2 Physiochemical Characterization of Bioactive Bacterial Isolates.

4.2.2.1. Growth at Different pH.

Figure **4.12** shows varied growth of bacteria isolates at different pH. Despite the isolates being obtained from an alkaline environment, the bacterial isolates showed growth at pH 5.0 and 7.0. Most isolates grew at alkaline pH (7.0, 8.5, and 10.0), which recorded the largest isolates with optical density (OD) reading above 1.0. The highest OD was recorded at pH 7.0, and the lowest OD was recorded at pH 10.0. The growth trend at pH 5.0, pH 7.0, and pH 10.0 increased significantly. On the other hand, the growth trend at pH 8.5 was uniform (

Figure 4.12).

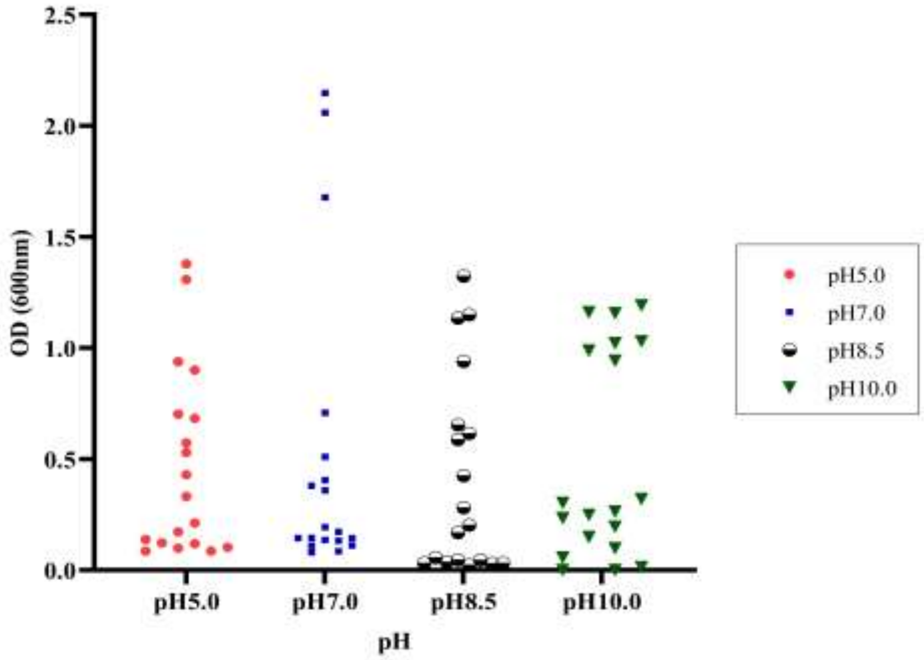


Figure 4.12: The Growth of Bacteria at Different pH values.

Analysis of the relationship among the bacteria isolates with respect to different pH showed four cluster groups of the bioactive isolates (

Figure **4.13**). Additionally, there three cluster groups among the different pH tested. Most of the isolates were able to grow at neutral pH (7.0), followed by pH 10.0, pH 8.5, and the least grow observed at pH5.0. The most acidic isolates were M16, M60, the most alkaline isolate was B39, and the most neutral isolates were M9 and M50. Isolate B7 and B21 did not grow well across the pH. It was also observed that isolate M9 grew well in > pH 7.0 compared to the rest of the isolates (

Figure 4.13).

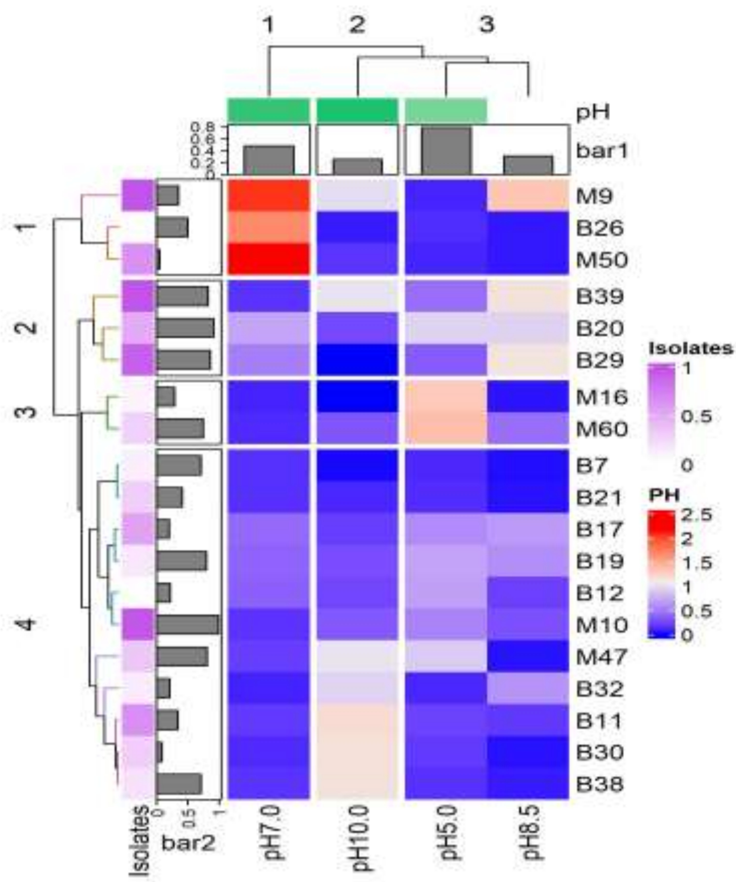


Figure 4.13: Annotated Hierarchical Clustergram of Assayed Bacteria Isolates at Varied pH.

Hierarchical clustergram generated using means of optical density of bioactive bacteria at different pH. The Annotated heatmap (Euclidean matric) shows the relationship between selected bioactive bacteria isolates and varied pH. The colored scale bar indicates the significant quantified strength of the pH.

4.2.2.2 Growth at Different Salinity

Bacteria showed varied growth at different concentrations of sodium chloride concentration (0.0 M-2.0 M). The number of bacteria isolates that grew decreased with an increase in salt concentration. 0.0M recorded the highest OD as an indicator of highest growth followed by 0.5M NaCl and gradually decreased towards 2.0M with

minimal growth. Additionally, the trend of growth decreased with an increase in salt concentration (Figure 4.14).

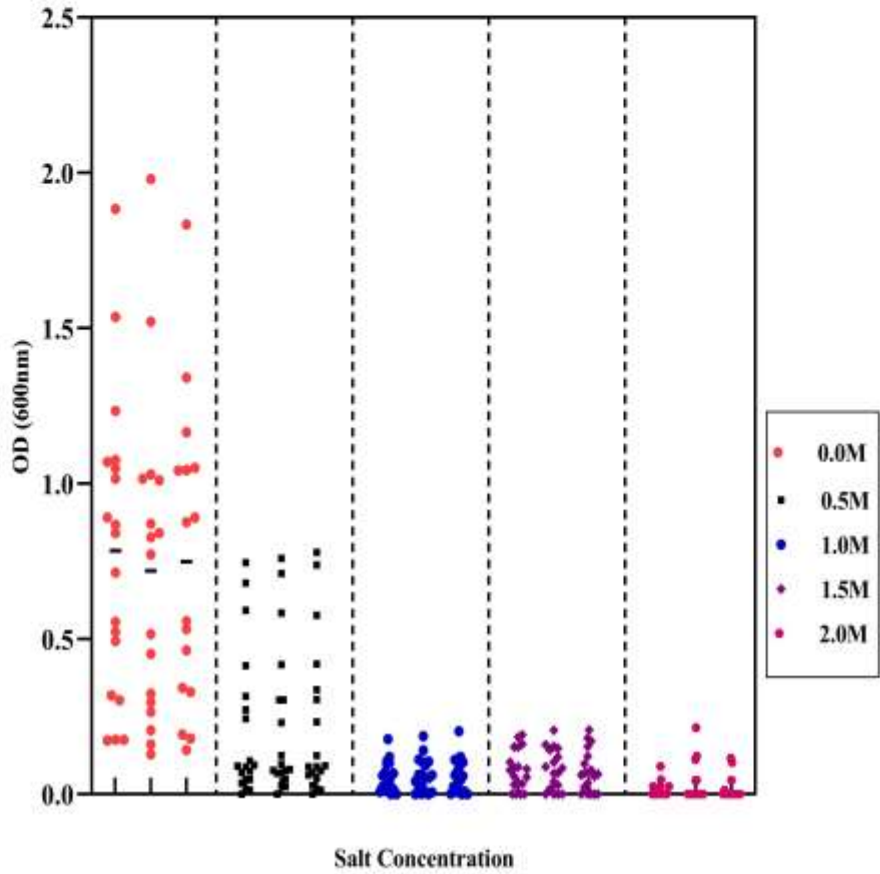


Figure 4.14: The Growth of Bioactive Bacteria at Different Salt Concentrations.

The correlation profile between the salt concentration and the isolates showed four functional cluster groups among the isolates. Additionally, there were four cluster groups (1, 2, 3 and 4) amongst the salt concentrations tested. Most of the bacteria were able to grow in neutral media (No salt concentration). However, other isolates were halophilic; for instance, B30 and B32 could grow at a higher salt concentration of 2.0M (Figure 4.15). Some isolates were halotolerant (grow and multiply in the presence of

high salt but do not require it for growth), such as B39, B21, B32, M47, and M16, though their growth varied at different salt concentrations (Figure 4.15).

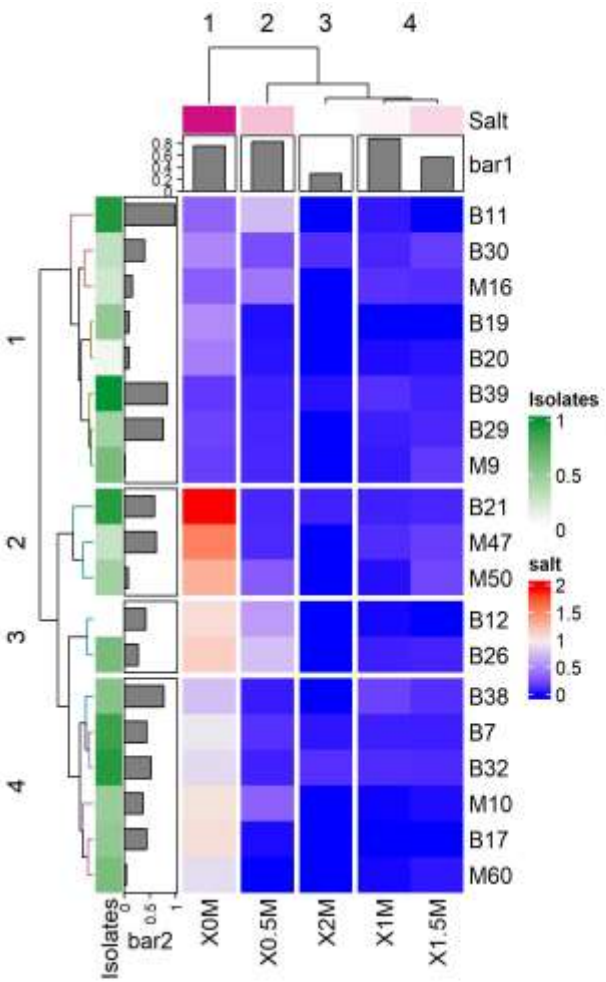


Figure 4.15: Annotated Hierarchical Clustergram of Assayed Bioactive Isolates at Varying Salt Concentrations.

Hierarchical clustergram generated using means of optical density of bioactive bacteria at different salt concentrations. The heatmap (Euclidean matric) shows the relationship between selected bioactive bacteria isolates and varying salt concentrations. The colored scale bar indicates the significant quantified strength of the salt concentration.

4.2.2.3 Growth at Different Temperature

All the bioactive bacterial isolates grew at a wide range of temperatures (20°C-60°C). The optimum growth was observed between 30-45°C (Figure 4.16). However, good growth was observed at 35°C. Some isolates had excellent growth at 60°C (Figure 4.16)

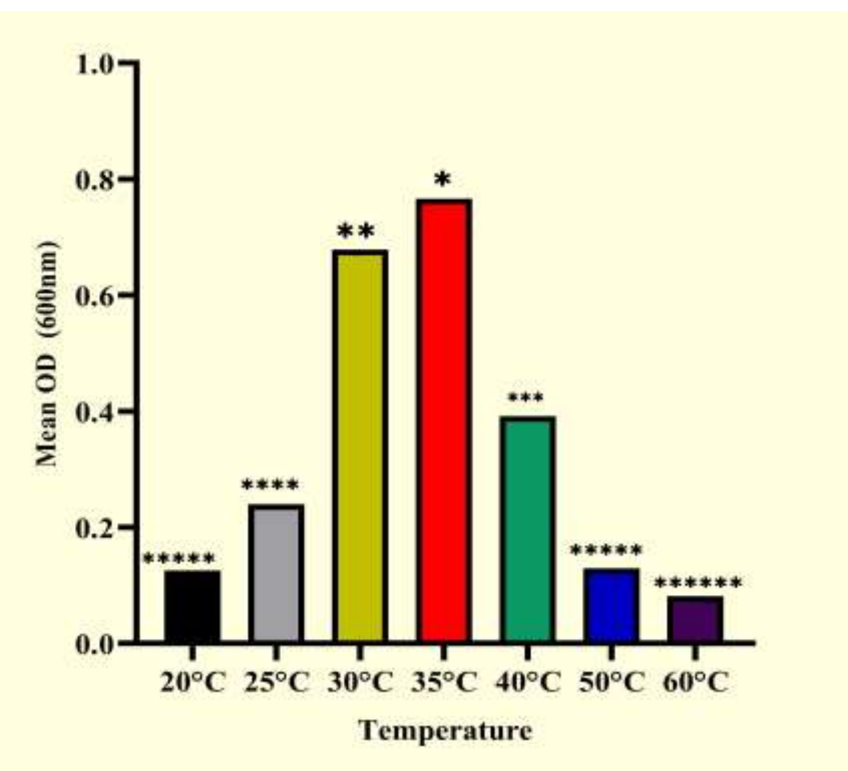


Figure 4.16: Growth of Bacterial Isolates at Different Temperatures

Analysis of the relationship among the bioactive isolates with respect to different temperatures showed five cluster groups of the bioactive isolates. Additionally, there were four cluster groups amongst the different temperatures tested. (Figure 4.17). Most of the isolates were able to grow at 35°C, followed by 30°C, 25°C and least grow observed in both 20°C and 60°C. The most thermophilic isolates were B32 and B30. Mesophilic bacteria at 20°C recorded seven (7) bacteria. M9 grew at a wide range of temperatures (30°C-60°C).

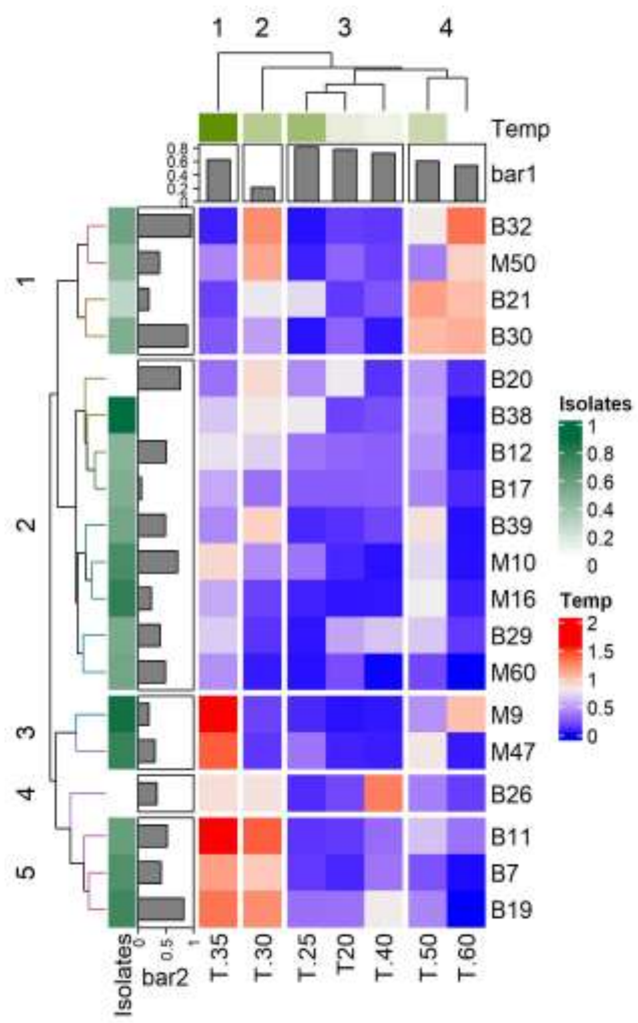


Figure 4.17: Annotated Hierarchical Clustergram of Assayed Bioactive Isolates at Varying Temperatures.

Hierarchical clustergram generated using means of optical density of bioactive bacteria at different salt concentrations. The heatmap (Euclidean matric) shows the relationship between selected bioactive bacteria isolates and varied temperatures. The colored scale bar indicates the significant quantified strength of the temperature.

4.2.3. Enzymatic Bioassays of Bacterial Isolates.

Bioactive bacteria transferred on skim milk agar showed a clear zone of inhibition formed around the isolates, indicating a protease-producing strain. For pectin, the

bioactive isolates were cultured on a pectin agar medium, and the clear zone of inhibition indicated pectinase-producing strains. The isolates were also cultured in a phosphate solubilization media, and the ability of the isolates to release phosphate was indicated by the presence of a clear zone around the isolate. The production of the HCN by the isolates was detected by the ability of the isolates to turn yellow disc paper into a light reddish color

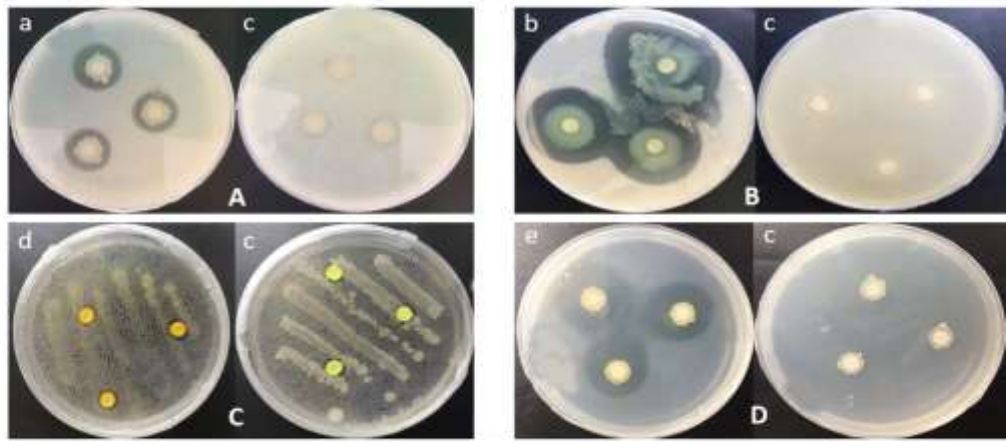


Plate 6.6: Enzymatic Bioassay of Bacterial Isolates from L. Bogoria and L. Magadi against Fusarium Solani and Rhizoctonia Solani.

A) Protease, **B)** Pectinase, **C)** Hydrogen Cyanide, **D)** Phosphatase. **a)** B7, **b)** B121, **c)** Control, **d)** B12 and **e)** B17.

From the results, 14 bacterial isolates formed a clear zone of inhibition around the colonies in an agar medium supplemented with chitin after 72hrs. This indicates that they are chitinase-producing strains. Seventeen bacteria isolates formed a clear zone of inhibition on skim milk agar after 48hrs indicating that they protease-producing strain (Figure 4.18). On pectinase activity, three isolates formed a clear zone of inhibition on medium supplemented with pectinase after 48hrs indicating they are a pectinase-producing strain. B12 and B17 induced a change of filter paper color (light reddish)

compared to control (Yellow); hence, indicating ability to produce Hydrogen cyanide on nutrient agar medium. Fourteen isolates changed the media color to indicate presence of IAA (Table 4.7). Hence, it can produce IAA. Thirteen isolates were able to solubilize the phosphate as indicated by forming a clear zone of inhibition around the colonies (Figure 4.18). Isolate B12, and B17 tested positive for all the enzymatic parameters tested.

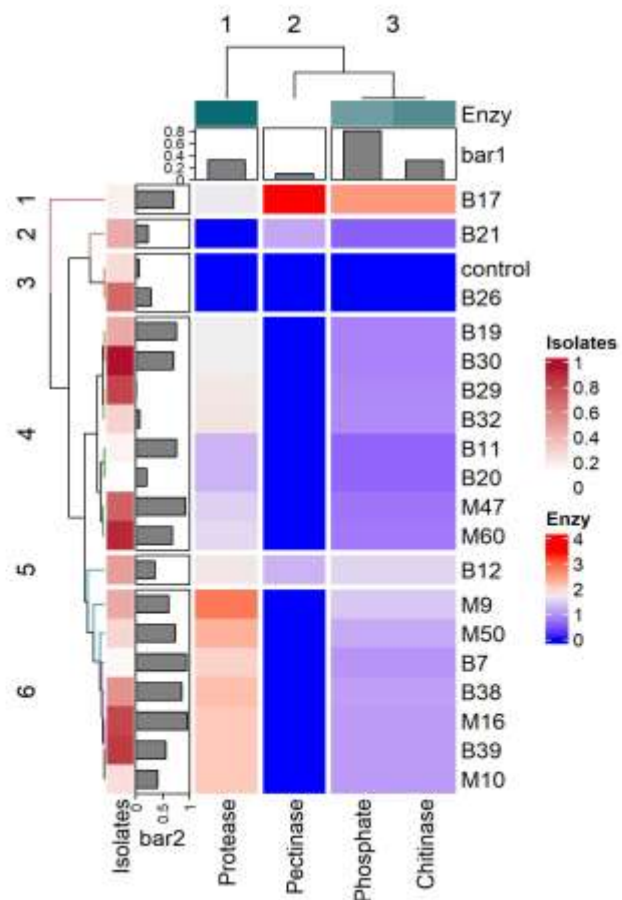


Figure 4.18: Annotated Hierarchical Clustergram of Assayed Bacterial Isolates at Different Enzymes.

Hierarchical clustergram generated using means of optical density of bioactive bacteria at different enzymes. The heatmap (Euclidean matric) shows the relationship between

selected bioactive bacteria isolates and different enzymes. The colored scale bar indicates the significant quantified strength of the enzymes.

Table 4.7: Enzymatic Bioassay of Bioactive Bacteria Isolates rfom L. Bogoria and L. Magadi against *F. Solani* and *R. Solani*.

Isolates	Hydrogen Cyanide Activity	Indole-3-acetic acid
B7	Negative	Negative
B11	Negative	Negative
B12	Negative	Positive
B17	Positive	Positive
B19	Positive	Positive
B20	Negative	Negative
B21	Negative	Positive
B26	Negative	Positive
B29	Negative	Positive
B30	Negative	Positive
B32	Negative	Positive
B38	Negative	Positive
B39	Negative	Positive
M9	Negative	Positive

Isolates	Hydrogen Cyanide Activity	Indole-3-acetic acid
M10	Negative	Positive
M16	Negative	Positive
M47	Negative	Negative
M50	Penctiform	Negative
M60	Circular	Negative

4.2.4 Molecular Characterization of Bacterial Isolates.

4.2.4.1 DNA Extraction.

Genomic DNA was extracted from all the selected 19 bioactive bacterial isolates (Figure 4.19). All of the bands were linear and approximate to the well of the loading.

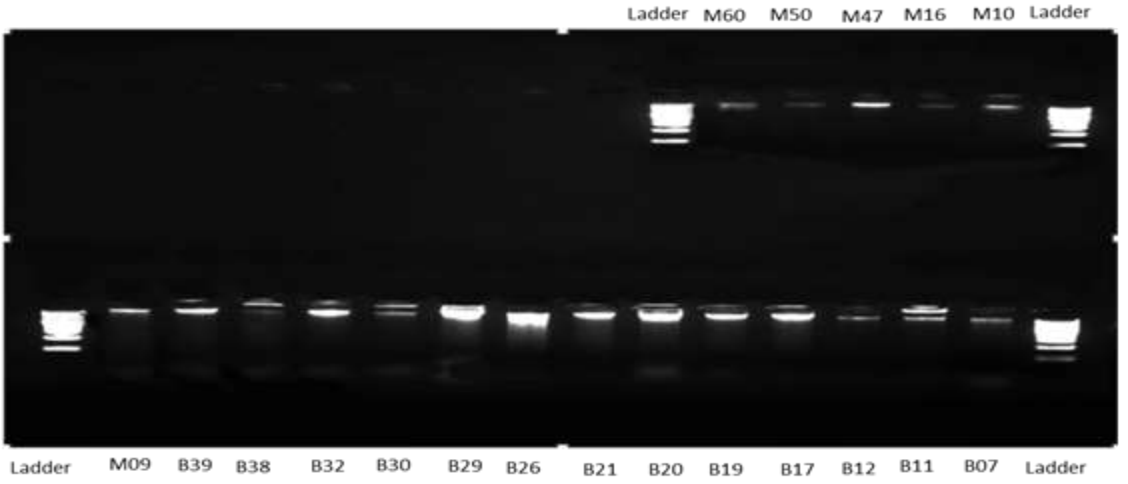


Figure 4.19: Gel Showing Genomic DNA Extracted from Bioactive Bacterial Isolates

4.2.4.2 PCR Amplification of 16s rRNA Genes.

The Genomic DNA extracted from all the 19 bacteria isolates were subjected to PCR by use of 16S rRNA gene amplification with universal bacterial primers yielding the product of approximately 1500bp (Figure 4.20).

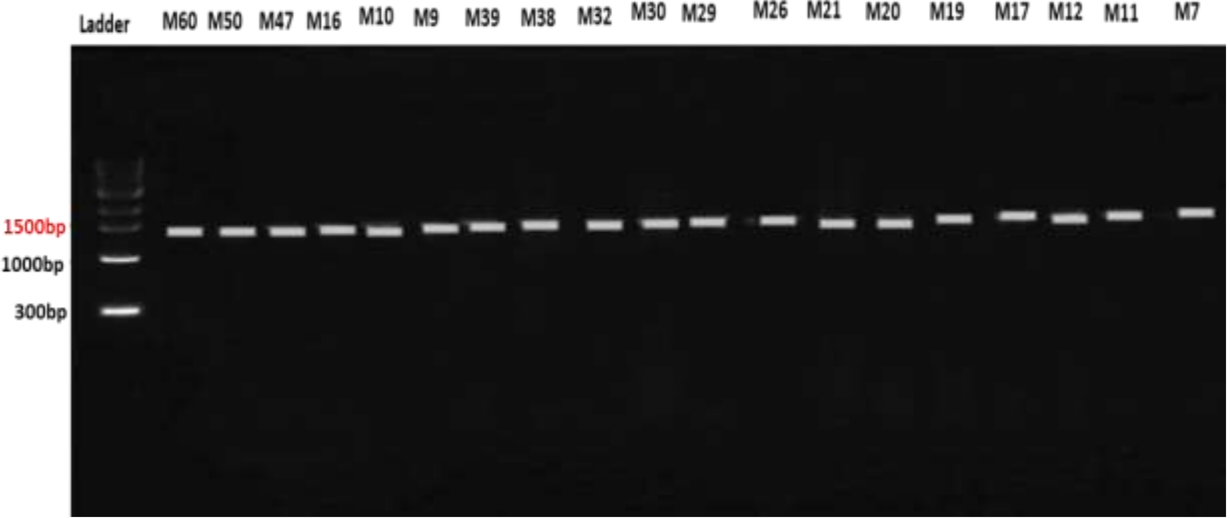


Figure 4.20: A 1% Agarose Gel Showing the Size of the PCR Amplicons of the 19 Bioactive Bacterial Isolates Visualized after adding Ethidium Bromide Stain and Size Estimated using 1kb Ladder

4.2.4.3 Phylogenetic Analysis of Bacteria Isolates from L. Bogoria.

From the partial sequences, the BLAST analysis showed ten isolates (76.9%) were from the *Bacillus genus* within the Firmicutes bacteria domain with a percentage identity between 98.71% and 100%. The isolates included *Bacillus tequilensis, Brevibacillus brevis, Bacillus velezensis, Bacillus subtilis, Bacillus amyloliquefaciens* and *Bacillus licheniformis (*Figure 4.21*).* Three isolates belonged to Gammaproteobacteria in the proteobacteria phylum with a 99.59% and 100% similarity index. Among the proteobacteria groups were *Alcaligenaceae bacterium, Pseudomonas sp* and *Pseudomonas aeruginosa.* No novel isolates were identified as a potential biocontrol

since all the isolates had a similarity percentage of above 98% with the reference sequence from the NCBI database (Table 4.8).

Table 4.8: Blast Results of Lake Bogoria Bioactive Isolates and their Respective Closest Relatives

Isolate code	Max Score	Total Score	Query Coverage	Acc No.	Next Neighbor in Blast	%ID
B7	2279	2279	100%	MZ314516.1	*Bacillus tequilensis strain* IKAK46	100%
B11	2684	2684	100%	NR_041524.1	*Brevibacillus brevis strain* NBRC 15304	100%
B12	1345	1345	100%	MT295372.1	*Alcaligenaceae bacterium strain* S10	99.73%
B17	1781	1781	100%	JQ014351.1	*Pseudomonas sp.* LC128	99.59%
B19	2732	2732	100%	DQ777865.1	*Pseudomonas aeruginosa strain* PAO1	100%
B20	2287	2287	100%	MT072102.1	*Bacillus velezensis strain* QH03-23	100%
B21	1406	1406	99%	LN556353.1	*Bacillus subtilis subsp. Inaquosorum*	99.36%
B26	2645	2645	100%	MH265986.1	*Bacillus amyloliquefaciens*	100%

Isolate code	Max Score	Total Score	Query Coverage	Acc No.	Next Neighbor in Blast	%ID
					strain K2-2	
B29	1552	1552	100%	MT641226.1	*Bacillus subtilis strain* CFR07	100%
B30	2187	2187	99%	KX129842.1	*Bacillus velezensis strain* JS39D	98.71%
B32	2608	2608	100%	MT538513.1	*Bacillus subtilis strain* 3645	100%
B38	2603	2603	100%	MT111040.1	*Bacillus subtilis*	99.93%
B39	2765	2765	100%	MT367712.1	*Bacillus licheniformis strain* AP6	100%

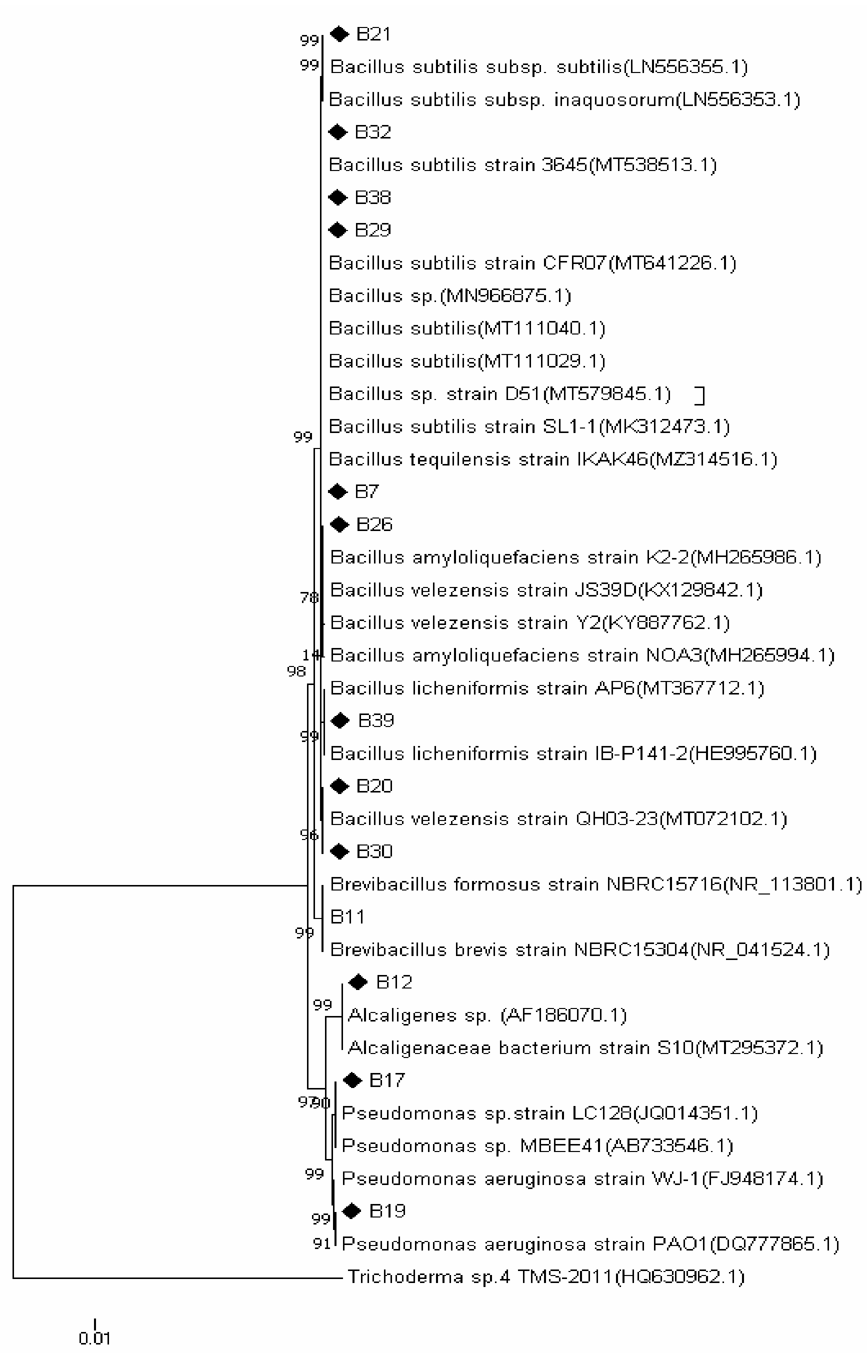


Figure 4.21: Phylogenetic Tree of Bacterial Isolates from L. Bogoria Based on 16S Rrna Sequences.

The scale bar of 0.01 was used. Evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura 3-parameter model.

4.2.4.4 Phylogenetic Analysis of Bacteria Isolates from L. Magadi

From the partial sequences, the BLAST analysis showed six isolates (100%) were obtained from the *Bacillus genus* within the Firmicutes bacteria domain with a percentage identity between 99.39% and 100% (Table 4.9). Among the isolates were *Bacillus velezensis, Bacillus subtilis* and *Bacillus pumilus (*

Figure **4.22***)*. No novel isolates were identified as a potential biocontrol since all the isolates had a similarity percentage of above 98% with the reference sequence from the NCBI database.

Table 4.9: Blast Results of Lake Magadi Bioactive Isolates and their Respective Closest Relatives

Isolate code	Max Score	Total Score	Query Coverage	Acc No.	Next Neighbor in Blast	%ID
M9	2608	2608	100%	MT538513.1	*Bacillus subtilis strain 3645*	100%
M10	2658	2658	100%	MT012197.1	*Bacillus velezensis strain CLT81*	100%
M16	2649	2649	99%	KY206830.1	*Bacillus subtilis strain Q235*	99.93%
M47	1981	1981	99%	MT538489.1	*Bacillus subtilis strain 3617*	99.39%
M50	1958	1958	100%	MN966875.1	*Bacillus sp. (in: Bacteria) strain S*	100%
M60	2693	693	100%	*EU379282.1*	*Bacillus pumilus strain 4RS-5b 16S*	100%

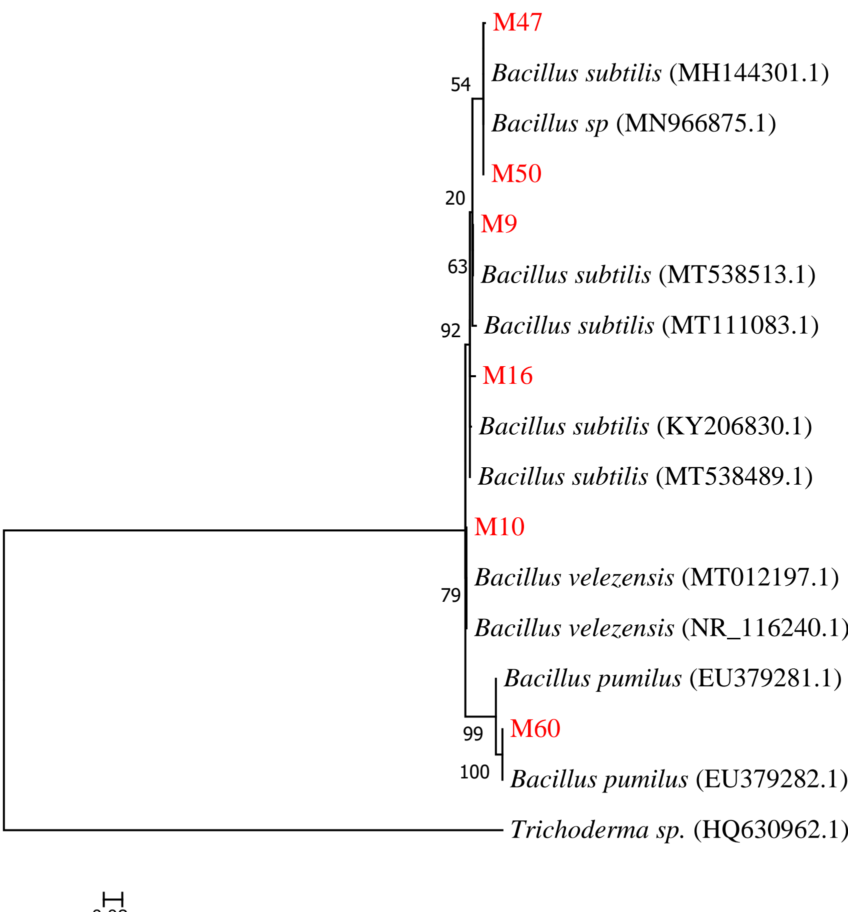


Figure 4.22: Phylogenetic Tree of Bacterial Isolates from L. Magadi Based on 16S Rrna Sequences

The scale bar of 0.02 was used. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura 3-parameter model.

4.3 To Determine the Efficacy of Bacterial Isolates against *Fusarium solani* and *Rhizoctonia solani* on Bean Plantlets.

4.3.1 Root Mortality Rate

During pathogen pre-challenged conditions, the lowest root mortality (15%) was observed in the treatment (P5) and (18%) treatment (P1). The highest root mortality was observed in treatments P3 and P4 (56% and 50%, respectively), having pathogen inoculation alone (Figure 4.23). However, seed biopriming with antagonistic bacteria significantly lowered ($p<0.05$) root tissue mortality in the presence of the pathogen. In comparing the antagonistic bacteria and Trichotech, there was a significant difference in that the bacteria strains had the lowest root mortality compared to Trichotech treatments (Figure 4.23).

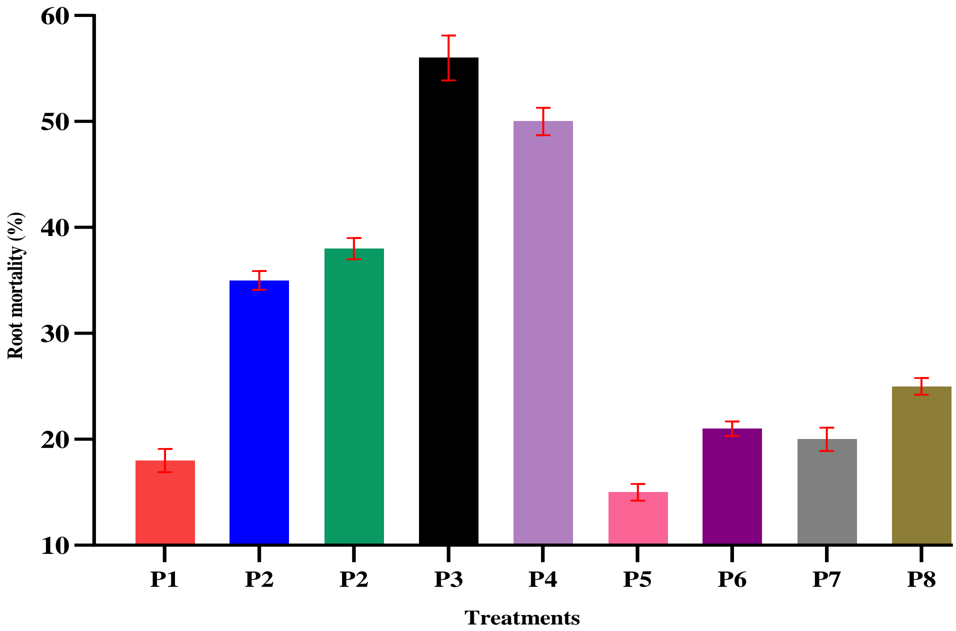
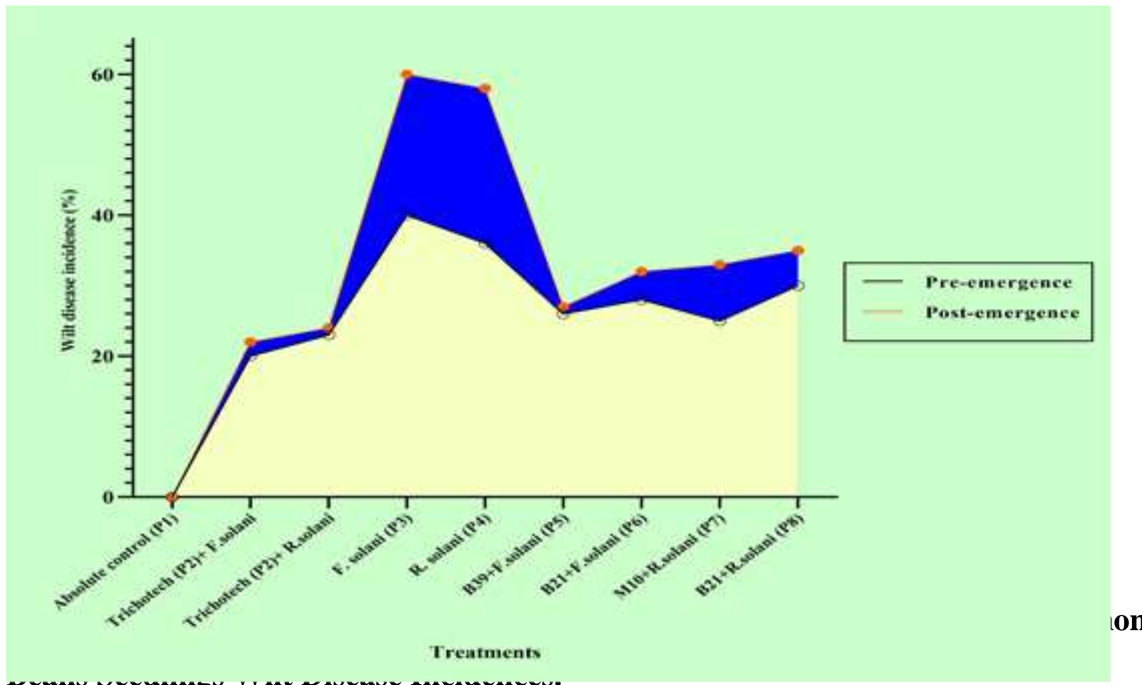


Figure 4.23: Effect of Seed Biopriming with B39, B21, and M10 on Common Beans Seedlings Root Mortality.

4.3.2 Effect on Pre-and Post-Emergence Wilt Disease Incidences

The pathogen-challenged conditions showed that P2 (Trichotech) on both pathogens *(F. solani* and *R. solani*) had the lowest (20-23%) pre-emergence wilt disease incidence in comparison to P3 (*F. solani*) and P4 (*R. solani*). Bio-primed seedlings (P5-P8) significantly ($p<0.05$) lower pre-emergence wilt disease incidence (25-36%). Additionally, in absolute control (P1), no pre-and post-emergence wilt disease incidence was recorded (Figure 4.24). Regarding the post-emergence wilt incidence, there was an increase of wilt across all the treatments. P3 and P4 recorded the highest post-emergence wilt disease incidence (60-58%, respectively). Trichotech treatment showed the lowest post-emergence wilt disease incidence of 22-24%. Additionally, seed bio-priming seeds (P5-P8) showed relatively lower post-emergence wilt disease incidence compared to P3 and P4 (Figure 4.24).



on

Beans Seedlings Wilt Disease Incidences.

4.3.3 Effect of Bacterial Isolates on the Germination Rate, Chlorophyll, Shoot, and Root Length, Shoot and Root Biomass

The highest germination rate was recorded on absolute control (P1) (99%). There was no significant difference in effect on germination among the pre-inoculated treatments (P2, P5, P6, and P7). However, they showed a relatively high germination rate than pathogen-only inoculation treatments (P3 and P4) (Figure 4.25). The lowest germination rate of 68% was observed at P3. Seed biopriming also had an effect on the fresh root weight and shoot fresh weight. The highest shoot weight of 1.97g was recorded in the P5 treatment (B39+ *F. solani*). However, there was a significant improvement ($p<0.05$) in the seed biopriming treatment compared to the pathogen-only inoculated treatment (P3 and P4). The lowest shoot fresh weight was recorded in P3 (*F. solani* only) and P4 (*R. solani* only). B39+F. solani (P5) and P2+ *F. solani* showed a maximum root fresh weight of 0.39g compared to other treatments. P3 and P4 treatments had the lowest shoot fresh weight of 0.18g and 0.20g, respectively (Figure 4.25).

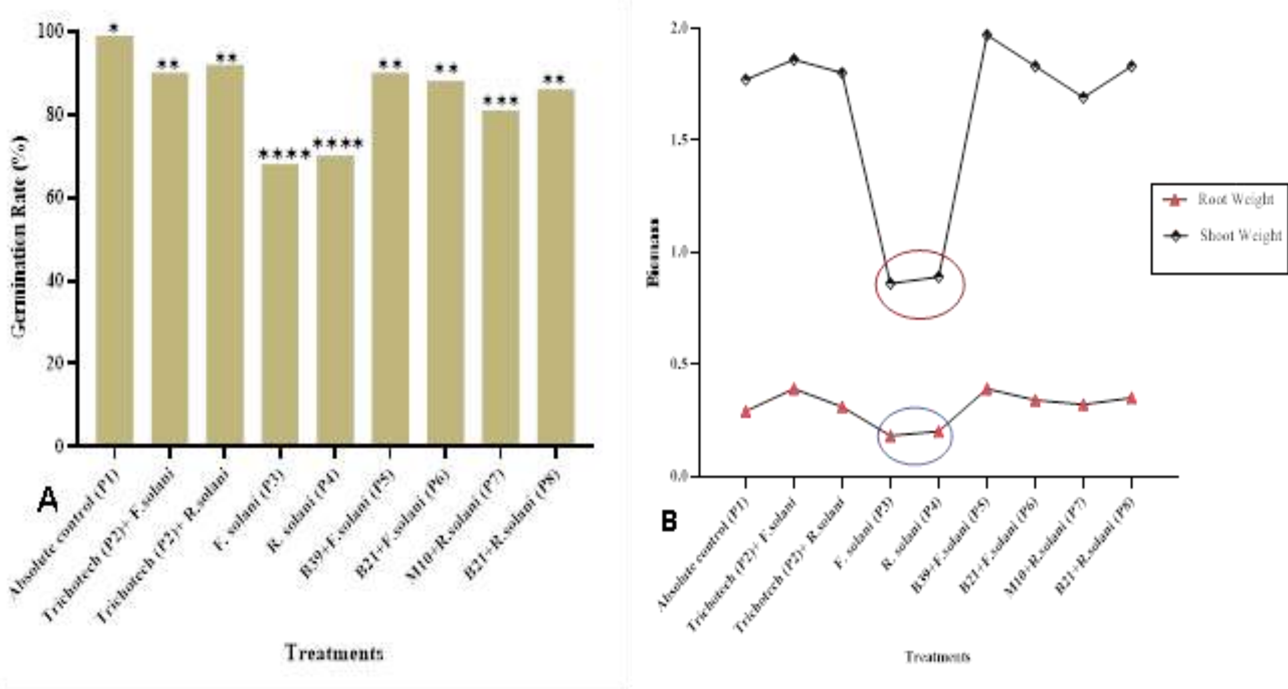


Figure 4.25: Effect of Seed Biopriming with B39, B21, and M10 on the Common Beans Seedlings Germination Rate and Biomass Observed under Pathogen Pre-Inoculation Conditions.

The correlation profile between the shoot, root length and chlorophyll, and the treatments showed three functional cluster groups (Figure 4.26). Additionally, there was a relationship between the shoot length and chlorophyll. The highest recorded length was observed for shoot length at treatment P5 (B39+ *F. solani*), with the highest chlorophyll content. It was followed by P1 (absolute control) and P2 (Trichotech + *F. solani* & Trichotech + *R. solani*), which also showed higher chlorophyll content. Treatments P3 (*F. solani*) and P4 (*R. solani*) recorded the lowest readings across the shoot, root length, and chlorophyll content compared to other treatments.

Additionally, there was varying significance among the treatments on the three parameters tested (Shoot, root length, and chlorophyll content.). There was no

correlation between the root length and both chlorophyll and shoot length. Treatment P1 clustered independently, P7 and P8 clustered together, P5 also clustered independently, P6 clustered independently, P2 for both *F. solani* and *R. solani* clustered together, and finally, P3 and P4 clustered together across the tested parameters (Figure 4.26).

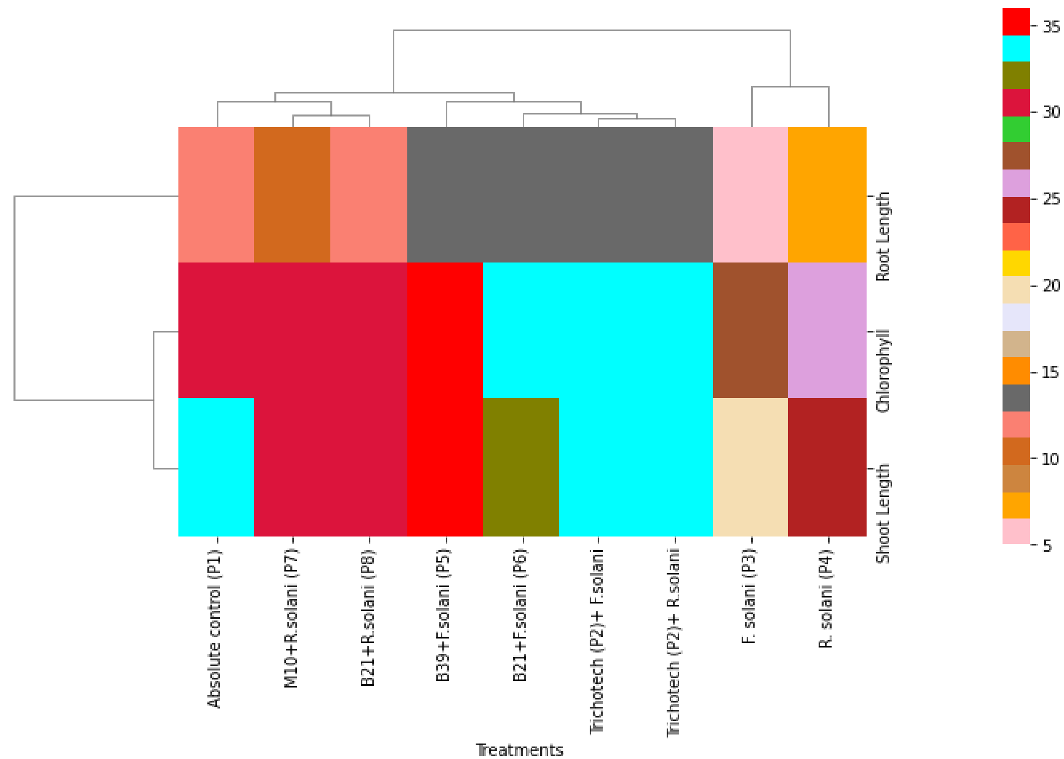


Figure 4.26: Hierarchical Clustergram of Assayed Seed Treatment on Shoot Length, Chlorophyll, and Root Length of the Common Bean Plantlets.

Hierarchical clustergram generated using means of shoot/root length and chlorophyll content at treatments. The heatmap (Euclidean matric) shows the relationship between treatments, shoot/ root length, and chlorophyll content. The colored scale bar indicates the significant quantified strength of the parameters tested (shoot, root, and chlorophyll). The red color in the heatmap indicates the highest, and pink indicates the lowest significance at $P \leq 0.05$ for the assayed treatments.

4.3.4 Induction of Defense Enzymes

In the presence of the pathogen, the highest level (343.21 µg cinnamic acid $hr^{-1}g^{-1}$ fresh wt.) of PAL activity was recorded in plant tissue of treatment P5 (B39+ *F. solani*) followed by treatment P6 (321.09 µg cinnamic acid $hr^{-1}g^{-1}$ fresh wt.). The lowest PAL activity was observed at P1 (141.08 µg cinnamic acid $hr^{-1}g^{-1}$ fresh wt.). For PPO enzymes, the highest absorbance (0.083 Changes in absorbance $min^{-1}g^{-1}$ fresh wt.) was obtained in treatment P7 (M10+ *R. solani*). There was no significant difference ($p<0.05$) in PPO enzymes for the bio-priming seeds treatments (**Table 4.10**). On the other hand, PO build-up was enhanced by the seed bio-priming with bacteria isolates. The highest PO (0.142 changes in OD min^{-1} g^{-1} fresh wt.) was recorded treatment P6 treatment (B21+ *F. solani*). Additionally, there was significant difference ($p<0.05$) for seed bio-priming treatments (**Table 4.10**). Treatment P1 recorded the lowest PO treatment (0.047 changes in OD min^{-1} g^{-1} fresh wt.) compared to other treatments. There was no significant difference ($p<0.05$) amongst pathogens for PAL, PPO, and PO enzymes (**Table 4.10**).

Table 4.10: Effect of Seed Bio-Priming with Bioactive Bacteria on Induction of Defense Enzymes in Common Bean Plantlets in Pathogen (*F. Solani* And *R. Solani*) Challenged Condition.

Treatments	Phenylalanine ammonia-lyase (PAL) (cinnamic acid hr-1g-1 fresh wet	Polyphenol oxidase (PPO) (Δ Changes in absorbance min−1g−1 fresh wt.)	Peroxidase (PO) (Δ Changes in absorbance min− 1g− 1 fresh wt.)
Absolute control (P1)	141.08±10.21f	0.015±0.002e	0.047±0.002f

Trichotech (P2) +F. solani	208.02±9.14de	0.032±0.001c	0.103±0.009d
Trichotech (P2) +R. solani	212.06±10.45d	0.029±0.002cd	0.100±0.002d
F. solani (P3)	230.01±11.23cd	0.027±0.002cd	0.096±0.005de
R. solani (P4)	241.13±14.07c	0.021±0.001d	0.980±0.002de
B39+ F. solani (P5)	343.21±15.78a	0.080±0.004a	0.119±0.003c
B21+F. solani (P6)	321.04±14.98ab	0.075±0.002b	0.142±0.005a
M10+ R. solani (P7)	301.09±13.82b	0.083±0.003a	0.102±0.002d
B21+ R. solani (P8)	311.05±10.23	0.071±0.003b	0.127±0.006b

*s*Means in a column followed by the same letter do not significantly differ according to the Tukey HSD test (P<0.001)*

4.3.5 Total Phenolic Content

The total phenolic content significantly increased in leaf and root tissues of biocontrol bacteria (B39, B21, and M10) compared to P1 and P2 (controls). The highest phenolic content of 142 μg catechol^{-1}g fresh leaf wt. and 98 μg catechol^{-1}g fresh root wt. was observed in P5 (B39+ F. *solani*) followed by P6 (B21+ *F. solani*) with 139 μg catechol^{-1}g fresh leaf wt. and 90 μg catechol−1g fresh root wt. (Figure 4.27). The least phenolic content was recorded in P3-(*F. solani*) with 95 μg catechol^{-1}g fresh leaf wt. and 79 μg catechol^{-1}g fresh root wt. followed by P4 (*R. solani*) with 98 μg catechol^{-1}g fresh leaf wt. and 80 μg catechol^{-1}g fresh root wt. The amount of phenolic content obtained from the leaves was higher than that obtained from the roots (Figure 4.27).

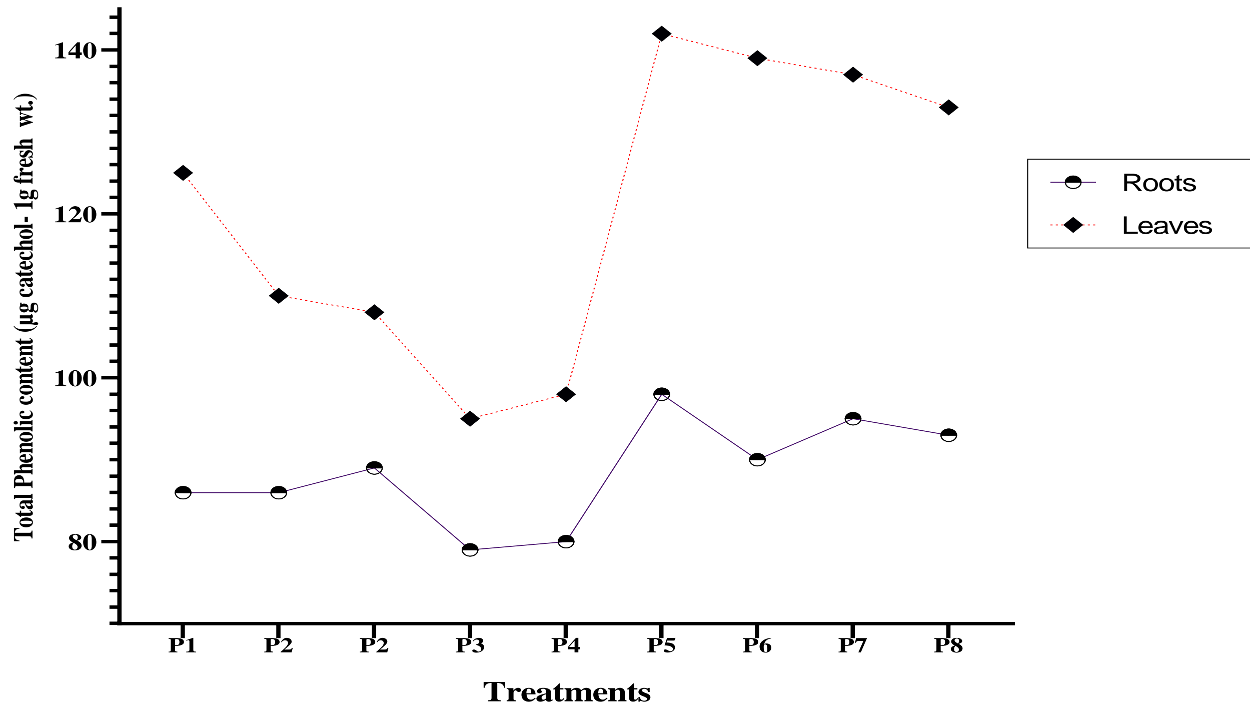


Figure 4.27: Effect of Seed Bio-Priming with Bioactive Bacteria on Phenolic Content in Common Beans.

CHAPTER FIVE

DISCUSSION, CONCLUSION, AND RECOMMENDATIONS

5.1 Discussion

The study aimed to isolate bacterial from L. Bogoria and L. Magadi for subsequent antifungal activity using dual culturing and disc diffusion method, characterization of the bioactive isolates morphologically, physiochemical, biochemical, and molecular approaches. Lastly, assessment of bioactive bacterial isolates for the effectiveness in controlling both *Fusarium solani* and *Rhizoctonia solani* in greenhouse experiments.

The physical parameters of L. Bogoria showed that it is thermophilic with a high-temperature range recorded. Its location also indicates that it is a highland lake with 1004-1010M above sea level. The results also indicated that L. Bogoria has low salt concentration and pH compared to L. Magadi, which is recorded the highest pH value and salt concentration. Lake Magadi is a lowland lake with a range of 604-618M above sea level, with high conductivity and TDS. The result agrees with earlier studies on both L. Bogoria and L. Magadi. For instance, Renaut & Tiercelin, (1994) described L. Bogoria as a highland lake with a surface elevation of >990M above sea level. The lake water is alkaline and saline, an ideal condition and home of the world's largest populations of lesser flamingoes (Harper et al. 2003). The alkalinity (due to Na-HCO3-CO3 composition) of the water enables the growth of blue-green algae, which feeds the flamingoes (Harper et al. 2003; Kambura et al. 2013; Simasi 2013). The results showed few hot springs sampled with a range temperature of 60-90°. The result does not agree with the past finding of the number of hot springs, which initially had approximately 200 hot springs with a water temperature range of 40 to 110°C (Harper et al. 2003). Over the past 8 years, the water level has increased, affecting most geysers and the number of hot springs (Scoon 2018). Since 2013, the lake has been experiencing a high increase in

water level (Scoon 2018). The result for L. Magadi agrees with AW Duckworth (1996), who described L. Magadi as an extensive Salt Lake and second-largest source of sodium carbonate. According to Barnabás et al., (2008); Eunice et al., (2020), the salinity level goes up to 30% w/v with a pH range of 8.8-12.0 and a low temperature of 22°C-34°C apart from the hot springs. The lake is unique and has earned itself the tag of an 'alkaline saline pan.' This is because it has the largest deposit of solid NaCl, Na2CO3, and trona (NaHCO3.Na2CO3.12H2O). The lake has an elevation of 600m above sea level since it is in the southern part of Kenya and closely borders Tanzania. According to Antony et al., (2012); AW et al., (2000); Eunice et al., (2020); Kambura et al., (2013); Nyakeri et al., (2018); Scoon, (2018), L. Bogoria and L. Magadi are classified as soda lakes due to the unique characterization they have. Despite that, the two lakes are different in terms of their physical characteristics, such as temperature, pH, salinity, TDS, and conductivity (Duckworth et al. 1996; Scoon 2018).

Soda lake has become the center stage of research in exploiting the microbial diversity among the Kenya lakes. Many culturable bacteria isolates were obtained from both L. Magadi and L. Bogoria. The results indicate that most bacteria were obtained from soil samples compared to sediment and water. Additionally, a reported number of bacteria isolates were obtained from either two-sample type or three sample types. The results agree with previous studies on soda lakes where diverse culturable bacteria isolates were identified. For instance, Nyakeri et al., (2018) isolated thirty-three bacteria isolates to assess the ability of L. Magadi isolates to produce enzymes. Some of the genera identified are bacillus, clostridium, and halomonas (Nyakeri et al. 2018). Other studies such as Anne et al., (2016); Eunice et al., (2020); Mulango et al., (2020) show diverse exploitation and identification of various bacteria communities in L. Magadi. They identified that the lake is rich in the microbial community (bacteria and fungus), and they have played a significant role, especially in the biotechnology field. The study Bett (2020) shows that L. Magadi comprises diverse bacterial communities utilizing different substrates. Lastly, the study done by Ngetha Edwin, (2019) indicates that L. Magadi contains phylogenetic and morphological diversity of culturable cyanobacteria.

For L. Bogoria, various studies have indicated that the lake is rich in microbial communities. For instance, Harper et al., (2003); *How Kenya's Lake Bogoria Is Feeding the Global Biotech Industry*, n.d., Kambura et al., (2013), reported the diversity of the microbial community of the lake that ranges from bacillus and *Gammaproteobacteria* species. Additionally, the study done by Tom et al., (2015) indicates that L. Bogoria has a wide range of bacillus species that have antibiotic-producing properties. The lake being saline and thermophilic, has a wide range of enzyme-producing bacteria that has been exploited, identified, and used in various biotechnological fields (Duckworth et al. 1996, 2000; Simasi 2013). Regarding the distribution of the isolates regarding sample type, the results agree with previous studies that reported that soil has a large number of microbes compared to water and sediment (Wu et al. 2021). Soil microbial communities are responsible for nutrient cycling, stabilizing the ecosystem, and maintaining the soil structure. Approximately 90% of the bacteria are found in the soil, contributing to the soil's nutrient content compared to water and sediment. Grasslands have also been shown to have a more diverse bacterial community due to the stability of biomass compared to water and sediments (Lawlor et al. 2000). Studies have shown relations amongst the microbes and environmental factors, geographical location (Fierer and Jackson 2006; Lauber et al. 2009), pH (Fierer and Jackson 2006; Lauber et al. 2009), nutrients (Fierer and Jackson 2006; Lawlor et al. 2000) sand temperature. L. Magadi and L. Bogoria are both saline lakes. The water pH is more alkaline compared to the soil, which is offshore of the lake. Different pH and temperature contributed to diversity composition in the number of bacterial communities among the sample types. The incidences of some bacteria reported to have been isolated from all sample types agree with the study done by AW Duckworth, (1996); Eunice et al., (2020); Kambura et al., (2013); Scoon, (2018), where different species of haloalkaliphilic bacteria were identified.

In terms of plant pathogenicity, the results indicate that both *F. solani* and *R. solani* elicited narrow root and red-brown lesions on the hypocotyl of the bean seedling. Lesions extend down to the central taproot, which caused the wilting and death of the

plantlet for both pathogens tested compared to control. The results agree with the study by Leep (2016), who reported the narrow roots, red-brown lesion on the stem, and the lesion extending down to the main taproots as the symptoms associated with *Fusarium solani* and *Rhizoctonia solani*. Additionally, the same results were reported by (Aydi et al. 2016) on *fusarium* wilt in tomatoes; (Abbas et al. 2019) on *fusarium* wilt rot of safflowers. After the pathogen has colonized the whole root system, in some case extend up the hypocotyl to the soil surface (Leep 2016). The infected plants are stunted, grow slowly compared with healthy plants, and light green to yellow for the leaves (Amare Ayalew 2015; Naseri 2014; Teixeira et al. 2015; Toghueo et al. 2016). The symptoms also agree with previous studies reporting the pathogenicity of *F. solani* and *R. solani*. For instance, Jabnoun-Khiareddine H, (2018) reported the pathogenicity of *R. solani* on pepper (*Capsicum annum*) which caused *Rhizoctonia* root rot and *R. solani* inducing pre- and post-emergence root rot diseases and plant growth reduction (Jabnoun-Khiareddine H 2018). Amare Ayalew, (2015); Belete, (2015) reported the same symptoms on black root rot of faba beans and seed rot, post-emergence damping-off, and root rot by *R. solani.*

The results showed that both *F. solani* and *R. solani* impact germination rates, plant height, the severity of the plant, length of root, root fresh weight, and shoot fresh weight. This could be because, during germination, pathogens have colonized the soil. It is easy for the pathogen to attack the young seedlings due to weak rooting, preventing germination. Additionally, it causes poor root function depriving plants of nutrients and water. Therefore, it results in stunted plant growth in terms of height, causing the death of the plant and affecting the plant biomass (fresh root weight and shoot fresh weight). The modes of action for the *F. solani* and *R. solani* were different. For instance, *F. solani* produces spore that help it from spreading and compromise the plant's immune system. It was able to colonize the stem of the plants depriving the plant of a sufficient supply of nutrients and water. For *R. solani*, they were classified as mushroom-like due to their inability to produce spores. Instead, they have a high and faster growth rate. This mode of action makes the mycelium deprive the plant of nutrients, resulting in a

decrease in plant height and biomass (Dukare, Paul, and Arambam 2020; Rathna Priya and Manickavasagan 2020; Toghueo et al. 2016). The production of common beans has been affected due to the *Fusarium* root rot and *Rhizoctonia* root rot which causes the death of the plant and reduces the yield of production to approximately 50% (Fatiha Lazreg et al. 2014; Sippell and Hall 2009).

Soda lakes, are the most exploited lakes in Kenya, have contributed a significant impact in the industry, for instance, in the production of enzymes (Nyakeri et al. 2018). Exploitation in terms of genetic diversity also has made various discoveries on the importance of isolated and identified microbes. In agriculture, limited research has been done to quantify the significant impact of soda lakes microbes in managing and controlling plant pathogens. The screening of soda lakes bacteria will help detect biocontrol potential, which acts against damaging fungal root pathogens. In the present research, the dual plate and disc diffusion assay showed that bacteria isolates obtained from L. Bogoria and L. Magadi inhibit mycelium growth of phytopathogenic agents (*F. solani* and *R. solani*). This can probably be because of the synthesis of lytic enzymes produced by bacteria involved in cell degradation during antagonism.

Additionally, mycelial inhibition rate could have been as a result of bacterial diffusible inhibitory antibiosis substances, which could have suppressed and restricted the growth of the pathogen. Biocontrol employ different mechanisms in which the biocontrol employs in the management of the pathogen are mycoparasitism, characterized by the active growth along the host hyphae and the production of enzymes that degrade or break the host cell wall. The bioactive isolates might have employed this mechanism by penetrating the *F. solani* and *R. solani* cytoplasm, resulting in the pathogen's death. Still, the bioactive isolates could produce hydrolytic enzymes, such as chitinase and cellulases, which reduce the mycelial length. This mechanism has been reported by (Gerbore et al. 2014) on the biological control of plant-pathogen a cases study of *Pythium oligandrum*. Despite this mechanism being reported, further investigation is needed to determine whether bioactive isolates (L. Magadi and L. Bogoria) employed

the same mechanism in inhibiting the mycelium of the pathogen tested. Antibiosis is a second mechanism employed by most microbes. Antibiosis is the specific interaction in which the prey is destroyed by toxic secondary metabolites produced by antagonistic microbes. The result shows the antibiosis mechanism. They are responsible for the suppression and restriction of the growth of the pathogen. According to Asaka & Shoda (1996), different bacteria such as *Bacillus spp*. are well known to produce diverse antibiotics used in the biological control of plant pathogens. Additionally, endophytic bacteria have been reported to have antibiosis effects against *fusarium* (Alabouvette et al. 2007; Amare Ayalew 2015; Baazeem et al. 2021; Gerbore et al. 2014; Muriungi et al. 2014; Rocha et al. 2017). Therefore, this has resulted in antibiosis as the most important mechanism to limit pathogen invasion in plants and inhibition of the development of plant pathogenic organisms by producing secondary metabolites (Gerbore et al. 2014).

The results show the formation of a colorless zone of inhibition, which suggests the production of colorless metabolites by the isolates that diffuse in the media and possibly inhibit the radial growth of fungal pathogen tested. The results agree with the findings reported by (Fravel 2005). The study shows varying levels of antagonism from different isolates, which indicates the possibility of different mechanisms of antibiosis exhibited by antagonistic agents against different pathogens. Similar effects were reported by Belete (2015) using Native Bacillus isolates to manage black root rot diseases caused by *F. solani* in faba beans; (Jabnoun-Khiareddine H 2018) the use of fungal and bacterial agents in control of *Rhizoctonia* root rot of pepper. Aydi et al., (2016) reported the use of endophytic bacteria from *Datura stramonium* to suppress *Fusarium* wilt disease in tomatoes. Mahmoudi & Naderi, (2017) reported anti-fungal and bio-control properties of chitinolytic bacteria in control of *Fusarium* root rot in safflower, among others.

The results also indicate the variation of *F. solani* and *R. solani* in an isolated location. This might be due to the different physical characteristics of the lakes. For instance, L. Bogoria is a hot spring lake with a pH of 9.0 and a temperature of approximately 76-

90ºC. Additionally, it has low salt concentration, conductivity, and total dissolved solvent. L. Magadi is known as a saline lake with a pH>9.0. It has the highest salt concentration, conductivity, and total dissolved solvent. This variation contributes to different mechanisms of action. This result agrees with the previous studies done by Gerbore et al., (2014), indicating that the mode of action for most antibiosis microbes is affected by environmental factors. Additionally, the variation may have been due to microbe's growth being depended on temperature, nutrition, and light, prerequisites for heavy parasitism. Still, in a harsh environment, this mode is consistently attenuated.

Nutrient and space competition can also be attributed to the suppression of spore germination around the bacteria. According to Gerbore et al., (2014); Jabnoun-Khiareddine H, (2018), nutrition and space competition is a general phenomenon of regulating the population dynamics of microbe sharing the same ecological niche and physiological requirements when resources are limited. This phenomenon can be quantified by the elevated chloride concentration, as reported by (Gerbore et al. 2014). A similar model of action was reported for *T. harzanium* against *R. solani* on radish (Lui and Baker 1980*) Fusarium oxysporum* against fusarium wilt on melon (Alabouvette et al. 1983). Despite this mode of action being principal in microbial antagonism, especially when suppressing the pathogen causing decays, it is considered difficult to exploit for biological control Alabouvette et al. (2006) for soilborne pathogens whose interactions are numerous. Since various studies described mycoparasitism and/or antibiosis as the main mode of biocontrol, space competition and space are probably minor mechanisms used by most biological control agents.

Physiochemical characterization of the bioactive isolates showed that they could grow in a wide pH range. The highest growth was observed at pH7.0, 8.5, and 10.0, respectively. However, the results also indicated that the bioactive isolates could grow at acidic pH 5.0. The results concurred with a previous study by Morita et al., (1999) which indicated that a pH range of 5.7 to 9.0 favors the growth of alkaliphiles. However, the pH range of 7.0 to 11.0 serves as selective optimum pH. Soda lakes have a high range of

pH. Hence, the microbes can only survive by maintaining their cytoplasm at the same pH as their mesophilic relatives. The mechanism through which this achieved is secondary proton uptake mediated by membrane-associated antiporters (Kambura et al., 2016). They also use passive mechanisms, for instance, negatively charged cell wall polymers in alkaliphiles, unusual bioenergetics, positive surface charges, and high internal buffer capacity. Alkaliphiles are also able to maintain an internal pH >7,5 regardless of the environmental pH. Most bacteria activate the sodium-ion pump at this high pH, which lowers the internal pH by transporting hydrogen ions into the cells (Abo-Bakr et al. 2020). According to DeLong and Pace (2001), alkaliphiles have cell membranes with a special composition of tissues that protect them from highly alkaline conditions. The results showed that M16 and M60 had the highest growth at acidic conditions; B39 was alkaliphiles, and M9 and M50 were neutrophiles. The study concurred with the previous findings by Krulwich et al., (1985), who reported the wide range of pH (4.5-10.0) which supports bacillus can grow. The result agrees with the findings of Kambura et al. (2016) Mulango et al. (2020); Kambura et al., (2013); Nyakeri et al., (2018), reported the varied bacterial growth at different pH.

The bacteria isolates were able to tolerate different concentrations of salinity. Most of the bioactive isolates had a maximum growth at 0.0M of NaCl. This is because most of them were isolated from soil samples. The ability of the bioactive isolates to grow at different salt concentrations indicates they are tolerant to salinity and as an adaptive strategy to survive in adverse growth conditions. This is in agreement with earlier studies on *Bacillus spp*. (Li et al. 2002). Soda lake bacteria can survive for millions of years in the fluid inclusions of salt deposits, including the evaporates, by adaptation to these potentially deadly ecosystems (Mwirichia, et al. 2010). To prevent water loss from their cell membrane, halophile offsets the high salt in the environment by accumulating potassium and glycine-betaine compounds. This mechanism balances the salt concentration inside the cell and the environment preventing the cell from bursting due to the diffusion of water. Studies done on Magadi bacteria agree with the findings of diverse growth of bacteria at different salt concentration (Nyakeri et al. 2018). The

ability of bacterial isolates to grow at different salt concentrations has also been reported by (Bett 2020; Duckworth et al. 1996, 2000; Kambura et al. 2013, 2016; Kiplimo et al. 2019; Nyakeri et al. 2018; Scoon 2018) for lake Magadi and (Anon n.d.; Antony et al. 2012; Harper et al. 2003; Scoon 2018) for lake Bogoria isolates.

The growth of the bioactive bacteria at varied temperatures indicates that Bogoria and L. Magadi harbors diverse microbes that can adapt to a wide temperature range. This property is ideal for biocontrol since different environments have different temperatures. The study agrees with previous studies that show the ability of L. Magadi and L. Bogoria bacterial isolates to adapt at different temperatures (Duckworth et al. 1996, 2000; Kambura et al. 2013; Scoon 2018).

The BLAST results showed diversity of L. Bogoria bacterial isolates were from the *Bacillus* genus within the Firmicutes domains. Others belong to the *Gammaproteobacteria* class in the phylum *proteobacteria* with a 98.71-100% similarity index range. This results concur with the previous studies on L. Bogoria, where phylogenetic analysis of the bacteria partial 16S rRNA gene sequence showed Firmicutes and *Gammaproteobacteria* as the dominant bacteria domains (Mwirichia et al. 2010; Simasi 2013; Szilagyi-Zecchin et al. 2016; Torome* et al. 2015). The BLAST result for L. Magadi bacteria isolates showed (100%) were obtained from the Bacillus genus within the Firmicutes bacteria domain with a percentage identity between 99.39% and 100%. This results concur with the previous findings on the diversity of microbes on L. Magadi (Kambura et al. 2013; Kiplimo et al. 2019; Mulango et al. 2020; Nyakeri et al. 2018). From the study of the two lakes, firmicutes with low G+C content were more diverse and abundant than the proteobacteria. This agrees with the study where cultivation-dependent analysis of microbial diversity of soda lakes, revealed two major cluster groups of the established lineage of bacteria; Gram-positive bacteria (*Bacillus* & relative) and Gram-negative subdivision proteobacteria.

From the study, 84.2% of the bioactive bacteria belong to the genus Bacillus. Among these were; *Bacillus subtilis, Bacillus tequilensis, Brevibacillus brevis, Bacillus*

velezensis, Bacillus amyloliquefaciens and Bacillus licheniformis and Bacillus pumilus. *Bacillus sp* are most abundance found in aerobic, eubacterial alkaliphiles in soda lakes and normal environments (Antony et al. 2012; Duckworth et al. 1996; Morita et al. 1999; Scoon 2018). The *Bacillus spp* identified; *Bacillus subtilis* (seven), *Brevibacillus brevi* (One), *Bacillus velezensis* (Three), *Bacillus tequilensis* (One), *Bacillus amyloliquefacien* (One), *Bacillus licheniformis* (One), *Bacillus pumilus* (One) and *Bacillus sp* (One) have been previously isolated from L. Bogoria and L. Magadi, clustering with Bacillus members that are alkalitolerant and alkaliphilic. Hence, they are heterogenic in physiology, ecology, and genetics. They have a wide diversity in physiological types, such as antibiotic production, nitrifiers, nitrogen fixation, acidophiles, thermophiles, etc. According to a study done by Anne et al., (2016); Eunice et al., (2020), soda lakes comprise diverse microbes not only in firmicutes but other domains.

B. subtilis have biotechnological importance due to its ability to produce extracellular alkaline enzymes like amylase, protease, and pectinase that are resistant to adverse temperature and at high pH (Sokorin et al., 2014). From the result, these strains produce different enzymes. The enzymes produced include pectinase, phosphatase, chitinase, produce, and IAA. The production of the secondary metabolites by the *B. subtilis* strains varied. *Bacillus* produces different diffusible metabolites with potent growth inhibitory activity against several phytopathogens (Hernandez-Leon et al., 2015; Hossain et al., 2016; Nam et al., 2016). The antagonistic potential of diffusible and volatile metabolites producing avocado rhizobacterial strains of Bacillus has been reported against *Fusarium kuroshium* using dual plate culture assay (Guevara-Avendano ˜ et al., 2020). The production of different hydrolytic enzymes (such as chitinases, amylase, cellulase, protease, pectinase, and lipase) is another trait associated with Plant growth promoting rhizobacteria (PGPR), enabling them to restrict fungal pathogen growth from disintegrating their cell wall (Dhar et al., 2018). Chitin lytic enzyme production is not only a bio-control mechanism related to these bacteria but is also the most important mechanism (Chang et al., 2003). It has been reported that there is a high correlation

between the anti-fungal activity and chitinase-producing ability of chitinolytic bacteria (Compant et al., 2005). Chitinase production has been reported in different species of *Bacillus* such as *B. megaterium, B. circulans, B. cereus* (Huang, 2005), *B. subtilis* (Wang 2006).

Bacillus amyloliquefaciens strain K2-2(MH265986.1) is Gram-positive bacterium, creamy-white in color and rough with irregular edge and single rod-shaped. The findings agree with the morphological description of previously isolated *Bacillus amyloliquefaciens* as a Gram-positive, non-pathogenic endospore-forming soil-inhabiting prokaryotes (Belbahri et al. 2017a; Hwang et al. 2012). Naturally, these bacteria are found in soil and reported to from temperature range of 14.5 to 57.7°C (Berendsen et al. 2016). The results indicate that it can grow at a low saline condition of below 0.5M. The results concur with the findings (Marach et al. 2020), indicating the ability to grow in nutrient broth with or without 5% NaCl for 24h. *Bacillus amyloliquefaciens* promotes the growth of a plant. For instance, the bacteria has the ability of the bacteria to produce chitinase and Indole-3-acetic acid (IAA). Belbahri et al., (2017); Borriss et al., (2011) also demonstrated that *B. amyloliquefaciens subsp. Plantarum* can colonize plant roots and produce plant growth hormone known as indole-3-acetic acid. Other studies also established that *B. amyloliquefaciens* could produce numerous antimicrobial and bioactive metabolites such as surfactin, iturin, and fencing, which have well established in vitro activity (Belbahri et al., 2017). Its antifungal activity is due to the non-ribosomal synthesis of the cyclic lipopeptides bacillomycin and fengycin. Its antibacterial activity is mainly due to non-ribosomally synthesized polyketides (Chowdhury et al. 2013). However, these metabolites were not assayed in this study. The production of these compounds highlights *B. amyloliquefaciens* and its close relative in the study (MH265986.1) as good candidates for the development of biocontrol agents.

Bacillus tequilensis strain IKAK46 (MZ314516.1) is a Gram-positive and rod-shaped bacterium. with wide temperature range of 30-40°C and grows in a wide range of pH

from 5.5-8. The findings concur with previous studies describing *Bacillus tequeilensis* as a Gram-positive and rod-shaped bacterium (Li et al. 2018). Bacillus tequilensis is capable of producing enzymes such as chitinase, phosphatase, and protease. The production of secondary metabolites helps degrade cell walls and is therefore used as an antifungal against various pathogens. They produce extracellular enzymes, which makes it possible to have the capacity to demonstrate multiple mechanisms against *F. solani* and *R. solani*. First, chitinase, cellulase, and protease might cause an abnormal hyphal morphology of pathogens. Therefore, chitinase and protease secreted by *Bacillus tequilensis* can cause hyphal deformation and growth suppression of pathogens. Secondly, phosphatase secreted by *Bacillus tequilensis* can promote plant growth by reducing the adverse effects of ethylene. As we know, plant growth promoters might improve plant disease resistance indirectly. Maybe, it could be a result of the antagonism of *Bacillus* in the protection of host rice against pathogens by promoting growth. In addition, further studies aiming to identify the compounds responsible for the antifungal activity of B7 (*Bacillus tequilensis*) should be done. *Bacillus tequilensis* has been used as a biocontrol for plant pathogens. The ability to decrease the radial mycelium growth of most fungi has been an upper advantage of being used as a potential biocontrol agent.

The results concur with the previous findings, which have described *Brevibacillus brevis* as a Gram-positive, aerobic, motile, spore-forming, and rod-shaped bacteria (Ray, Patel, and Amin 2020). *Brevibacillus* species are isolated from soil, water, and air, and therefore, they have a wide spectrum of different species. It is also difficult to distinguish between species due to the complexity of the genus *Brevibacillus* and poor response in conventional biochemical tests (Goto et al. 2004). The complexity of the bacteria also has been identified to be isolated in a different environment. Some of the characters found are halophilic and thermophilic, growing at 30-50°C with an optimum range of 35°C. *Brevibacillus brevi*s grow at an alkaline pH and with or without saline conditions (Ray et al. 2020).

Brevibacillus brevis produces secondary metabolites such as protease, phosphatase, chitinase, HCN, and IAA. The results concur with the finding of Ray et al., (2020), who described the *Brevibacillus* as a PGPR bacteria. Additionally, Nehra et al., (2016) reported that *B. brevis* is a potent PGPR in cotton crops and, therefore, encouraged the use of *B. brevis* on a large scale for enhancing the growth and productivity of the cotton crop. It is observed that the ability to produce phosphatase, IAA, acetylene, and antifungal can of good impact on the agriculture sector. The production of secondary metabolites that can inhibit the mycelium growth of *F. solani* and *R. solani* makes it a potential biocontrol. *B. brevis* synthesized a potential peptide, antibiotic gramicidin S, which is responsible for attacking the lipid bilayer of the membrane of organisms (Ahmed 2017). It has potential antifungal properties that act as biocontrol agents. For instance, *B. brevis* was active against *fusarium* wilt in pigeon pea (Ray et al. 2020). Edwards & Seddon, (2000, 2001) reported that gramicidin S produced by *B. brevis* is highly sporicidal toward conidia of *B. cinerea* and is less inhibitory toward the growth of mycelium. Omar, (2014), investigated the inhibitory and antagonistic impact of several rhizobacteria against various isolates of *Fusarium* on sage plants and found that *B. brevis, B. agri*, and *B. formosus* have a high effect in the suppression of Salvia officinalis wilt and root rot disease effectiveness, probably due to the production of several inhibitory metabolites like HCN, chitinase, and siderophore. (Barnabás et al. 2008; Tamiru and Muleta 2018; Tekner et al. 2019) Investigated the potential effect of *B. formosus strain DSM 9885* and *B. brevis strain NBRC 15304* as a biocontrol agent on potato against potato brown leaf spot disease caused by *Alternaria alternata* and reported that the use of the tested *Brevibacillus* strains could enhance resistance to brown leaf spot in potato by inhibiting the linear mycelia growth and spore germination of *A. alternata* as well as checking the in vitro hypersensitive response through detached leaves. Protein profiles obtained through SDS-PAGE revealed a unique protein band pattern due to *Brevibacillus* as a possible biocontrol agent. (Nehra et al. 2016) reported that the *B. brevis IPC11* and other strains provided maximum protection to tomatoes against bacterial canker disease. (Nehra et al. 2016), discovered that *B. brevis* is a

potential biologic control agent for reducing the impact of *Fusarium oxysporum f.sp. lycopersici* on tomato. Ahmed, (2017); Edwards & Seddon, (2000, 2001) reported the largescale use of *Brevibacillus brevis* and *Bacillus polymyxa* as biocontrol of pre- and postharvest strawberry from gray mold Disease. *B. brevis DZQ7* was isolated from the tobacco rhizosphere soil in Guizhou, China, and reported to show broad-spectrum antimicrobial activity to soilborne disease-causing pathogens, i.e., *Ralstonia solanacearum, Phytophthora nicotianae*, and *Fusarium spp*., and has been widely used in biologic control of soilborne.

The use of *B. velezensis* as a potential control against *F. solani* and *R. solani* has been reported by (Elmahdi et al. 2015; Jiang et al. 2018; JM et al. 2016; Kim et al. 2021; Myo et al. 2019; Rabbee et al. 2019). Therefore, more exploitation needed to be evaluated to quantify its mode of action. Additionally, B. *velezensis* is attracting attention as a valuable biocontrol agent. Accordingly, to develop and formulate bio-based products, it is increasingly important to understand the antifungal potential of biosynthesis of *B. velezensis*. Furthermore, the elucidation of genes responsible for bioactive secondary metabolites and the ability to control such genes are additional important steps for increasing the production of metabolites by beneficial microbes and for facilitating metabolic engineering. *B. velezensis* may represent a practical and powerful biocontrol agent that can be used as an effective alternative to synthetic agro-chemicals.

Bacillus licheniformis strain AP6 (MT367712.1) is a bacterium mostly found in soil and birds' feathers. It also survives at a high temperature of 50°C and high alkaline pH of 8-10. Additionally, the presence of flamingo birds at L. Bogoria contributes to the colonization of the *B. licheniformis*. *B. licheniformis* produces different enzymes such as protease, IAA, phosphatase chitinase. The results agree with previous findings, which indicate that it produces alkaline protease used in industry due to its ability to withstand high temperatures (Nyakeri et al. 2018). The ability of the isolate to produce secondary metabolites can therefore be linked to its ability to prevent *R. solani* and *F. solani*. It is in the same class as *Bacillus sp*. It is classified as a PGPR bacteria and is, therefore, able

to be used as a biocontrol agent. Different research has been reported for different strains of *B. licheniformis* in control of different plant pathogens. For instance, Zhaolin et al., (2015) reported the biocontrol effect of *B. licheniformis W10* on peach brown rot caused by Monilinia fructicola in the storage of peach fruits. It was observed that *B. licheniformis strain W10* had an antifungal activity that reduced peach fruit rot hence recorded as a potential biocontrol agent (Zhaolin et al. 2015). *B. licheniformis* have been reported as biocontrol of *vitis vinifera cv. Glera* (Nigris et al. 2018). The ability to produce systematic induced defense (ISR) via specific synthetic pathways that can produce metabolites protects the plant against pathogens. *B. licheniformis strain N1* has been linked as a biocontrol in managing tomato gray mold caused by *Botrytsis cinerea* (Lee et al. 2006). Lastly, *B. licheniformis* has been reported as a potential biocontrol of *Fusarium* root rot of coastal pine (Won et al. 2018; Y et al. 2020).

Bacillus pumilus is a spore-forming bacteria that is rod-shaped, Gram-positive, and aerobic. It resides in soils, and some colonize in the root area of some plants where *B. pumilus* has antibacterial and antifungal activity. *Bacillus pumilus* participates in a wide range of symbiotic relationships. *B. pumilus* can function as plant growth-promoting rhizobacteria within the rhizosphere of agriculturally significant plants such as red peppers (*Capsicum annuum L.*) and wheat (*Triticum aestivum*). In wheat, *B. pumilus* also induces plant resistance to Take-all (*Gaeumannomyces graminis*), a fungal disease that can significantly damage wheat crops (Hill, Baiano, and Barnes 2009; Sari, Etebarian, and Aminian 2007). Additionally, *B. pumilus* is thought to function as a plant growth-promoting endophyte in *Vitis vinifera* grape plants (Thomas 2004).

The study also obtained bacteria affiliated to members of the class Gammaproteobacteria. The genera in this class include *Alcaligenes* and *pseudomonas*. This concurs with earlier studies on Lake Elmenteita, where BLAST analysis of the partial sequences shows that 60 % of isolates belonged to the class Gammaproteobacteria. These were affiliated with Halomonas, Marinospirillum, and Idiomarina species (Mwirichia et al., 2010). In another study by Grant (2004), forty

cloned sequences were found to be like that of known bacterial isolates (>97 % sequence similarity), represented by the species of the genera Gammaproteobacteria.

Alcaligenaceae bacterium strain S10 (MT295372.1) is a gram-negative, aerobic, rod-shaped bacteria and capable of growing in different conditions. For instance, at pH 5.0-7.0, the temperature of 25-40°C and salinity of 0-0.5M. The results agree with previous research findings, which identified similar physiochemical characteristics (Kambura et al. 2013; Mulango et al. 2020; Nyakeri et al. 2018). In terms of enzymatic production, the results indicate the ability to produce protease, pectinase, phosphatase, chitinase, HCN, and IAA. The metabolites are used as a defense mechanism and are therefore capable of inhibiting the growth of the pathogen. Similarly, they play a big role in the growth and development of the plant. For instance, the ability to produce phosphatase and IAA for enhancement of plant growth and development. Similar results have been reported by (Mulango et al. 2020; Ngetha et al. 2019; Nyakeri et al. 2018). In terms of biocontrol, limited research has been done to quantify the *Alcaligenaceae bacterium's* ability to inhibit the fungal pathogen's growth. However, they have been used as potential nitrifiers (Kalniņš et al. 2020). Additionally, the whole genome sequences *alicaligene sp* contain protein-coding genes responsible for denitrification pathways, a network associated with phenolic compounds degradation, and HCN and siderophores synthesis (Felestrino et al. 2020).

Pseudomonas spp has been previously isolated in L. Bogoria (Antony et al. 2012; Duckworth et al. 1996, 2000) and L. Magadi (Eunice et al. 2020; Nyakeri et al. 2018). They are gram-negative and rod-shaped bacteria with colony morphology of large, opaque, flat with irregular margins, and distinctively fruity odor colonies. The results show that *pseudomonas spp* can grow at salinity, varied pH, and temperature range of 20-40°C. This agrees with previous findings, indicating that *pseudomonas* can induce salinity tolerance (Egamberdieva et al., 2015) and grow at a 5.5-10 (Xiong et al. 1996). The results (Figure 4.18;

Table **4.7**) indicate that they can produce different enzymes such as IAA, HCN, protease, chitinase, phosphatase, and pectinase. *Pseudomonas spp* produces phosphatases catalyzing the hydrolysis of phytic acid, thereby releasing a usable form of inorganic Phosphatase for the plants (Weller 2007a). Bacteria with phytase activity have been isolated from the rhizosphere and proposed to promote plant growth in soils with high content of organic Phosphatase. Studies have revealed that phytase-producing rhizobacteria not only harbor the ability to mineralize phytate but also harbor other PGPR activities, such as the production of indole acetic acid, siderophore, volatiles, and ammonia.

They are capable of producing HCN and HCN by beneficial rhizobacteria have been studied as a biocontrol mechanism (i.e., antibiosis) displayed by several biocontrol agents (Cernava et al. 2015). The ability of pseudomonas to be used as biocontrol have been reported by various research findings (Egamberdieva, Jabborova, and Hashem 2015; Gómez-Lama Cabanás et al. 2018; Weller 2007a).

The ability to substantially lowered the root mortality is evident for the presence of fewer root dead cells in this strain treatment due to the presence of inhibitory enzymes associated with the killing of pathogen and diminishing population in the rhizosphere and thus curbing their entry inside the root tissue (Abbas et al. 2019; Ajayi-Oyetunde and Bradley 2018; Akrami et al. 2013; Aydi et al. 2016; Chang et al. 2008; Grady et al. 2019; Kim et al. 2021). *Bacillus spp* can produce dehydrogenase enzymes mainly linked with the mitochondrial respiratory function in the living tissue, hence determining the viability of the plant survival (Dukare and Paul 2021; Jiang et al. 2018; Nigris et al. 2018; Zhao et al. 2017). *Bacillus licheniformis* had the lowest root rot mortality, due to its ability to secrete chitinase that degrades chitin. Chitin is the major component of fungal cell walls, makes it easier to protect the seedling (Nigris et al. 2018; Won et al. 2018). The results agree with previous findings, which reported the ability of *Bacillus licheniformis* to have a lower root rot mortality on hyphae of *F. oxysporum* (Idris,

Labuschagne, and Korsten 2007). Like *bacillus subtilis* and *bacillus velezensis*, the mechanism of defense against the tested pathogen is similar.

Further, *Bacillus licheniformis, Bacillus subtilis*, and *Bacillus velezensis* reduced wilt disease incidence in bean plantlets. The previous studies have reported the ability of the Bacillus species to reduce fusarium and Rhizoctonia wilt diseases (Abbas et al. 2019). According to Abbas et al., (2019); Mahmoudi & Naderi, (2017), many bacillus species can reduce wilt incidence due to the ability to produce antifungal arsenals and priming host immunity against harmful pathogens. *Bacillus* species can synthesize 45 antimicrobial compounds majorly comprising antifungal cyclic lipopeptides. The metabolites can curb the growth, metabolism, and pathogenesis of many fungal phytopathogens. The results are supported by a report from which can reduce wilt incidence caused by various pathogens in the host plant (Ashraf et al. 2020; Edwards and Seddon 2000; Gossen et al. 2016; Jiao et al. 2021; Mahmoudi and Naderi 2017; Won et al. 2018). Further, the bacteria's inoculation can improve the growth of the host plant compared to untreated control.

In planta assay results, the evaluated Plant growth promoting rhizobacteria (PGPR) considerably improved plant growth in shoot and root elongation, plant biomass, and chlorophyll content compared to the other treatments. The increase in plant height and biomass for biopriming seeds can be due to growth regulator hormones and phosphatase production. Seed biopriming results in quantitative changes in biochemical content of the seed and, therefore, improved membrane integrity. Seed coating with a bio-control agent is the most effective treatment for controlling root rot disease and increasing the plant's growth. The chlorophyll content was observed highest in bio-primed seeds compared to uninoculated. Therefore, the increase in chlorophyll content indicates the possibility of enhanced nutrient uptake that could have led to an observed increase in biomass and height of the bean plantlets. The improved chlorophyll content is also an indicator of increased fruit production. *Bacillus licheniformis, Bacillus subtilis*, and *Bacillus velezensis* have been linked to increasing plant biomass, height, and chlorophyll

content. Additionally, the suppression of root pathogens in the rhizosphere improves the host plant's rooting and growing condition. Similar findings have been demonstrated by Mahmood et al. (2020), wherein the inoculation of biocontrol microbes improved seedling germination and survival, vigor index, shoot/root elongation, and fresh/dry biomasses.

Elucitation of the host plants' defense system is another indirect way through biocontrol that makes plants more tolerant towards invading phytopathogens. Along these lines, the results the induction of systemic resistance (ISR) in common bean by the antagonistic species of bioactive bacteria (*Bacillus licheniformis, Bacillus subtilis*, and *Bacillus velezensis*) in response to wilt pathogen *F. solani* and *R. solani*. In the absence of bioactive bacterial bioagents, the bean plants inoculated with *F. solani* and *R. solani* had reduced defense-related antioxidants enzymes (PAL, PPO, and PO). Like the antioxidant enzyme, a higher phenolics compound was highly accumulated in bioactive bacterial primed common beans seedlings. The enhanced activity of the host plant defense system (PAL, PPO, and PO) is probably due to the secretion of siderophores, chitinase, and protease, which act as signaling molecules in the activation of systemic resistance (Rais et al. 2017)

5.2. Conclusion

The study showed that L. Bogoria and L. Magadi harbors diverse bacteria species. A total of 49 and 61 bacteria isolates were obtained from L. Bogoria and L. Magadi, respectively. For L. Bogoria, out of 49 bacteria isolates, 17 bacteria isolates (34.7%) had antifungal activity against *F. solani* and *R. solani*. On the other hand, for L. Magadi, out of 61 bacterial isolates, 25 bacterial isolates (41.0%) had antifungal activity against *F. solani* and *R. solani*. A number of bacteria isolated from L. Bogoria and L. Magadi showed antifungal activity against *F. solani* and *R. solani*.

The selected bioactive bacteria were characterized and identified. The bioactive isolates grew well at pH 7-10 though some grew at pH 5.0, the temperature of 20-60 with an optimum temperature of 30-35, Salinity concentration of 0-2M with an optimum of 0M.

The bioactive isolates produced different enzymes; protease, pectinase, phosphatase, chitinase, IAA, and HCN which are indicators of biocontrol agents.

Molecular characteristics of bioactive isolates indicated that all of them belong to the bacteria domain. 84.2% of the bioactive isolates were affiliated to genus *Bacillus*, phylum Firmicutes, and 15.8% affiliated to Gammaproteobacterial. Firmicutes were obtained from both Lakes, while Gammaproteobacterial was from L. Bogoria only.

Isolates tested in vivo (greenhouse) showed the ability to reduce root rot mortality, increase the plant's length, increase the biomass, reduce pre-and post-emergence wilt incidence, and produced phenolic and antioxidants enzymes compared to uninoculated control.

Therefore, tested isolates showed potential biocontrol activities and can be used to manage *F. solani* and *R. solani* in beans plantlets.

5.3 Recommendations

Soda lakes harbor diverse microorganisms that are important not only in managing plant pathogens but also in industrial use. And to obtain diverse variety, the need for modification of the protocol is necessary to allow the isolation of more diverse genera.

Further exploitation in the use of soda-lakes microbes for different plant pathogens needs to be done. Since there is a wide range of plant-pathogen affecting agriculture sectors, soda-lakes can be part of the solution.

Research should also be narrowed to isolating specific secondary metabolites (enzymes and growth promoters) produced by these microorganisms for biocontrol, biofertilizers

or industrial use. It will also help to elucidate the structure and biochemical characteristics of any novel bioactive metabolites detected.

Further research on the mode of action for the bioactive isolates should be done to determine the interaction between the biocontrol agent and the pathogen

Field trials should be conducted for those isolates tested in greenhouses to determine their effectiveness in the field on the tested plant pathogens. Additionally, toxicity tests should be conducted on the products and the isolates to assess if they are harmful to the human consumption of animals.

Whole-genome sequences of the bioactive isolates should assess some of the genes responsible for producing different secondary metabolites or salinity and drought-induced genes since they were obtained from harsh environments and whether the genes can be useful in the biotechnological field.

REFERENCES

Abbas, Aqleem, Shahid Ullah Khan, Wasim Ullah Khan, Tawfik A. Saleh, Muhammad Hafeez Ullah Khan, Sana Ullah, Ahmad Ali, and Muhammad Ikram. 2019. “Antagonist Effects of Strains of Bacillus Spp. against Rhizoctonia Solani for Their Protection against Several Plant Diseases: Alternatives to Chemical Pesticides.” *Comptes Rendus - Biologies* 342(5–6):124–35.

Abo-Bakr, Aya, Eman Mahmoud Fahmy, Fatma Badawy, Ashraf Oukasha Abd El-latif, and Saad Moussa. 2020. “Isolation and Characterization of the Local Entomopathogenic Bacterium, Bacillus Thuringiensis Isolates from Different Egyptian Soils.” *Egyptian Journal of Biological Pest Control* 30(1):1–9. doi: 10.1186/S41938-020-00250-Z/FIGURES/4.

Ahmed, Ahmed IS. 2017. “Biological Control of Potato Brown Leaf Spot Disease Caused by Alternaria Alternata Using Brevibacillus Formosus Strain DSM 9885 and Brevibacillus Brevis Strain NBRC 15304.” *Journal of Plant Pathology & Microbiology* 08(06). doi: 10.4172/2157-7471.1000413.

Ajayi-Oyetunde, O. O., and C. A. Bradley. 2018. “Rhizoctonia Solani: Taxonomy, Population Biology and Management of Rhizoctonia Seedling Disease of Soybean.” *Plant Pathology* 67(1):3–17.

Akrami, Mohammad, Hossein Karbalaei Khiavi, Haji Shikhlinski, and Hossein Khoshvaghtei. 2013. *Bio Controlling Two Pathogens of Chickpea Fusarium Solani and Fusarium Oxysporum by Different Combinations of Trichoderma Harzianum, Trichoderma Asperellum and Trichoderma Virens under Field Condition*. Vol. 1.

Akrami, Mohammad, Mohsen Sabzi, Farhad Baghbani Mehmandar, and Ehsan Khodadadi. 2012. “Effect of Seed Treatment with Trichoderma Harzianum and Trichoderma Asperellum Species for Controlling Fusarium Rot of Common Bean.” *Scholars Research Library Annals of Biological Research* 3(5):2187–89.

Al-Hazmi, A. S., and S. N. Al-Nadary. 2015. “Interaction between Meloidogyne Incognita and Rhizoctonia Solani on Green Beans.” *Saudi Journal of Biological Sciences* 22(5):570–74. doi: 10.1016/J.SJBS.2015.04.008.

Alabouvette, Claude, Chantal Olivain, Floriane L’Haridon, Sébastien Aimé, and Christian Steinberg. 2007. “Using Strains of Fusarium Oxysporum to Control Fusarium Wilts: Dream or Reality?” *NATO Security through Science Series A: Chemistry and Biology* 157–77. doi: 10.1007/978-1-4020-5799-1_8.

Amare Ayalew, Eshetu Belete. 2015. “Evaluation of Local Isolates of Trichoderma Spp. against Black Root Rot (Fusarium Solani) on Faba Bean.” *Journal of Plant Pathology & Microbiology* 06(06):1–5. doi: 10.4172/2157-7471.1000279.

Amongi, W., F. Kato, A. Male, S. Musoke, S. Sebuliba, B. Nakyanzi, C. Naluwooza, C. Acam, and C. Mukankusi. 2020. “Resistance of Andean Beans and Advanced Breeding Lines to Root Rots in Uganda.” *African Crop Science Journal* 28(3):389–409. doi: 10.4314/ACSJ.V28I3.5.

Andrés, Javier A., Nicolás A. Pastor, Mauricio Ganuza, Marisa Rovera, María Marta Reynoso, and Adriana Torres. 2016. “Biopesticides: An Eco-Friendly Approach for the Control of Soilborne Pathogens in Peanut.” Pp. 161–79 in *Microbial Inoculants in Sustainable Agricultural Productivity: Vol. 1: Research Perspectives*. Springer India.

Anon. n.d. “How Kenya’s Lake Bogoria Is Feeding the Global Biotech Industry.” Retrieved June 23, 2021 (https://www.unep.org/news-and-stories/story/how-kenyas-lake-bogoria-feeding-global-biotech-industry).

Antony, Chakkiath Paul, Deepak Kumaresan, Sindy Hunger, Harold L. Drake, J. Colin Murrell, and Yogesh S. Shouche. 2012. “Microbiology of Lonar Lake and Other Soda Lakes.” *The ISME Journal 2013 7:3* 7(3):468–76. doi: 10.1038/ismej.2012.137.

Asad-Uz-Zaman, Md, Mohammad Rejwan Bhuiyan, Mohammad Ashik Iqbal Khan, Md Khurshed Alam Bhuiyan, and Mohammad Abdul Latif. 2015. “Integrated Options for

the Management of Black Root Rot of Strawberry Caused by Rhizoctonia Solani Kuhn." *Comptes Rendus - Biologies* 338(2):112–20. doi: 10.1016/j.crvi.2014.11.006.

Asaka, Orie, and Makoto Shoda. 1996. "Biocontrol of Rhizoctonia Solani Damping-off of Tomato with Bacillus Subtilis RB14." *Applied and Environmental Microbiology* 62(11):4081–85. doi: 10.1128/aem.62.11.4081-4085.1996.

Ashraf, Hina, Tehmina Anjum, Saira Riaz, and Shahzad Naseem. 2020. "Microwave-Assisted Green Synthesis and Characterization of Silver Nanoparticles Using Melia Azedarach for the Management of Fusarium Wilt in Tomato." *Frontiers in Microbiology* 11:238. doi: 10.3389/fmicb.2020.00238.

Atafar, Zahra, Alireza Mesdaghinia, Jafar Nouri, Mehdi Homaee, Masoud Yunesian, Mehdi Ahmadimoghaddam, and Amir Hossein Mahvi. 2008. "Effect of Fertilizer Application on Soil Heavy Metal Concentration." *Environmental Monitoring and Assessment 2008 160:1* 160(1):83–89. doi: 10.1007/S10661-008-0659-X.

Aydi, Rania, Ben Abdallah, Hayfa Jabnoun-Khiareddine, Ahlem Nefzi, Sonia Mokni-Tlili, and Mejda Daami-Remadi. 2016. "Endophytic Bacteria from Datura Stramonium for Fusarium Wilt Suppression and Tomato Growth Promotion." *J Microb Biochem Technol* 8(1):30–041. doi: 10.4172/1948-5948.1000259.

Baazeem, Alaa, Abdulaziz Almanea, Palanisamy Manikandan, Mohammed Alorabi, Ponnuswamy Vijayaraghavan, and Ahmed Abdel-Hadi. 2021. "In Vitro Antibacterial, Antifungal, Nematocidal and Growth Promoting Activities of Trichoderma Hamatum FB10 and Its Secondary Metabolites." *Journal of Fungi (Basel, Switzerland)* 7(5). doi: 10.3390/jof7050331.

Babirye, Immaculate, Florence Nakazi, Eliud Abucheli Birachi, Jackline Bonabana Wabbi, Michael Adrogu Ugen, and Gabriel Elepu. 2023. "Exploring Processed Common Beans Market in Kenya: Implications for the Business Community."

Http://Www.Editorialmanager.Com/Cogentagri 9(1). doi: 10.1080/23311932.2023.2175538.

Barnabás, Beáta, Katalin Jäger, and Attila Fehér. 2008. "The Effect of Drought and Heat Stress on Reproductive Processes in Cereals." *Plant, Cell and Environment* 31(1):11–38. doi: 10.1111/j.1365-3040.2007.01727.x.

Belbahri, Lassaad, Ali Chenari Bouket, Imen Rekik, Faizah N. Alenezi, Armelle Vallat, Lenka Luptakova, Eva Petrovova, Tomasz Oszako, Semcheddine Cherrad, Sébastien Vacher, and Mostafa E. Rateb. 2017a. "Comparative Genomics of Bacillus Amyloliquefaciens Strains Reveals a Core Genome with Traits for Habitat Adaptation and a Secondary Metabolites Rich Accessory Genome." *Frontiers in Microbiology* 8(AUG). doi: 10.3389/FMICB.2017.01438.

Belbahri, Lassaad, Ali Chenari Bouket, Imen Rekik, Faizah N. Alenezi, Armelle Vallat, Lenka Luptakova, Eva Petrovova, Tomasz Oszako, Semcheddine Cherrad, Sébastien Vacher, and Mostafa E. Rateb. 2017b. "Comparative Genomics of Bacillus Amyloliquefaciens Strains Reveals a Core Genome with Traits for Habitat Adaptation and a Secondary Metabolites Rich Accessory Genome." *Frontiers in Microbiology* 8(AUG):1438. doi: 10.3389/FMICB.2017.01438.

Belete, Eshetu. 2015. "Antagonistic Effect of Native Bacillus Isolates against Black Root Rot of Faba Bean." *African Crop Science Journal;23,(2015) Pagination 249,259*.

Berendsen, Erwin M., Rosella A. Koning, Jos Boekhorst, Anne de Jong, Oscar P. Kuipers, and Marjon H. J. Wells-Bennik. 2016. "High-Level Heat Resistance of Spores of Bacillus Amyloliquefaciens and Bacillus Licheniformis Results from the Presence of a SpoVA Operon in a Tn1546 Transposon." *Frontiers in Microbiology* 0(DEC):1912. doi: 10.3389/FMICB.2016.01912.

Bett, Sylvya Chepkemoi. 2020. "Bacteria Isolated from Lake Magadi Soil with Potential for Utilization of Different Substrates."

Beveridge, T. J. 2001. “Use of the Gram Stain in Microbiology.” *Biotechnic and Histochemistry* 76(3):111–18. doi: 10.1080/bih.76.3.111.118.

Birachi, E. A., J. Ochieng, D. Wozemba, C. Ruraduma, M. C. Niyuhire, and D. Ochieng. 2011. “FACTORS INFLUENCING SMALLHOLDER FARMERS’ BEAN PRODUCTION AND SUPPLY TO MARKET IN BURUNDI.” *African Crop Science Journal* 19(4):335–42.

Birnbaum, Linda S. 2008. “The Effect of Environmental Chemicals on Human Health.” *Fertility and Sterility* 89(2):e31. doi: 10.1016/J.FERTNSTERT.2007.12.022.

Blair, Matthew W., Paulo Izquierdo, Carolina Astudillo, and Michael A. Grusak. 2013. “A Legume Biofortification Quandary: Variability and Genetic Control of Seed Coat Micronutrient Accumulation in Common Beans.” *Frontiers in Plant Science* 4(JUL):275. doi: 10.3389/FPLS.2013.00275.

Borriss, Rainer, Xiao Hua Chen, Christian Rueckert, Jochen Blom, Anke Becker, Birgit Baumgarth, Ben Fan, Rüdiger Pukall, Peter Schumann, Cathrin Spröer, Helmut Junge, Joachim Vater, Alfred Pühler, and Hans Peter Klenk. 2011. “Relationship of Bacillus Amyloliquefaciens Clades Associated with Strains DSM 7 T and FZB42 T: A Proposal for Bacillus Amyloliquefaciens Subsp. Amyloliquefaciens Subsp. Nov. and Bacillus Amyloliquefaciens Subsp. Plantarum Subsp. Nov. Based on Complete Genome Sequence Comparisons.” *International Journal of Systematic and Evolutionary Microbiology* 61(8):1786–1801. doi: 10.1099/IJS.0.023267-0.

Castro-Guerrero, Norma A., Mariel C. Isidra-Arellano, David G. Mendoza-Cozatl, and Oswaldo Valdés-López. 2016. “Common Bean: A Legume Model on the Rise for Unraveling Responses and Adaptations to Iron, Zinc, and Phosphate Deficiencies.” *Frontiers in Plant Science* 7:391–417. doi: 10.3389/FPLS.2016.00600.

Cawoy, Hélène, Martin Mariutto, Guillaume Henry, Christophe Fisher, Natallia Vasilyeva, Philippe Thonart, Jacques Dommes, and Marc Ongena. 2014. “Plant Defense

Stimulation by Natural Isolates of Bacillus Depends on Efficient Surfactin Production." *Http://Dx.Doi.Org/10.1094/MPMI-09-13-0262-R* 27(2):87–100. doi: 10.1094/MPMI-09-13-0262-R.

Celmeli, Tugce, Hatice Sari, Huseyin Canci, Duygu Sari, Alper Adak, Tuba Eker, and Cengiz Toker. 2018. "The Nutritional Content of Common Bean (Phaseolus Vulgaris l.) Landraces in Comparison to Modern Varieties." *Agronomy* 8(9):166. doi: 10.3390/agronomy8090166.

Cernava, Tomislav, Ines A. Aschenbrenner, Martin Grube, Stefan Liebminger, and Gabriele Berg. 2015. "A Novel Assay for the Detection of Bioactive Volatiles Evaluated by Screening of Lichen-Associated Bacteria." *Frontiers in Microbiology* 0(MAY):398. doi: 10.3389/FMICB.2015.00398.

Chang, K. F., S. F. Hwang, B. D. Gossen, G. D. Turnbull, H. Wang, and R. J. Howard. 2008. "Effects of Inoculum Density, Temperature, Seeding Depth, Seeding Date and Fungicidal Seed Treatment on the Impact of Rhizoctonia Solani on Lentil." Pp. 799–809 in *Canadian Journal of Plant Science*. Vol. 88. Agricultural Institute of Canada.

Chowdhury, Soumitra Paul, Kristin Dietel, Manuela Rändler, Michael Schmid, Helmut Junge, Rainer Borriss, Anton Hartmann, and Rita Grosch. 2013. "Effects of Bacillus Amyloliquefaciens FZB42 on Lettuce Growth and Health under Pathogen Pressure and Its Impact on the Rhizosphere Bacterial Community." *PLOS ONE* 8(7):e68818. doi: 10.1371/JOURNAL.PONE.0068818.

DeLong, and Pace. 2001. "Environmental Diversity of Bacteria and Archaea." *Systematic Biology* 50(4):470–78. doi: 10.1080/10635150118513.

Duckworth, AW, WD Grant, BE Jones, D. Meijer, MC Márquez, and A. Ventosa. 2000. "Halomonas Magadii Sp. Nov., a New Member of the Genus Halomonas, Isolated from a Soda Lake of the East African Rift Valley." *Extremophiles : Life under Extreme Conditions* 4(1):53–60. doi: 10.1007/S007920050007.

Duckworth, AW, WD Grant, BE Jones, and R. Van Steenbergen. 1996. "Phylogenetic Diversity of Soda Lake Alkaliphiles." *FEMS Microbiol Ecol* 19(3):181–91. doi: 10.1111/j.1574-6941.1996.tb00211.x.

Dukare, Ajinath, and Sangeeta Paul. 2021. "Biological Control of Fusarium Wilt and Growth Promotion in Pigeon Pea (Cajanus Cajan) by Antagonistic Rhizobacteria, Displaying Multiple Modes of Pathogen Inhibition." *Rhizosphere* 17:100278. doi: 10.1016/j.rhisph.2020.100278.

Dukare, Ajinath, Sangeeta Paul, and Asha Arambam. 2020. "Isolation and Efficacy of Native Chitinolytic Rhizobacteria for Biocontrol Activities against Fusarium Wilt and Plant Growth Promotion in Pigeon Pea (Cajanus Cajan L.)." *Egyptian Journal of Biological Pest Control* 30(1). doi: 10.1186/s41938-020-00256-7.

Duku, Confidence, Annemarie Groot, Teferi Demissie, Joseph Muhwanga, Oscar Nzoka, and John Recha. 2020. "Common Beans Kenya: Climate Risk Assessment."

Ebrahim, Saboki, K. Usha, and Bhupinder Singh. 2011. "Pathogenesis-Related (PR)-Proteins: Chitinase and β-1,3-Glucanase in Defense Mechanism against Malformation in Mango (Mangifera Indica L.)." *Scientia Horticulturae* 130(4):847–52. doi: 10.1016/J.SCIENTA.2011.09.014.

Edwards, SG., and B. Seddon. 2001. "Mode of Antagonism of Brevibacillus Brevis against Botrytis Cinerea in Vitro." *Journal of Applied Microbiology* 91(4):652–59. doi: 10.1046/J.1365-2672.2001.01430.X.

Edwards, SG, and B. Seddon. 2000. "Selective Medium Based on Tyrosine Metabolism for the Isolation and Enumeration of Brevibacillus Brevis (Bacillus Brevis)." *Letters in Applied Microbiology* 31(5):395–99. doi: 10.1046/J.1472-765X.2000.00838.X.

Egamberdieva, Dilfuza, Dilfuza Jabborova, and Abeer Hashem. 2015. "Pseudomonas Induces Salinity Tolerance in Cotton (Gossypium Hirsutum) and Resistance to Fusarium Root

Rot through the Modulation of Indole-3-Acetic Acid." *Saudi Journal of Biological Sciences* 22(6):773. doi: 10.1016/J.SJBS.2015.04.019.

El-Mohamedy, R., and M. A. Alla. 2013. "Bio-Priming Seed Treatment for Biological Control of Soil Borne Fungi Causing Root Rot of Green Bean (Phaseolus Vulgaris L.)." *Undefined*.

Elmahdi, Salha, Jugah Kadir, Mahmud Tengku Muda Mohamed, Ganesan Vadamalai, and Shamima Akter. 2015. "Isolation, Screening and Characterization of Effective Microbes with Potential for Biological Control of Fusarium Wilt of Rock Melon." *World Journal of Agricultural Research* 3(1):11–16. doi: 10.12691/WJAR-3-1-3.

Etesami, Hassan, Hossein Ali Alikhani, and Hossein Mirseyed Hosseini. 2015. "Indole-3-Acetic Acid (IAA) Production Trait, a Useful Screening to Select Endophytic and Rhizosphere Competent Bacteria for Rice Growth Promoting Agents." *MethodsX* 2:72. doi: 10.1016/J.MEX.2015.02.008.

Eunice, Mulango, Kasili Remmy, Mwirichia Romano, Kambura Anne-Kelly, and Muhonja Christabel. 2020. "Isolation and Characterization of Haloalkaliphilic Bacteria from the Hot Springs of Lake Magadi." *African Journal of Microbiology Research* 14(7):294–302. doi: 10.5897/ajmr2018.8953.

FAO. 2011. "Food Agricultural Organization of United Nations, Statistical Databases FAOSTAT."

Fatiha Lazreg, Lakhdar Belabid, Jose Sanchez, Gallego Eduardo, and Bassam Bayaa. 2014. "Pathogenicity of Fusarium Spp. Associated with Diseases of Aleppo-Pine Seedlings in Algerian Forest Nurseries." *Journal of Forest Science* 60(3):115–20.

Felestrino, Érica Barbosa, Angélica Bianchini Sanchez, Washington Luiz Caneschi, Camila Gracyelle de Carvalho Lemes, Renata de Almeida Barbosa Assis, Isabella Ferreira Cordeiro, Natasha Peixoto Fonseca, Morghana Marina Villa, Izadora Tabuso Vieira, Luciana Hiromi Yoshino Kamino, Flávio Fonseca do Carmo, Aline Maria da Silva, Andrew Maltez Thomas, José Salvatore Leister Patané, Fernanda Carla Ferreira,

Leandro Grassi de Freitas, Alessandro de Mello Varani, Jesus Aparecido Ferro, Robson Soares Silva, Nalvo Franco Almeida, Camila Carrião Machado Garcia, João Carlos Setubal, and Leandro Marcio Moreira. 2020. "Complete Genome Sequence and Analysis of Alcaligenes Faecalis Strain Mc250, a New Potential Plant Bioinoculant." *PLOS ONE* 15(11):e0241546. doi: 10.1371/JOURNAL.PONE.0241546.

Fierer, Noah, and Robert B. Jackson. 2006. "The Diversity and Biogeography of Soil Bacterial Communities." *Proceedings of the National Academy of Sciences* 103(3):626–31. doi: 10.1073/PNAS.0507535103.

Fravel, D. R. 2005. "Commercialization and Implementation of Biocontrol." *Annual Review of Phytopathology* 43:337–59.

Gang, Shraddha, Sheetal Sharma, Meenu Saraf, Martin Buck, and Jörg Schumacher. 2019. "Analysis of Indole-3-Acetic Acid (IAA) Production in Klebsiella by LC-MS/MS and the Salkowski Method." *BIO-PROTOCOL* 9(9). doi: 10.21769/BIOPROTOC.3230.

Gerbore, J., N. Benhamou, J. Vallance, G. Le Floch, D. Grizard, C. Regnault-Roger, and P. Rey. 2014. "Biological Control of Plant Pathogens: Advantages and Limitations Seen through the Case Study of Pythium Oligandrum." *Environmental Science and Pollution Research* 21(7):4847–60. doi: 10.1007/s11356-013-1807-6.

Gómez-Lama Cabanás, Carmen, Garikoitz Legarda, David Ruano-Rosa, Paloma Pizarro-Tobías, Antonio Valverde-Corredor, José L. Niqui, Juan C. Triviño, Amalia Roca, and Jesús Mercado-Blanco. 2018. "Indigenous Pseudomonas Spp. Strains from the Olive (Olea Europaea L.) Rhizosphere as Effective Biocontrol Agents against Verticillium Dahliae: From the Host Roots to the Bacterial Genomes." *Frontiers in Microbiology* 0(FEB):277. doi: 10.3389/FMICB.2018.00277.

Gossen, Bruce D., Robert L. Conner, Kan Fa Chang, Julie S. Pasche, Debra L. McLaren, Maria A. Henriquez, Syama Chatterton, and Sheau Fang Hwang. 2016. "Identifying and

Managing Root Rot of Pulses on the Northern Great Plains." *Plant Disease* 100(10):1965–78. doi: 10.1094/PDIS-02-16-0184-FE.

Goto, K., R. Fujita, Y. Kato, M. Asahara, and A. Yokota. 2004. "Reclassification of Brevibacillus Brevis Strains NCIMB 13288 and DSM 6472 (=NRRL NRS-887) as Aneurinibacillus Danicus Sp. Nov. and Brevibacillus Limnophilus Sp. Nov." *International Journal of Systematic and Evolutionary Microbiology* 54(Pt 2):419–27. doi: 10.1099/IJS.0.02906-0.

Grady, Elliot Nicholas, Jacqueline MacDonald, Margaret T. Ho, Brian Weselowski, Tim McDowell, Ori Solomon, Justin Renaud, and Ze-Chun Yuan. 2019. "Characterization and Complete Genome Analysis of the Surfactin-Producing, Plant-Protecting Bacterium Bacillus Velezensis 9D-6." *BMC Microbiology 2019 19:1* 19(1):1–14. doi: 10.1186/S12866-018-1380-8.

Hammerschmidt, R., E. M. Nuckles, and J. Kuć. 1982. "Association of Enhanced Peroxidase Activity with Induced Systemic Resistance of Cucumber to Colletotrichum Lagenarium." *Physiological Plant Pathology* 20(1):73–82. doi: 10.1016/0048-4059(82)90025-X.

Harper, David M., R. Brooks Childress, Maureen M. Harper, Rosalind R. Boar, Phil Hickley, Suzanne C. Mills, Nickson Otieno, Tony Drane, Ekkehard Vareschi, Oliver Nasirwa, Wanjiru E. Mwatha, Joanna P. E. C. Darlington, and Xavier Escuté-Gasulla. 2003. "Aquatic Biodiversity and Saline Lakes: Lake Bogoria National Reserve, Kenya." *Aquatic Biodiversity* 259–76. doi: 10.1007/978-94-007-1084-9_19.

Hartman, Diane. 2011. "Perfecting Your Spread Plate Technique." *Journal of Microbiology & Biology Education* 12(2):204–5. doi: 10.1128/JMBE.V12I2.324.

Hill, J. E., J. C. F. Baiano, and A. C. Barnes. 2009. "Isolation of a Novel Strain of Bacillus Pumilus from Penaeid Shrimp That Is Inhibitory against Marine Pathogens." *Journal of Fish Diseases* 32(12):1007–16. doi: 10.1111/J.1365-2761.2009.01084.X.

Hwang, Sun Kyoung, Chang Gi Back, Nang Kyu Kyu Win, Myung Kyum Kim, Hee Dae Kim, In Kyu Kang, Sang Chul Lee, and Hee Young Jung. 2012. “Occurrence of Bacterial Rot of Onion Caused by Bacillus Amyloliquefaciens in Korea.” *Journal of General Plant Pathology* 78(3):227–32. doi: 10.1007/S10327-012-0376-8.

Idris, H. Ahmed, N. Labuschagne, and L. Korsten. 2007. “Screening Rhizobacteria for Biological Control of Fusarium Root and Crown Rot of Sorghum in Ethiopia.” *Biological Control* 40(1):97–106. doi: 10.1016/j.biocontrol.2006.07.017.

Jabnoun-Khiareddine H. 2018. “Rhizoctonia Root Rot of Pepper (Capsicum Annuum): Comparative Pathogenicity of Causal Agent and Biocontrol Attempt Using Fungal and Bacterial Agents.” doi: 10.4172/2157-7471.1000431.

Jacobsen, S. E., C. R. Jensen, and F. Liu. 2012. “Improving Crop Production in the Arid Mediterranean Climate.” *Field Crops Research* 128:34–47.

Jangir, Monika, Ritika Pathak, Satyawati Sharma, and Shilpi Sharma. 2018. “Biocontrol Mechanisms of Bacillus Sp., Isolated from Tomato Rhizosphere, against Fusarium Oxysporum f. Sp. Lycopersici.” *Biological Control* 123:60–70. doi: 10.1016/J.BIOCONTROL.2018.04.018.

Jiang, Chun Hao, Meng Jie Liao, Hong Kai Wang, Ming Zi Zheng, Jian Jun Xu, and Jian Hua Guo. 2018. “Bacillus Velezensis, a Potential and Efficient Biocontrol Agent in Control of Pepper Gray Mold Caused by Botrytis Cinerea.” *Biological Control* 126:147–57. doi: 10.1016/J.BIOCONTROL.2018.07.017.

Jiao, Xiurong, Yoko Takishita, Guisheng Zhou, and Donald L. Smith. 2021. “Plant Associated Rhizobacteria for Biocontrol and Plant Growth Enhancement.” *Frontiers in Plant Science* 12:634796. doi: 10.3389/fpls.2021.634796.

JM, Palazzini, Dunlap CA, Bowman MJ, and Chulze SN. 2016. “Bacillus Velezensis RC 218 as a Biocontrol Agent to Reduce Fusarium Head Blight and Deoxynivalenol Accumulation:

Genome Sequencing and Secondary Metabolite Cluster Profiles." *Microbiological Research* 192:30–36. doi: 10.1016/J.MICRES.2016.06.002.

Kalniņš, Mārtiņš, Andrejs Bērziņš, Dita Gudrā, Kaspars Megnis, Dāvids Fridmanis, Pavel Danilko, Olga Muter, Mārtiņš Kalniņš, Andrejs Bērziņš, Dita Gudrā, Kaspars Megnis, Dāvids Fridmanis, Pavel Danilko, and Olga Muter. 2020. "Selective Enrichment of Heterotrophic Nitrifiers <em>Alcaligenaceae</Em> and <em>Alcanivorax</Em> Spp. from Industrial Wastewaters." *AIMS Microbiology 2020 1:32* 6(1):32–42. doi: 10.3934/MICROBIOL.2020002.

Kambura, AK, RK Mwirichia, J. Ngaira, and HI Boga. 2013. "Isolation and Characterization of Bacterial Isolates from Lake Magadi." *Journal of Tropical Microbiology and Biotechnology* 8(1). doi: 10.4314/jtmb.v8i1.

Kambura, Anne Kelly, Kachiuru Mwirichia Romano, Wekesa Kasili Remmy, Nderitu Karanja Edward, Mae Makonde Huxley, and Iddi Boga Hamadi. 2016. "Diversity of Fungi in Sediments and Water Sampled from the Hot Springs of Lake Magadi and Little Magadi in Kenya." *African Journal of Microbiology Research* 10(10):330–38. doi: 10.5897/AJMR2015.7879.

Katungi, E., d karanja, D. Wozemba, t Mutuoki, and JC Rubyogo. 2012. "A Cost Benefit Analysisof Farmer Based Seed Production for Common Bean in Kenya." *African Crop Science Journal* 19(4):409–15. doi: 10.4314/acsj.v19i4.

Katungi, Enid, and Andrew Farrow Geagrofía. 2009. "Common Bean in Eastern and Southern Africa: A Situation and Outlook Analysis RUFORUM POTATO MARKET ENHANCEMENT View Project Seed Security Assessments View Project."

KATZNELSON, H., and H. BOSE. 1959. "Metabolic Activity and Phosphate-Dissolving Capability of Bacterial Isolates from Wheat Roots, Rhizosphere, and Non-Rhizosphere Soil." *Canadian Journal of Microbiology* 5(1):79–85. doi: 10.1139/M59-010.

Kim, Young Soo, Younmi Lee, Wonsu Cheon, Jungwook Park, Hyeok-Tae Kwon, Kotnala Balaraju, Jungyeon Kim, Yeo Jun Yoon, and Yongho Jeon. 2021. "Characterization of Bacillus Velezensis AK-0 as a Biocontrol Agent against Apple Bitter Rot Caused by Colletotrichum Gloeosporioides." *Scientific Reports 2021 11:1* 11(1):1–14. doi: 10.1038/s41598-020-80231-2.

Kiplimo, Denis, Julius Mugweru, Sarah Kituyi, Alex Kipnyargis, and Romano Mwirichia. 2019. "Diversity of Esterase and Lipase Producing Haloalkaliphilic Bacteria from Lake Magadi in Kenya." *Journal of Basic Microbiology* 59(12):1173–84. doi: 10.1002/JOBM.201900353.

Knodel, J. J., P. B. Beauzay, D. W. Franzen, H. J. Kandel, S. G. Markell, J. M. Osorno, and R. K. Zollinger. 2002. *Dry Bean Grower Survey of Pest Problems and Pesticide Use in Minnesota and North Dakota*.

Krulwich, T. A., R. Agus, M. Schneier, and A. A. Guffanti. 1985. "Buffering Capacity of Bacilli That Grow at Different PH Ranges." *Journal of Bacteriology* 162(2):768.

Lauber, Christian L., Micah Hamady, Rob Knight, and Noah Fierer. 2009. "Pyrosequencing-Based Assessment of Soil PH as a Predictor of Soil Bacterial Community Structure at the Continental Scale." *Applied and Environmental Microbiology* 75(15):5111–20. doi: 10.1128/AEM.00335-09.

Lawlor, Kirsten, Bruce P. Knight, Vera L. Barbosa-Jefferson, Peter W. Lane, Andrew K. Lilley, Graeme I. Paton, Steve P. McGrath, Síle M. O'Flaherty, and Penny R. Hirsch. 2000. "Comparison of Methods to Investigate Microbial Populations in Soils under Different Agricultural Management." *FEMS Microbiology Ecology* 33(2):129–37. doi: 10.1111/J.1574-6941.2000.TB00735.X.

Lee, Jae Pil, Seon Woo Lee, Choul Sung Kim, Ji Hee Son, Ju Hee Song, Kwang Youll Lee, Hyun Ju Kim, Soon Je Jung, and Byung Ju Moon. 2006. "Evaluation of Formulations of Bacillus Licheniformis for the Biological Control of Tomato Gray Mold Caused by

Botrytis Cinerea.” *Biological Control* 37(3):329–37. doi: 10.1016/J.BIOCONTROL.2006.01.001.

Leep, R. 2016. “Fusarium Root Rot of Common Beans (E2876) - MSU Extension.” Retrieved July 17, 2021 (https://www.canr.msu.edu/resources/fusarium_root_rot_of_common_beans_e3876).

Li, Hui, Ying Guan, Yilun Dong, Lu Zhao, Songhao Rong, Wenqian Chen, Miaomiao Lv, Hong Xu, Xiaoling Gao, Rongjun Chen, Lihua Li, and Zhengjun Xu. 2018. “Isolation and Evaluation of Endophytic Bacillus Tequilensis GYLH001 with Potential Application for Biological Control of Magnaporthe Oryzae.” *PLoS ONE* 13(10). doi: 10.1371/JOURNAL.PONE.0203505.

Li, Z., Y. Kawamura, O. Shida, S. Yamagata, T. Deguchi, and T. Ezaki. 2002. “Bacillus Okuhidensis Sp. Nov., Isolated from the Okuhida Spa Area of Japan.” *International Journal of Systematic and Evolutionary Microbiology* 52(Pt 4):1205–9. doi: 10.1099/00207713-52-4-1205.

Mahmoud, A., and A. F. A. Mahmoud. 2016. “Evaluation of Certain Antagonistic Fungal Species for Biological Control of Faba Bean Wilt Disease Incited by Fusarium Oxysporum.” *Journal of Phytopathology and Pest Management* 3(2):1–14.

Mahmoudi, Esmaeil, and Davood Naderi. 2017. *Anti-Fungal and Bio-Control Properties of Chitinolytic Bacteria against Safflower Fusarium Root Rot*. Vol. 2017. Journal of Crop Protection.

Maina, Michuki, Paul Mwaniki, Edwin Odira, Nduku Kiko, Jacob McKnight, Constance Schultsz, Mike English, and Olga Tosas-Auguet. 2020. “Antibiotic Use in Kenyan Public Hospitals: Prevalence, Appropriateness and Link to Guideline Availability.” *International Journal of Infectious Diseases* 99:10–18. doi: 10.1016/J.IJID.2020.07.084.

Manna, M. C., A. Swarup, R. H. Wanjari, H. N. Ravankar, B. Mishra, M. N. Saha, Y. V. Singh, D. K. Sahi, and P. A. Sarap. 2005. “Long-Term Effect of Fertilizer and Manure

Application on Soil Organic Carbon Storage, Soil Quality and Yield Sustainability under Sub-Humid and Semi-Arid Tropical India." *Field Crops Research* 93(2–3):264–80. doi: 10.1016/J.FCR.2004.10.006.

Marach, Sasiprapha, Tiyakhon Chatnaparat, Supot Kasem, and Sutruedee Prathuangwong. 2020. "K2 a Newly Isolated Strain of Bacillus Amyloliquefaciens Regulates Responsive Proteins for Its Survival and Promotes Plant Growth of Rice Seedlings against Bacterial Leaf Blight and Salt Stresses." *Thai Agricultural Research Journal* 38(3). doi: 10.14456/THAIDOA-AGRES.2020.24.

Mayer, AM, E. Harel, R. Ben-Shaul- Phytochemistry, and Undefined 1966. 1965. "Assay of Catechol Oxidase—a Critical Comparison of Methods." *Elsevier*.

Miller, Gail Lorenz. 1959. "Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar." *Analytical Chemistry* 31(3):426–28. doi: 10.1021/AC60147A030/ASSET/AC60147A030.FP.PNG_V03.

Morita, Richard Y., Koki Horikoshi, and William D. Grant. 1999. "Extremophiles: Microbial Life in Extreme Environments." *BioScience* 49(3):245.

Mulango, Eunice, Remmy Kasili, Romano Mwirichia, Anne-Kelly Kambura, and Christabel Muhonja. 2020. "Isolation and Characterization of Haloalkaliphilic Bacteria from the Hot Springs of Lake Magadi." *African Journal of Microbiology Research* 14(7):294–302. doi: 10.5897/AJMR2018.8953.

Mullane, Patrick. 2010. "Research Guides: BIO 2410: Microbiology: Bacterial Colonial Morphology."

Muriungi JS, Mutitu EW, and Siboe MG. 2013. *Biocontrol of Fusarium Root Rot in Beans by Antagonistic Trichoderma Fungi*. Vol. 3. University of Nairobi.

Muriungi, SJ, Mutitu Ew, J. W. Muthomi, and Joseph Muriungi. 2014. *EFFICACY OF CULTURAL METHODS IN THE CONTROL OF RHIZOCTONIA SOLANI STRAINS CAUSING TOMATO DAMPING OFF IN KENYA*. Vol. 14. doi: 10.4314/ajfand.v14i2.

Mutari, Bruce, Julia Sibiya, Eileen Bongweh Nchanji, and Kennedy Simango. 2020. "Farmers' Perceptions of Navy Bean (Phaseolus Vulgaris L.) Production Constraints, Preferred Traits, Farming Systems and Their Implications on Bean Breeding: A Case Study from South East Lowveld Region of Zimbabwe." doi: 10.21203/rs.3.rs-116798/v1.

Mwaniki, A. 2002. "Assessment of Bean Production Constraints and Seed Quality and Health of Improved Common Bean Seed." *Undefined*.

Mwirichia, R., S. Cousin, A. W. Muigai, H. I. Boga, and E. Stackebrandt. 2011. "Bacterial Diversity in the Haloalkaline Lake Elmenteita, Kenya." *Current Microbiology* 62(1):209–21. doi: 10.1007/S00284-010-9692-4.

Mwirichia, Romano, A. W. Muigai, B. Tindall, H. I. Boga, and E. Stackebrandt. 2010. "Isolation and Characterisation of Bacteria from the Haloalkaline Lake Elmenteita, Kenya." *Extremophiles : Life under Extreme Conditions* 14(4):339–48. doi: 10.1007/S00792-010-0311-X.

Mwirichia, Romano, AW Muigai, B. Tindall, HI Boga, and E. Stackebrandt. 2010. "Isolation and Characterisation of Bacteria from the Haloalkaline Lake Elmenteita, Kenya." *Extremophiles : Life under Extreme Conditions* 14(4):339–48. doi: 10.1007/S00792-010-0311-X.

Myo, Ei Mon, Binghua Liu, Jinjin Ma, Liming Shi, Mingguo Jiang, Kecheng Zhang, and Beibei Ge. 2019. "Evaluation of Bacillus Velezensis NKG-2 for Bio-Control Activities against Fungal Diseases and Potential Plant Growth Promotion." *Biological Control* 134:23–31. doi: 10.1016/J.BIOCONTROL.2019.03.017.

Naseri, B. 2014. “Bean Production and Fusarium Root Rot in Diverse Soil Environments in Iran.” *Journal of Soil Science and Plant Nutrition* 14(1):177–88. doi: 10.4067/S0718-95162014005000014.

Nehra, Vibha, Baljeet Singh Saharan, and Madhu Choudhary. 2016. “Evaluation of Brevibacillus Brevis as a Potential Plant Growth Promoting Rhizobacteria for Cotton (Gossypium Hirsutum) Crop.” *SpringerPlus* 5(1). doi: 10.1186/S40064-016-2584-8.

Ngetha, Edwin, Akhwale Julia Khayeli, and Romano Mwirichia. 2019. “Phylogenetic and Morphological Diversity of Culturable Cyanobacteria from Lake Magadi in Kenya.” *African Journal of Biological Sciences* 01(04):24. doi: 10.33472/afjbs.1.4.2019.24-31.

Nigris, Sebastiano, Enrico Baldan, Alessandra Tondello, Filippo Zanella, Nicola Vitulo, Gabriella Favaro, Valerio Guidolin, Nicola Bordin, Andrea Telatin, Elisabetta Barizza, Stefania Marcato, Michela Zottini, Andrea Squartini, Giorgio Valle, and Barbara Baldan. 2018. “Biocontrol Traits of Bacillus Licheniformis GL174, a Culturable Endophyte of Vitis Vinifera Cv. Glera.” *BMC Microbiology 2018 18:1* 18(1):1–16. doi: 10.1186/S12866-018-1306-5.

Nyakeri, Evans Manyara, Romano Mwirichia, and Hamadi Boga. 2018. “Isolation and Characterization of Enzyme Producing Bacteria from Lake Magadi, an Extreme Soda Lake in Kenya.” *Journal of Microbiology & Experimentation* 6(2). doi: 10.15406/jmen.2018.06.00189.

Omar, A. 2014. “Antagonistic and Inhibitory Effect of Some Plant Rhizo-Bacteria Against Different Fusarium Isolates on Salvia Officinalis.” *Undefined*.

Pandey, V. N., and N. K. Dubey. 1994. “Antifungal Potential of Leaves and Essential Oils from Higher Plants against Soil Phytopathogens.” *Soil Biology and Biochemistry* 26(10):1417–21. doi: 10.1016/0038-0717(94)90226-7.

Panpatte, Deepak G., Yogeshvari K. Jhala, Harsha N. Shelat, and Rajababu V. Vyas. 2016. “Pseudomonas Fluorescens: A Promising Biocontrol Agent and PGPR for Sustainable

Agriculture.” Pp. 257–70 in *Microbial Inoculants in Sustainable Agricultural Productivity: Vol. 1: Research Perspectives*. Springer India.

Qadri, Masroor, Sarojini Johri, Bhahwal A. Shah, Anamika Khajuria, Tabasum Sidiq, Surrinder K. Lattoo, Malik Z. Abdin, and Syed Riyaz-Ul-Hassan. 2013. “Identification and Bioactive Potential of Endophytic Fungi Isolated from Selected Plants of the Western Himalayas.” *SpringerPlus* 2(1):1–14. doi: 10.1186/2193-1801-2-8.

Rabbee, Muhammad Fazle, Md. Sarafat Ali, Jinhee Choi, Buyng Su Hwang, Sang Chul Jeong, and Kwang-hyun Baek. 2019. “Bacillus Velezensis: A Valuable Member of Bioactive Molecules within Plant Microbiomes.” *Molecules* 24(6). doi: 10.3390/MOLECULES24061046.

Rais, Afroz, Zahra Jabeen, Faluk Shair, Fauzia Yusuf Hafeez, and Muhammad Nadeem Hassan. 2017. “Bacillus Spp., a Bio-Control Agent Enhances the Activity of Antioxidant Defense Enzymes in Rice against Pyricularia Oryzae.” *PLOS ONE* 12(11):e0187412. doi: 10.1371/JOURNAL.PONE.0187412.

Rathna Priya, T. S., and A. Manickavasagan. 2020. “Common Bean.” Pp. 77–97 in *Pulses: Processing and Product Development*. Springer International Publishing.

Ray, Sanket, Nafisa Patel, and Dhruti Amin. 2020. “Brevibacillus.” *Beneficial Microbes in Agro-Ecology* 149–67. doi: 10.1016/B978-0-12-823414-3.00009-5.

Renaut, RW, and JJ Tiercelin. 1994. “Lake Bogoria, Kenya Rift Valley—a Sedimentological Overview.”

Richardson, Kenneth Va. 2012. *GLADSTONE ROAD AGRICULTURAL CENTRE CROP RESEARCH REPORT NO. 7 EVALUATION OF FOUR GREEN BEAN VARIETIES (PHASEOLUS VULGARIS L.) FOR PEST AND DISEASE TOLERANCE*.

Rijavec, Tomaž, and Aleš Lapanje. 2016. “Hydrogen Cyanide in the Rhizosphere: Not Suppressing Plant Pathogens, but Rather Regulating Availability of Phosphate.” *Frontiers in Microbiology* 0(NOV):1785. doi: 10.3389/FMICB.2016.01785.

Rocha, Francine Yuriko Otsuka, Cristiana Maia de Oliveira, Paula Renata Alves da Silva, Leona Henrique Varial de Melo, Margarida Goréte Ferreira do Carmo, and José Ivo Baldani. 2017. “Taxonomical and Functional Characterization of Bacillus Strains Isolated from Tomato Plants and Their Biocontrol Activity against Races 1, 2 and 3 of Fusarium Oxysporum f. Sp. Lycopersici.” *Applied Soil Ecology* 120:8–19. doi: 10.1016/j.apsoil.2017.07.025.

Saran, Saurabh, Jasmine Isar, and Rajendra Kumar Saxena. 2007. “A Modified Method for the Detection of Microbial Proteases on Agar Plates Using Tannic Acid.” *Journal of Biochemical and Biophysical Methods* 70(4):697–99. doi: 10.1016/J.JBBM.2007.03.005.

Sari, E., H. R. Etebarian, and H. Aminian. 2007. “The Effects of Bacillus Pumilus, Isolated from Wheat Rhizosphere, on Resistance in Wheat Seedling Roots against the Take-All Fungus, Gaeumannomyces Graminis Var. Tritici.” *Journal of Phytopathology* 155(11–12):720–27. doi: 10.1111/J.1439-0434.2007.01306.X.

Schwartz, Howard F., and Pastor Corrales Marcial A. 1989. *Bean Production Problems in the Tropics*. 2nd ed. Cali, Colombia: Centro Internacional de Agricultura Tropical (CIAT).

Scoon, Roger N. 2018. “Lakes of the Gregory Rift Valley: Baringo, Bogoria, Nakuru, Elmenteita, Magadi, Manyara and Eyasi.” *Geology of National Parks of Central/Southern Kenya and Northern Tanzania* 167–80. doi: 10.1007/978-3-319-73785-0_15.

Sharma, Sushma, Divjot Kour, Kusam Lata Rana, Anu Dhiman, Shiwani Thakur, Priyanka Thakur, Sapna Thakur, Neelam Thakur, Surya Sudheer, Neelam Yadav, Ajar Nath Yadav, Ali A. Rastegari, and Karan Singh. 2019. “Trichoderma: Biodiversity,

Ecological Significances, and Industrial Applications." *Recent Advancement in White Biotechnology Through Fungi* 85–120. doi: 10.1007/978-3-030-10480-1_3.

Simasi, Lily. 2013. "Isolation, Identification and Characterization of Alkalithermophiles from the Hot Springs of Lake Bogoria of the Kenyan Rift Valley."

Singh, Shree P., and Howard F. Schwartz. 2010. "Breeding Common Bean for Resistance to Diseases: A Review." *Crop Science* 50(6):2199–2223. doi: 10.2135/CROPSCI2009.03.0163.

Sippell, David W., and Robert Hall. 2009. "Effects of Fusarium Solani Phaseoli, Pythium Ultimum, and F. Oxysporum on Yield Components of White Bean." *Https://Doi.Org/10.1080/07060668209501337* 4(1):54–58. doi: 10.1080/07060668209501337.

Sorokin, Dimitry Y., Tom Berben, Emily Denise Melton, Lex Overmars, Charlotte D. Vavourakis, and Gerard Muyzer. 2014. "Microbial Diversity and Biogeochemical Cycling in Soda Lakes." *Extremophiles* 18(5):791–809. doi: 10.1007/S00792-014-0670-9/FIGURES/4.

Stefańczyk, Emil, and Sylwester Sobkowiak. 2018. "Isolation, Identification and Preservation of Fusarium Spp. Causing Dry Rot of Potato Tubers." *Plant Breeding and Seed Science* 76(1):45–51. doi: 10.1515/PLASS-2017-0020.

Strausbaugh, Carl A., Imad A. Eujayl, Leonard W. Panella, and Linda E. Hanson. 2011. "Virulence, Distribution and Diversity of Rhizoctonia Solani from Sugar Beet in Idaho and Oregon." *Canadian Journal of Plant Pathology* 33(2):210–26. doi: 10.1080/07060661.2011.558523.

Sundaramoorthy, S., T. Raguchander, N. Ragupathi, and R. Samiyappan. 2012. "Combinatorial Effect of Endophytic and Plant Growth Promoting Rhizobacteria against Wilt Disease of Capsicum Annum L. Caused by Fusarium Solani." *Biological Control* 60(1):59–67. doi: 10.1016/J.BIOCONTROL.2011.10.002.

Syed Ab Rahman, Sharifah Farhana, Eugenie Singh, Corné M. J. Pieterse, and Peer M. Schenk. 2018. "Emerging Microbial Biocontrol Strategies for Plant Pathogens." *Plant Science* 267:102–11.

Szilagyi-Zecchin, V. J., F. Mógor, and G. G. O. Figueiredo. 2016. "Strategies for Characterization of Agriculturally Important Bacteria." Pp. 1–21 in *Microbial Inoculants in Sustainable Agricultural Productivity: Vol. 1: Research Perspectives*. Springer India.

Tamiru, Gedyon, and Diriba Muleta. 2018. "The Effect of Rhizobia Isolates Against Black Root Rot Disease of Faba Bean (Vicia Faba L) Caused by Fusarium Solani." *The Open Agriculture Journal* 12(1):131–47. doi: 10.2174/1874331501812010131.

Tamrela, H., A. Sugiyanto, I. Santoso, and Q. G. Fadhilah. 2021. "The Qualitative Screening of Cellulolytic, Chitinolytic, IAA-Producing, and Phosphate Solubilizing Bacteria from Black Soldier Fly Larvae (Hermetia Illucens L.)." *IOP Conference Series: Earth and Environmental Science* 948(1):012065. doi: 10.1088/1755-1315/948/1/012065.

Tamura, Koichiro, Joel Dudley, Masatoshi Nei, and Sudhir Kumar. 2007. "MEGA7: Molecular Evolutionary Genetics Analysis (MEGA) Software Version 7.0." *Molecular Biology and Evolution* 24(8):1596–99. doi: 10.1093/molbev/msm092.

Tariq, Moh, Amir Khan, Mohd Asif, Faryad Khan, Taruba Ansari, Mohammad Shariq, and Mansoor A. Siddiqui. 2020. "Biological Control: A Sustainable and Practical Approach for Plant Disease Management." *Acta Agriculturae Scandinavica Section B: Soil and Plant Science* 507–24.

Teish, Chang Wen, Chin Shuh Chen, and San Lang Wang. 2003. "An Antifungal Chitinase Produced by Bacillus Cereus with Shrimp and Crab Shell Powder as a Carbon Source." *Current Microbiology* 47(2):102–8. doi: 10.1007/S00284-002-3955-7.

Teixeira, Hudson, Trazilbo J. Paula Júnior, Rogério F. Vieira, Miller S. Lehner, Renan C. Lima, and Emerson M. Del Ponte. 2015. "Seasonal Dynamics of Soil-Borne Inoculum and Severity of Fusarium Root Rot of Common Beans Affected by Sequential Planting of

Legume or Cereal Crops." *Tropical Plant Pathology 2015 40:5* 40(5):335–38. doi: 10.1007/S40858-015-0047-3.

Tekner, Nasibe, Recep Kotan, Elif Tozlu, and Fatih Dadasoglu. 2019. "Determination of Some Biological Control Agents Against Alternaria Fruit Rot in Quince." *Alınteri Zirai Bilimler Dergisi* 34(1):25–31. doi: 10.28955/ALINTERIZBD.578541.

Thomas, P. 2004. "Isolation of Bacillus Pumilus from in Vitro Grapes as a Long-Term Alcohol-Surviving and Rhizogenesis Inducing Covert Endophyte." *Journal of Applied Microbiology* 97(1):114–23. doi: 10.1111/J.1365-2672.2004.02279.X.

Thomas, Pious, Aparna C. Sekhar, Reshmi Upreti, Mohammad M. Mujawar, and Sadiq S. Pasha. 2015. "Optimization of Single Plate-Serial Dilution Spotting (SP-SDS) with Sample Anchoring as an Assured Method for Bacterial and Yeast Cfu Enumeration and Single Colony Isolation from Diverse Samples." *Biotechnology Reports* 8:45–55. doi: 10.1016/J.BTRE.2015.08.003.

Toghueo, Rufin Marie Kouipou, Pierre Eke, Íñigo Zabalgogeazcoa, Beatriz Rodríguez Vázquez de Aldana, Louise Wakam Nana, and Fabrice Fekam Boyom. 2016. "Biocontrol and Growth Enhancement Potential of Two Endophytic Trichoderma Spp. from Terminalia Catappa against the Causative Agent of Common Bean Root Rot (Fusarium Solani)." *Biological Control* 96:8–20. doi: 10.1016/j.biocontrol.2016.01.008.

Torome*, Tom Kintet, Lexa Gomezgani Matasyoh, George Orinda, and Francis Gakuya. 2015. "Isolation and Characterization of Antibiotic Producing Bacillus Species in Lake Bogoria, Kenya." *African Journal of Microbiology Research* 9(14):1037–43. doi: 10.5897/AJMR2015.7441.

Tripathi, Nishant, and Amit Sapra. 2020. "Gram Staining." *StatPearls*.

Trutmann, P., J. Voss, and J. Fairhead. 2008. "Management of Common Bean Diseases by Farmers in the Central African Highlands."

Http://Dx.Doi.Org/10.1080/09670879309371817 39(3):334–42. doi: 10.1080/09670879309371817.

Wangui, Patricia, Mworia Mugambi, and David Mushimiyimana. 2017. "Yield Potential Of Different Certified Common Bean Varieties Under Different Tillage Methods In Kenya A Case Of Laikipia County." *International Journal of Advanced Research and Publications*.

Weller, David M. 2007a. "Pseudomonas Biocontrol Agents of Soilborne Pathogens: Looking Back over 30 Years." *Phytopathology* 97(2):250–56. doi: 10.1094/PHYTO-97-2-0250.

Weller, David M. 2007b. "Pseudomonas Biocontrol Agents of Soilborne Pathogens: Looking Back Over 30 Years." *Http://Dx.Doi.Org/10.1094/PHYTO-97-2-0250* 97(2):250–56. doi: 10.1094/PHYTO-97-2-0250.

Won, Sang Jae, Vantha Choub, Jun Hyeok Kwon, Dong Hyun Kim, and Young Sang Ahn. 2018. "The Control of Fusarium Root Rot and Development of Coastal Pine (Pinus Thunbergii Parl.) Seedlings in a Container Nursery by Use of Bacillus Licheniformis MH48." *Forests* 10(1). doi: 10.3390/F10010006.

Wu, Bobo, Peng Wang, Adam T. Devlin, Shengsheng Xiao, Wang Shu, Hua Zhang, and Mingjun Ding. 2021. "Influence of Soil and Water Conservation Measures on Soil Microbial Communities in a Citrus Orchard of Southeast China." *Microorganisms 2021, Vol. 9, Page 319* 9(2):319. doi: 10.3390/MICROORGANISMS9020319.

Xiong, Y. Q., J. Caillon, H. Drugeon, G. Potel, and D. Baron. 1996. "Influence of PH on Adaptive Resistance of Pseudomonas Aeruginosa to Aminoglycosides and Their Postantibiotic Effects." *Antimicrobial Agents and Chemotherapy* 40(1):35.

Y, Chen, Xu Y, Zhou T, Akkaya MS, Wang L, Li S, and Li X. 2020. "Biocontrol of Fusarium Wilt Disease in Strawberries Using Bioorganic Fertilizer Fortified with Bacillus Licheniformis X-1 and Bacillus Methylotrophicus Z-1." *3 Biotech* 10(2). doi: 10.1007/S13205-020-2060-6.

Yehia, Suzan M., Iriny M. Ayoub, Masato Watanabe, Hari Prasad Devkota, and Abdel Nasser B. Singab. 2023. “Metabolic Profiling, Antioxidant, and Enzyme Inhibition Potential of Iris Pseudacorus L. from Egypt and Japan: A Comparative Study.” *Scientific Reports* 13(1):5233. doi: 10.1038/S41598-023-32224-0.

Zhao, H., D. Shao, C. Jiang, J. Shi, Q. Li, Q. Huang, MRS Rajoka, H. Yang, and M. Jin. 2017. “Biological Activity of Lipopeptides from Bacillus.” *Applied Microbiology and Biotechnology* 101(15):5951–60. doi: 10.1007/S00253-017-8396-0.

Zhaolin, Ji, He Huiwen, Zhou Huijuan, Han Feng, Tong Yunhui, Ye Zhengwen, and Xu Jingyou. 2015. “The Biocontrol Effects of the Bacillus Licheniformis W10 Strain and Its Antifungal Protein Against Brown Rot in Peach.” *Horticultural Plant Journal* 1(3):131–38. doi: 10.16420/J.ISSN.2095-9885.2016-0011.

Zieslin, N., R. Ben-Zaken-Plant physiology and Biochemistry, and Undefined 1993. 1993. “Peroxidase Activity and Presence of Phenolic Substances in Peduncles of Rose Flowers.” *Pascal-Francis.Inist.Fr*.

Zitnick-Anderson, Kimberly, Atena Oladzadabbasabadi, Shalu Jain, Chryseis Modderman, Juan M. Osorno, Phillip E. McClean, and Julie S. Pasche. 2020. “Sources of Resistance to Fusarium Solani and Associated Genomic Regions in Common Bean Diversity Panels.” *Frontiers in Genetics* 11:475. doi: 10.3389/fgene.2020.00475.

APPENDICES

Appendix I: RStudio Scripts for Physiochemical and Enzymatic Analysis

```
data <- read.csv(file.choose(),row.names = 1)

data <- data.frame(data)

dt <- as.matrix(data)

library(devtools)

library(ComplexHeatmap)

library(dendextend)

dend = as.dendrogram(hclust(dist(dt)))

dend = color_branches(dend)

rownames(dt)

colnames(dt)

column_ha = HeatmapAnnotation(Temp = runif(7), bar1 = anno_barplot(runif(7)))

row_ha = rowAnnotation(Isolates = runif(19), bar2 = anno_barplot(runif(19)))

Heatmap(dt, name = "Temp", row_split = 5, cluster_rows = dend,column_split = 4,
top_annotation = column_ha, left_annotation = row_ha)

image <- Heatmap(dt, name = "Temp", row_split = 5, cluster_rows = dend,column_split
= 4, top_annotation = column_ha, left_annotation = row_ha)
```

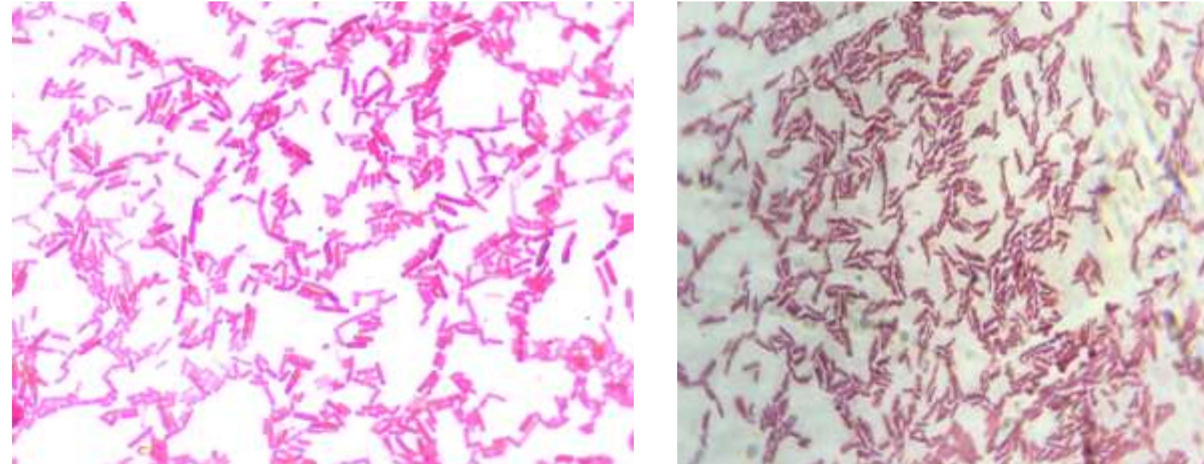
```
image

png("image.png",units = "in",width = 5, height = 7, res = 300)

image

dev.off()
```

Appendix II: Gram Staining of the Bioactive Bacteria



Appendix III: 50X TAE Electrophoresis Buffer

Contents and Storage

Contents	Amount	Storage
50X TAE Electrophoresis Buffer	1 litre	15 °C to 25 °C

1X Buffer Composition

40 mM Tris, 20 mM acetic acid, 1 mM EDTA.

Applications

Nucleic acid agarose and polyacrylamide gel electrophoresis.

Agarose and polyacrylamide gel preparation.

Note

Buffer concentrate should be diluted to a working concentration of 1X before use.

For each electrophoresis fresh 1X buffer should be used.

Appendix IV: Ethidium Bromide 10X

Dissolve 1.0 g of EtBr in a final volume of 100 ml ddH2O. Wrap the bottle in aluminum foil and stir several hours to get a true solution. Store at 4 °C. To make the 1× stock used to stain gels take 10 ml of the 10× stock and bring to a final volume of 100 ml using ddH2O. Wrap bottle in aluminum foil and store at room temp

Appendix V: PCR Program for 16S rRNA Primers

Cycle (35*X*)	**Temperature**	**Time**
Initial activation	96°C	10mins
Denaturation	95°C	45seconds
Annealing	53°C	45seconds
Extension	72°C	1mins
Store	4°C	~

Appendix VI: Chemical Used, Including Safety Data Information According to the Globally Harmonised System of Classification and Labelling of Chemicals (GHS)

Chemical	Producer	Substance	acronym	Formula	CAS-no.	Amount	Pictograms	H and P Statements
Sodium hydroxide	Merck		Soda caustic	NaOH	1310-73-2		Danger	H290 H314 P280 P301 + P330 + P331 P305 + P351 + P338 P308 + P310
Sodium chloride	Merck		NaCl	NaCl	7647-14-5	-	-	-
Hydrochloric acid	Merck		Hydrogen chloride solution	HCl	7647-01-0		Danger	H290 H314 H335 P280 P301 + P330 + P331 P305 + P351 + P338

Chemical	**Producer**	**Substance**	**acronym**	**Formula**	**CAS-no.**	**Amount**	**Pictograms**	**H and P Statements**
								P308 + P310
Chloramphenicol	Roth		Chloromycetin	$C_{11}H_{12}Cl_2N_2O_5$	56-75-7	0.25 g	Danger	H350 H351 H361d P201 P202 P280
streptomycin	Roth		Streptomycin sulfate	$C_{42}H_{84}N_{14}O_{36}S_3$	3810-74-0	0.5g	Danger	H302 H361d P202 P280 P301 + P312 P308 + P313
TAE buffer	Bio-Rad laboratories	Tris base /Acetic Acid/EDTA buffer in distilled water	TAE	-	6381-92-6	-	Warning	H319 P280 P264

Chemical	Producer	Substance	acronym	Formula	CAS-no.	Amount	Pictograms	H and P Statements
2 x gel loading buffer	Bio-Rad laboratories	2 % bromophenol blue, 2 % xylene cyanol, 70 % glycerol in distilled water	Loading dye	-	N.A	-	See Annex 2A for bromophenol blue, xylene cyanol and glycerol	See Annex 2A for bromophenol blue, xylene cyanol and glycerol
Ethidium bromide	Roth		EtBr	C21H20BrN3	1239-45-8	3 µg	Danger	H341 H302 H332 + H330 P261-P281-P311

N.A: not available

Appendix VII: Equipment's Used

Name of device	**Model Name**	**Manufacturer**
Microscope	MT5000	BioImager
pH/Temp/TDs reader	ISOLAB	ISOLAB
Spectrophotometer	VERSA MAX	Thermo Fisher Scientific

Appendix VIII: Molecular Biological Kits

Kit Name	**Use**	**Manufacturer**
ISOLATE II Genomic DNA	Bacterial DNA extraction	Meridian Bioscience, UK/USA/Germany/ Australia
PCR Purification Kit	For PCR purification	Norgen Biotek. Corp, USA

Appendix IX: Antibiotics Preparation

Antibiotic	Initial conc.	Final conc.	Preparation
Ampicillin	100 mg/ml	100 µg/ml	1 g ampicillin was dissolved in 10 ml sterile distilled water and mixed thoroughly to dissolve, followed by sterilization through 0.2 µm membrane filter and was stored at 2-8°C.
Chlorampheni col	25 mg/ml	25 µg/ml	0.25 g chloramphenicol was added in 10 ml 100 % absolute ethanol and properly mixed to dissolve followed by filtration through 0.2 µm membrane filter and was stored at 2-8°C.
Erythromycin	50 mg/ml	50 µg/ml	0.5 g erythromycin was dissolved in 10 ml 100 % absolute ethanol followed by mixing and filtration through 0.2 µm membrane filter and was stored at 2-8°C.
Streptomycin	50 mg/ml	50 µg/ml	0.5 g streptomycin was dissolved in 10 ml 70 % ethanol followed by mixing and filtration through 0.2 µm membrane filter and was stored at 2-

			8°C.

Appendix X: List of Culture media, Suppliers, Uses, Composition, and Preparation

Name	Company	Uses	Composition (g/l)	Preparation
Nutrient broth	Himedia, Mumbai, India	Sterility testing and cultivation of non fastidious microorgan isms	Peptic digest of animal tissue 5.0; beef extract 1.5; sodium chloride 5.0g and yeast extract 1.5	13 g were suspended in 1 litre distilled water and autoclaved for 15 min at 121°C.
Potato dextrose agar	Himedia, Mumbai, India	Isolation and enumeratio n of yeasts and moulds from dairy and other food stuffs	Potatoes infusion from 200.0; dextrose 20.0; agar agar 15.0.	39 g were suspended in 1 litre distilled water (pH 5.6), heated to boil, and followed by autoclaving for 15 min at 121°C. After cooling to 45°C, sterile 10 % Tartaric acid (14 ml/l) was added before dispensing on plates
Nutrient Agar	Himedia, Mumbai, India	general purpose culture medium which may be used as enriched mediu	Peptone 10.0g/L Meat extract 10.0/L Sodium chloride 5.0/L Agar 12.0/L	Suspend 37.0 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 10lbs pressure(115°C) for 30 minutes or alternatively at 15 lbs pressure (121°C) for 15 minutes or as per

Name	Company	Uses	Composition (g/l)	Preparation
				validated cycle
LB	Himedia, Mumbai, India	used for routine cultivation	Casein enzymic hydrolysate 10.0/L Yeast extract 5.0/L Sodium chloride 10.0/L	Suspend 25 grams in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Dispense as desired
Skim Milk Agra	Himedia, Mumbai, India	Recommended for cultivation and enumeration of microorganisms encountered in dairy industry.	SM powder 28.0g/L Tryptone 5.0g/L Yeast extract 2.5g/L Dextrose (Glucose) 1.0g/L Agar 15.0g/L	Suspend 51.5 grams of in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates
Pikovskaya medium	Himedia, Mumbai, India	Pikovskaya Broth is recommended for cultivation of phosphate	Yeast extract 0.5g/L Dextrose 10.0g/L Calcium phosphate 5.0g/L Ammonium sulphate 0.5g/L	Suspend 16.3 grams in 1000 ml distilled water. Heat if necessary to dissolve the medium completely and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Dispense as

Name	Company	Uses	Composition (g/l)	Preparation
		solubilizing microorganisms.	Potassium chloride 0.2g/L Magnesium sulphate 0.1g/L Manganese sulphate 0.0001g/L Ferrous sulphate 0.0001g/L	desired.

Appendix XI: Green- House Assay

Appendix XII: Publications

No.	Tittle	Publisher
1.	Isolation and Characterization of Bacillus velezensis from LakeBogoria as a Potential Biocontrol of Fusarium solani inPhaseolus vulgaris L.	MDPI-Bacteria
2.	Taxonomical, functional, and cytopathological characterization of Bacillus spp. from Lake Magadi, Kenya, against Rhizoctonia solani Kühn in Phaseolus vulgaris L	Journal of Basic Microbiology-Wiley
3	Pathogenicity Test, Antifungal Mechanisms, and Secondary Metabolites of Bacillus spp from L. Bogoria as Biocontrol of Rhizoctonia solani in Phaseolus vulgaris L.	International Journal of Microbiology-Hindawi