MOLECULAR CHARACTERIZATION AND MINERALIZATION POTENTIAL OF PHOSPHORUS SOLUBILIZING BACTERIA COLONIZING COMMON BEAN (*Phaseolus vulgaris* L.) RHIZOSPHERE IN WESTERN KENYA

Kiprotich Kelvin

A Thesis Submitted in Partial Fulfillment for the requirements for the award of the Degree of Master of Science in Molecular Biology of Masinde Muliro University of Science and Technology

November, 2023

DECLARATION

This thesis is my own original research prepared with no other than indicated sources and has not been presented elsewhere for any other award.

Signature……. Date……

Kiprotich Kelvin

REG: SBB/G/01-53357/2018

CERTIFICATION

The undersigned certify that we have read and hereby recommend for acceptance of Masinde Muliro University of Science and Technology a thesis entitled; **'Molecular Characterization and Mineralization Potential of Phosphorus Solubilizing Bacteria Colonizing Common Bean (*Phaseolus vulgari L.)* Rhizosphere in Western Kenya'.**

Signature……Date……

Prof. John Omondi Muoma, PhD

Department of Biological Sciences

Masinde Muliro University of Science and Technology

Signature……. …… Date……..

Dr. Dennis Ondieki Omayio, PhD

Department of Biological Sciences

Masinde Muliro University of Science and Technology

COPYRIGHT

DEDICATION

This work is dedicated to my grandmother Rebecca Jeptanui Cheruiyot and my uncle Sammy Kipchirchir Rotich for their moral and financial support throughout my academic journey. May God bless you.

ACKNOWLEDGEMENTS

It is with my humble pleasure to thank all those who have made this thesis successful. First and foremost, I would like to express my sincere gratitude to the Director, Science Park, Incubation and Innovation, Prof. John Omondi Muoma who is also my first supervisor for his tremendous drive, funding and utter conviction throughout my Masters studies. Without his constant financial support and guidance; none of this would have been possible. I have been very fortunate in having a supervisory and mentorship team including Dr. Dennis Omayio, Dr. Clabe Wekesa, Dr. Okoth Patrick and Dr. Dennis Ochieno and wish to acknowledge them for their guidance and discussions, including comments that were important to the development of this thesis. At Masinde Muliro University of Science and Technology, I acknowledge my fellow colleagues for their support and academic friendship. To SPIIC-MMUST staff, I thank you all for providing a favorable academic environment. May God bless you. I would like to thank my wonderful grandmother Rebecca Jeptanui Cheruiyot and uncle Sammy Kipchirchir Rotich for not only providing moral and financial support during my Master's studies but also helped me to develop into a well-rounded scholar that I am today. Many thanks my family relatives and mentors for believing in me. Finally, I appreciate Dr. Hon Tecla Tum, Hon Paul Biego and Anglican Church of Kenya, Saint Mark's Mogoget for partially funding my school fees.

ABSTRACT

Phosphorous solubilizing bacteria (PSB) are a category of microbes that transform insoluble phosphates in soil into soluble forms that crops can utilize for growth and development. Phosphorous in natural soils is abundant, but since it is poorly soluble, it is not readily available to plants. Introducing phosphorous-solubilizing microbes, such as bacteria, is a safer way of improving soluble forms of phosphorous as compared to chemical fertilizers. Due to environmental issues and concerns about consumer health, the pervasive use of chemical fertilizers to provide nutrients in agriculture, especially the use of phosphorous and nitrogenous fertilizers, is currently under investigation. In soil and plant rhizospheres, multiple phosphorous solubilizing bacteria have been revealed, each with its own different capacity to solubilize phosphates. The solubilization potentials of these bacteria, on the other end, varies by genetic and molecular characteristics. The objective of this study were to determine the mineralization potential of phosphorus solubilizing bacteria, their molecular variations and plant growth promoting characteristics in growth and development of the common bean *Phaseolus vulgaris.*L, which were used as an indicator plant. The phosphate solubilization potential of each PSB isolates were evaluated under agar and broth medium of National Botanical Research Institute's phosphate (NBRIP) that was supplemented with Tricalcium Phosphate (TCP). The experimental design was complete randomized design and descriptive statistics was used to present the findings of the study. The strains, KV1 and KB5 (B5) were found to be the most effective phosphorus solubilizers with 3.69 solubility index and 4.16 solubility indices respectively: they converted total of amount soluble phosphate concentration in the broth medium (1471 P (ug/Ml) and 1395 P(ug/mL)) respectively. The least performing isolate was KBU with 2.34 solubility index. 16S ribosomal RNA gene sequencing and NCBI blasting closely identified the isolates KK3 as *Enterobacter mori*, KB5 as *Pseudomonas kribbensis*, KV1 as *Enterobacter asburiae*, KB3 as *Enterobacter mori*, KK1 as *Enterobacter cloacae*, KBU as *Enterobacter tabaci* and KB2 *as Enterobacter bugandensis*.The most efficient phosphate solubilizing isolate were used to test the improvement of plant growth parameters of Rosecoco and Mwetemania bean varieties and significant differences was determined using ANOVA and means were separated using Turkey Honest at 5 % level. PSB strains found in common bean rhizospheres varied in solubilization and genetically and that KVI and B5 were the most promising high efficiency strains that can be used to unravel the insufficiency of phosphorus and as a biofertilizer for sustainable crop production. Isolating and defining compatible PSB, along with comparing and analyzing the genetic factors would be a major step in developing an efficient biofertilizer for safer, economically sustainable agricultural systems, as well as protecting soil from hazardous chemical fertilizers.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
BLAST	Basic Local Alignment Sequence Tool
DNA	Deoxyribonucleic Acid
MMUST	Masinde Muliro University of Science and Technology
NBRIP	National Botanical Research Institute's Phosphate Growth Medium.
NCBI	National Center for Biotechnology Institute
NPK	Nitrogen, Phosphorus and Potassium
P	Phosphorous
PCR	Polymerase Chain Reactions
PDE	Phosphodiesterase
PME	Phosphomonoesterase
PSB	Phosphorous Solubilizing Bacteria
PSM	Phosphorous Solubilizing Microbe
PGPR	Plant Growth Promoting Rhizobacteria
TCP	Tricalcium Phosphates

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Phosphorus (P) is the second most essential macronutrient for plant growth and development after nitrogen. It plays a significant role in key metabolic pathways including nutrient uptake, biological oxidation and energy metabolism (Medici *et a*l., 2019). Crops need significant nutrients in order to grow and produce substantial yields in any production system (Fageria & Baligar, 2008; Kumar *et al.*, 2021). The majority of necessary plant nutrients, including phosphorus, are insoluble in soil and therefore must be solubilized into soluble forms before they can be available for plants (Goswami *et al*., 2019). Bacteria are examples of microorganisms that can solubilize phosphate, and as a group they are known as Phosphate Solubilizing Microorganisms (PSM) (Alori *et al.*, 2017). Phosphate solubilizing bacteria are among the Plant Growth Promoting Rhizobacteria (PGPR). Bacterial species in the soil and rhizosphere play an important role in plant growth and development, making them ideal phosphorus solubilizers. Despite numerous reports highlighting the current usage of phosphate-solubilizing bacteria in other plants, powerful novel bacteria colonizing common bean remain unclear especially in tropical sub -Saharan Africa. Among the most powerful and effective phosphate solubilizing microbes are bacterial strains from the genera *Pseudomonas*, *Bacillus* and *Rhizobium* (Rodríguez & Fraga, 1999a).

In agroecosystems, phosphorus-solubilizing bacteria (PSB) play a critical role in biogeochemical phosphorus cycling. Chelation, acidification, exchange reactions and the formation of polymeric substances are all used by phosphorus-solubilizing microbes to convert insoluble phosphorus to soluble forms. Agricultural land everywhere on the world is under tremendous pressure due to the urgent need to feed mankind's constantly expanding population. *(Alori et al.*, 2017). Because of increased land usage and the use of harmful inorganic fertilizers, the quality of ecosystems that produce food has declined as time has passed (Manzoor *et al.,* 2017). Inorganics fertilizers containing macronutrients (Nitrogen, Phosphorus, and Potassium (NPK)) have been extensively used in agronomic practice around the world to provide nutrients that promote plant growth and, as an outcome, increase crop productivity (Sharma e*t al.,* 2014*)*. Modern farming systems have clearly benefited much from these fertilizers, but their continued abuse has damaged agricultural soils and altered the vital plant growth-promoting rhizobacteria (PGPR), which has led to poorer production.(Bisht & Chauhan, 2020). Due to environmental and health concerns brought up by the pervasive usage of chemical fertilizers to deliver nutrients in agriculture (Tahir *et al*., 2018),the ultimate objective of current research is to help create alternative technologies that will enable the widespread implementation of organic fertilizers in agronomic operations while reducing dependency on synthetic phosphate fertilizers (Goswami *et al.*, 2019).Inoculating plants with rhizobacteria and mycorrhizae to boost plant growth and development is a popular modern application of microorganisms for crop production (Averill *et al*., 2019). Phosphorus solubilizing bacteria (PSB) are among well-known rhizobacteria that enrich plant growth characteristics (Kalayu, 2019).

The aforementioned microbes have been found to have a great capacity for solubilizing phosphorus (Satyaprakash *et al*., 2017). Numerous phosphorous-solubilizing microbes have been found in soil and crop rhizospheres, each having a unique capacity to solubilize phosphates (Toro, 2007). On the other hand, these bacteria's solubilization capacity differs genetically and environmentally (Alaylar e*t al.,* 2020). An emerging and sustainable field is the evaluation of potential phosphorus-solubilizing bacteria for specific zones that can be used as bio inoculants or biofertilizers to improve plant growth efficiency and yields. This is for the reason these bacterial inoculants might credibly moderate the excessive use of chemical fertilizers while preserving soil microflora (Alori *et al*., 2017; Pande *et al*., 2017).

Globally, phosphorus-solubilizing bacteria have been genetically characterized using the hypervariable sections of the 16S ribosomal RNA gene, a gene that is conserved across all prokaryotes (Alaylar *et al.*, 2020; Ayyaz *et al*., 2016; Javadi Nobandegani *et al.*, 2015) but limited has been reported in Kenya for microorganisms that promote plant growth, especially in Western Kenya, the Rift Valley and Central Kenya, where crops are grown. This study aimed to identify phosphorus-solubilizing bacteria, characterize them genetically, and assess how well they affected common bean growth and development. The study also aimed to quantify the amounts of phosphate solubilization in the broth and agar media. In spite of comparing and analyzing their phylogenetic relationships and mineralization potential, identifying potential PSB isolates linked to common beans in Western Kenya would be a significant step toward creating an effective inoculant and biofertilizers for safer, more prosperous agricultural systems that safeguard the soil from

harmful chemical fertilizers. (Chouhan *et al.*, 2021).

1.2 Statement of the Problem

The urgent need to feed the world's ever-growing population is pushing and immensely straining arable lands around the world to produce more yields (Fróna *et al.*, 2019). In recent years, there has been high usage chemical nutrient fertilizers, mainly for crop yield improvement and faster economic purposes (Krasilnikov *et al.*, 2022). Phosphate fertilizers have been commonly used in agricultural practice around the ecosphere to provide macro nutrients that promote plant growth and, as a result, increase crop productivity (Sharma *et al.*, 2014). Examples of these fertilizers used include Di-Ammonium Phosphate (DAP) and Triple Super Phosphate (TSP) fertilizers. These inorganic fertilizers have undoubtedly provided benefits to modern cropping systems, but their overuse has massively damaged and influenced the health of agricultural soils, resulting in long term lower production of yields (Krasilnikov *et al*., 2022).

In order to lessen dependency on chemical phosphate fertilizers and allow the widespread use of biofertilizers in agronomic operations, scientists are concentrating on creating suitable alternative technologies (Bhardwaj *et al*., 2014). In every agricultural system, crops require a lot of nutrients to grow and yield a quantity of enough food (Fageria *et al.*, 2008). One of the key nutrient required by plants is phosphorus (P) (White & Brown, 2010).To acquire adequate phosphorus for crop production, P fertilizer is applied to most agricultural lands in forms of inorganics, despite its effectiveness of P uptake by plants, it appears very low at approximately 15% owing to P fixation or loss from agricultural soils.

Phosphate anions easily forms complexes with metal cations like aluminum ions in soils which consequently result in an exceptionally very low content of available soil P for the demands of plants (Shen *et al*., 2011). Furthermore, unexploited P from fertilizer would be leached into groundwater in various forms including infiltration, while P left in the soil enters the water bodies through surface runoff, prompting to P fertilizer pollution to rivers and lakes (Gao *et al*., 2012). The P fertilizer pollution blowouts into farmland to an extensive kind of natural ecosystems leading to destruction of native microbes and loss of soil fertility (Bashir *et al*., 2020). Up to date, multiple strategies have been elevated to overcome or reduce the over dependence on chemical fertilizers and among them is employment of plant growth promoting microbes (García-Fraile *et al*., 2015; Tian *et al*., 2021). This study is part of the alternative product development using biotechnology aiming at isolating efficient PSB found in the common bean rhizosphere, characterizing their molecular variations and determining their potential in phenotypic effects in plant growth characteristics.

1.3 Justification and Significance of the Study

Continuous use of hazardous chemical fertilizers will degrade the soil fertility, destroy aquatic life and impose health hazard to humans (Pahalvi *et al*., 2021). Biofertilizers are sustainable and safer agricultural practice system and use of Phosphorous solubilizing microorganism is greatly beneficial (Silva *et al.*, 2023). The study thus gives insight on sustainable and safer agricultural system by use of Phosphorous solubilizing bacterial as an alternative method from inorganic fertilizers to biofertilizers. The use of phylogenetic and genomic studies to characterize these PSB provides a breakthrough for researchers in

terms of evolutionary relationships and the selection of novel bacteria for use as biofertilizers, thereby enhancing food security, maintaining consumer health, and preserving the environment (Odelade & Babalola, 2019). As more knowledge about PSB and the mechanisms that they employ come to be available, there is every reason to believe that their use as biofertilizers will become more efficient and essential mechanisms in the production of long-term soil management and soil amelioration systems aiming at boosting the soil fertility. Consumers of agricultural products are primarily concerned with the products' health, consistency, and nutritional value (Demi & Sicchia, 2021).Therefore, using PSB as possible biofertilizers is an environmentally friendly way to boost food production while also protecting the environment.. Uncovering the growth-promoting properties of these bacteria and providing evidence for the application of useful bio inoculants to leguminous crops for sustainable production in tropical regions require research on the effects of genetically diverse phosphorus solubilizing bacteria on the growth characteristics of plant varieties.

One of the fundamental strategies of maintaining soil health and improving crop production is by managing plant nutrition through use of appropriate methods. Proper nutrition can greatly influence the finer line between crop production and food insecurity. Hence, a healthy soil is a necessity for profitable, productive, and environmentally fit agricultural systems. Investing time in learning about soil processes and methods to boost soil quality through effective techniques can lead to a justifiable soil management system that enhances plant growth and environmental quality over a period. The management of soil microorganisms, a priceless and vital natural resource, can boost the availability of

nutrients. The rhizosphere of the soil is also seen as a complex ecosystem where live microbes and plant roots combine organic materials and mineral particles into a dynamic structure that regulates the quality of the air, water, and nutrients. According to Maeder *et al.,* (2002) organic systems emit 34 to 51% fewer greenhouse gases per hectare than conventionally managed systems as a result of nutrient inputs. As a result, nitrogen was decreased in a form that is susceptible to leaching losses and can increase greenhouse gas emissions. Additionally, two to three times as many beneficial soil microbes encouraging basic soil structure and fertility were present in organic soils, considerably boosting soil profile (Maeder *et al.,* 2002).

1.4 Objectives

1.4.1 General Objective

To determine molecular characteristics and mineralization potential of Phosphorous Solubilizing Bacteria colonizing common bean (*Phaseolus vulgaris*. L) Rhizosphere in Western Kenya.

1.4.2 Specific Objectives

1. To determine the mineralization potentials of phosphorus solubilizing bacteria colonizing common bean rhizosphere in Western Kenya.
2. To determine molecular variations and phylogeny of phosphorous solubilizing bacteria colonizing common bean rhizosphere in Western Kenya.
3. To determine the effects of selected high potential phosphorous solubilizing bacteria in promoting growth characteristics of common bean varieties.

1.5 Research Questions

1. What are the mineralization potentials of phosphorus solubilizing bacteria colonizing common bean rhizosphere in Western Kenya?
2. What are the molecular variations and phylogenetic relationships of phosphorous solubilizing bacteria colonizing common bean rhizosphere in Western Kenya?
3. What are the effects of selected high potential phosphorous solubilizing bacteria in promoting growth characteristics of common bean varieties?

CHAPTER TWO

LITERATURE REVIEW

2.1 Molecular Characterization of Bacteria using 16S rRNA Gene

Although not as accurate as genotypic identification, phenotypic characterization has enormously been used in bacterial identification (Franco-Duarte *et al.*, 2019). Regardless of other advanced genotypic and molecular methods of identifying and characterizing bacteria, the comparison of the 16S rRNA gene sequence for bacteria has recently emerged as the most sought after genetic method (Clarridge, 2004). Moreover, this form of gene sequencing has opened avenues making the isolation and identification of poorly described strains possible. The identification of strains such as *Rhizobia*, *Bacilli* and *Pseudomonas* has been made possible by use of this gene, making it possible to recognize pathogen that can be novel (Srinivasan *et al.,* 2015).

A total of 1550 base pairs make up the 16S rRNA gene sequence, which is divided into variable (V) and conserved portions (Figure 1). The 16S rRNA gene is big enough and has enough interspecific polymorphisms to allow for differentiated and statistically reliable molecular characterization. The gene is known to feature hyper variable and conserved sections that are useful for identifying and characterizing broad-length bacteria. In Figure 1, it displays a schematic structure of 16S rRNA gene and the ribosome complex of *Escherichia coli*. The conserved regions and hyper variable regions are shown with white and grey boxes respectively. In the figure, the bold arrows display the approximation of universal primers' positions on the 16S rRNA gene sequence (Fukuda *et al.*, 2016).

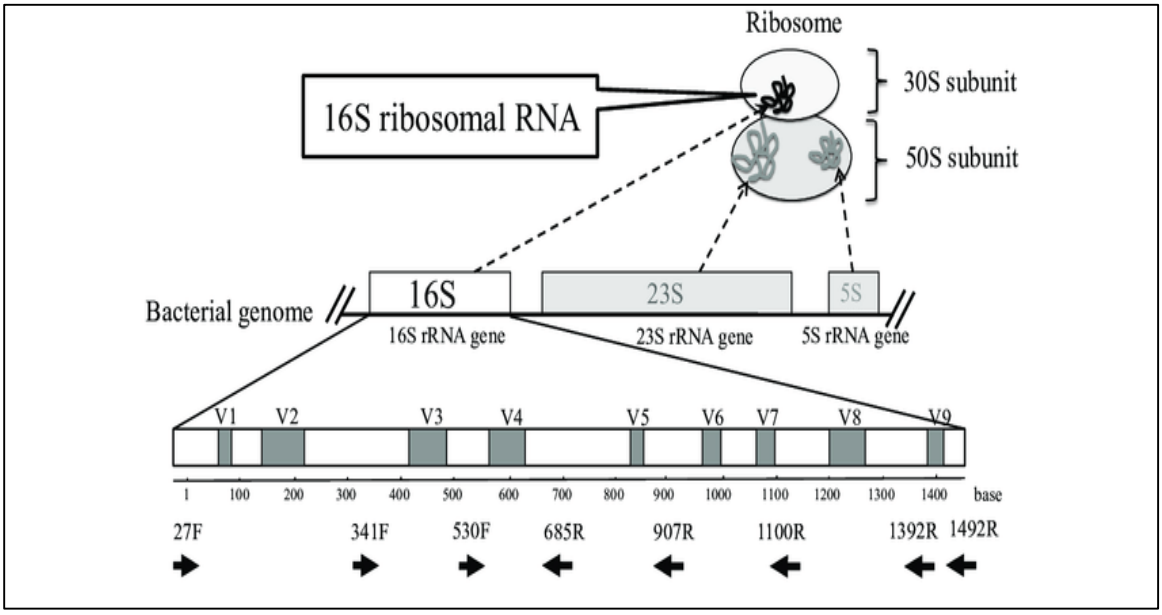


Figure 1. Structure of 16S rRNA gene with variable regions. Source: Fukuda *et al*.,(2016)

2.2 Phosphorus as a Plant Macronutrient

Phosphorus is recognized as the principal key element among all the elements needed for plant growth. It is the second most abundant element after nitrogen and mostly required by plants in early developmental stages. In soil, the diverse forms of phosphorus can further be broken down to soluble orthophosphates, insoluble organic and inorganic phosphates (Prabhu *et al.*, 2018). Moreover, the relative rate of decomposition of organic matter dictates the respective concentrations of P for plant uptake, and the ability of the inorganic constituents in soil to form respective soluble fractions. Plants takes up phosphorous by solubilization and mineralization (Manzoor *et al*., 2017). Soluble phosphates fertilizer is capable of increasing the number of orthophosphates in soil, when P-based fertilizers are added. This phenomenon can further enable the reaction of P with iron , aluminum and other elements like silicate clay hence becoming unavailable for plant use (Cole *et al*., 2016). Functions of phosphorus in all the plants include; energy transformations and

storage, improves cell structure components, plays major role in respiration and photosynthesis, cell division, elongation and root development (Kumar *et al*., 2021) (Figure 2). On the other hand, phosphorus deficiency leads to the following; reduced leaf expansion and number, reduced quality of fruits, seeds and forages, reduced shoot growth, improper nutrient uptake, delayed plant maturity and decrease disease and pathogen resistance (Meng *et a*l., 2021) (Figure 2).

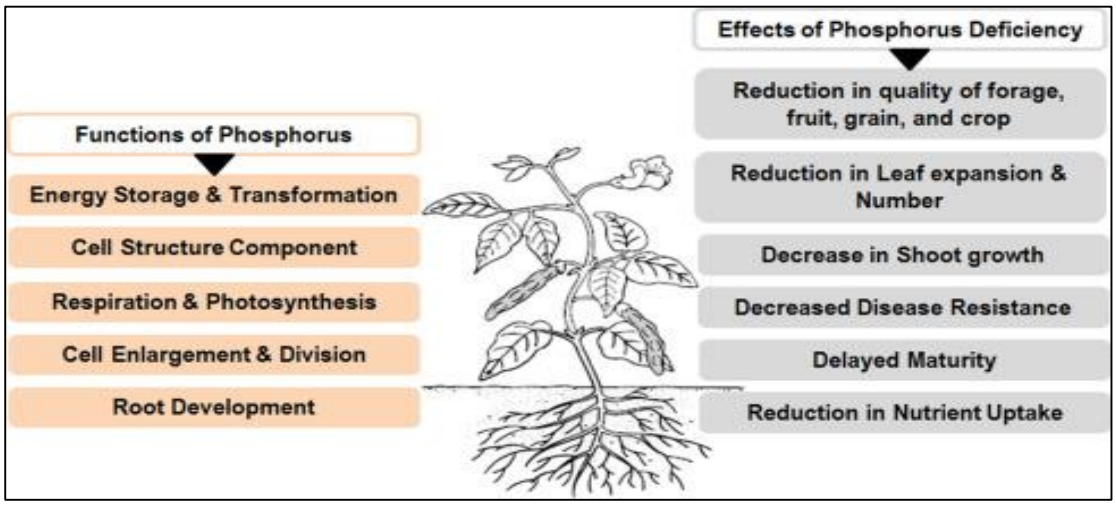


Figure 2. Functions of phosphorus and effects of P deficiency in plants. Source: Meng et al., (2021).

2.3 Biofertilizers in Agriculture

Biofertilizers are widely defined as organic fertilizers majorly bio-based, which could be from plants or animal source, or from dormant or living microbial masses. This has enormous capability of improving the bio-accessibility and biodiversity of nutrients in soil for plant use. Furthermore, it comprises of plant growth microbes, phosphorus solubilizing bacteria, nitrogen fixing bacteria, potassium solubilizes, among other beneficial fungi and bacteria (Figure 3.)

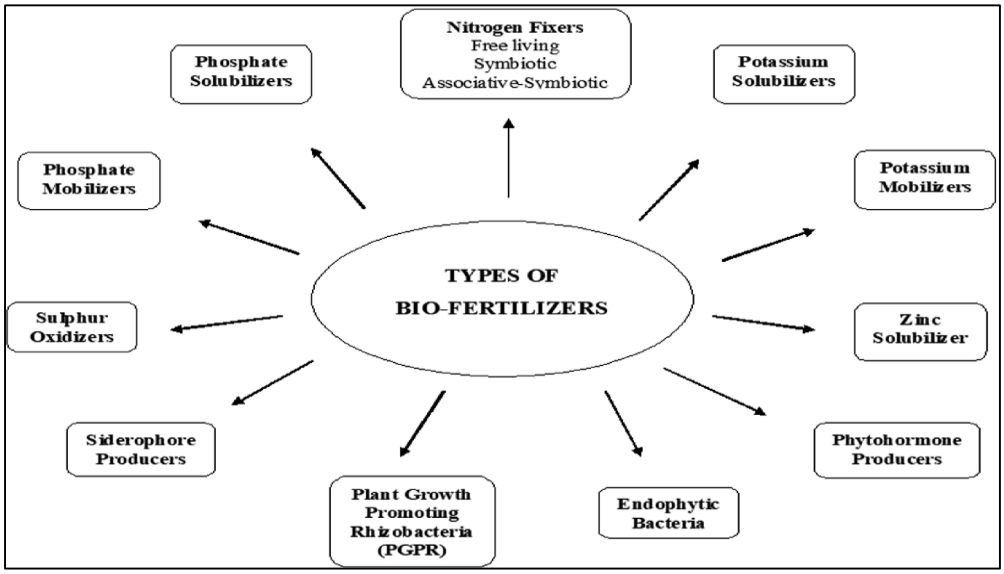


Figure 3. Types of Biofertilizers. Source :García-Fraile *et al.*, (2015).

Elsewhere, biofertilizers have been defined as a biological substance comprising of live microorganisms which are thought to have beneficial alteration of growth characteristics to plants (Mącik *et al*., 2020). Because of their capability in boosting enhancing food safety and boosting crop productivity, using microorganisms as biofertilizers is seen as a suitable alternative to chemical fertilizers in agriculture. In the agricultural sector, such microorganisms as plant growth promoting bacteria, fungus, rhizobacteria, cyanobacteria, and others have been discovered to have bio fertilizer-like capabilities. Bio fertilizers have been shown to be capable of giving vital nutrients to crops in appropriate proportions, resulting in increased agricultural yields, according to extensive research (Mahanty *et al.*, 2017). To improve soil fertility, nutrient uptake, and crop yields, microbial strains use a variety of biological mechanisms, including nitrogen fixation, potassium and phosphorus solubilization, phytohormone excretion, the production of substances that disarm phytopathogens, protection of plants from abiotic and biotic stresses, and the detoxification

of subsurface pollutants. Given the risks associated with the excessive use of chemical fertilizers and pesticides as well as the rising demand for food on Earth, biofertilizers are currently regarded as the most promising method and non-toxic alternative to synthetic agro-chemicals (Mącik *et al*., 2020). One of the key areas of scientific study for the advancement of sustainable agriculture is the widespread use of biofertilizers since it is thought that the use of microbial inoculants will eliminate the problems associated with chemical-based farming methods (Alori & Babalola, 2018).

Over the last few decades, there has been a rapid increase in global population, which poses a challenge to human food security (Maisonet-Guzman, 2011). As a result, in order to meet the enormous demand for food, agricultural production must be raised quickly and on the limited amount of available agricultural land in the world (Abebe *et al.*, 2022). Food security has made the agriculturalists globally to depend immensely on commercially accessible chemical-based fertilizers to improve agricultural production (Sasson, 2012). However, scientist have realized a tremendous improvement in agricultural production utilizing chemical fertilizers, which have proven to be harmful to our ecology, particularly in terms of human and animal health. The damaging effects of heavy chemical use in agricultural systems have made it difficult to sustainably produce crops and maintain the quality of the environment. Therefore, using biological fertilizers is a natural, affordable, and environmentally responsible option to try to solve this issue (Kumar *et al*., 2022). Biofertilizers include living microorganisms like PSB and other PGPM with ability of furnishing sufficient nutrients to the plants, while improving high yield and sustaining the environment (Chaudhary *et a*l., 2022). Numerous studies are attempting to describe the

need for biofertilizers, their preference over traditional synthetic ones, the various varieties, their uses in agriculture, how they are produced, how they work, and most significantly, the benefits and drawbacks of using them (Mitter *et a*l., 2021).

2.4 Phosphorus Solubilizing Bacteria (PBS)

Phosphate-solubilizing microbes (PSMs) are useful microorganisms capable of hydrolyzing or solubilizing both organic and inorganic insoluble P compounds, into soluble forms for easy plant uptake (Tian *et al*., 2021). These microbes are capable of availing natural phosphatases and important organic acids, which are thought to reduce the pH of soil while boosting the chelating mechanisms (Goswami *et al.,* 2019). The vast majority of these microorganisms are bacteria living in soil. It has been noted that the soil bacteria *Agrobacterium spp., Pseudomonas spp.* and *Bacillus circulans* can solubilize weakly accessible phosphorus (Babalola & Glick, 2012).

Various strains of bacteria that mineralize phosphorus include *Azotobacter* (A. Kumar *et al*., 2016), *Bacillus sp,* (Panneerselvam *et al.,* 2019) *Burkholderia sp,*.(Alori *et al.,* 2017), *Enterobacter sp,., Erwinia sp,.* (Ahmed *et al*., 2019), *Kushneria sp,*.(Zhu *et al*., 2011), *Paenibacillus* (Fernández Bidondo *et al*., 2011), *Ralstonia, Rhizobium* s*p,*.(Tajini *et al*., 2011), *Rhodococcus, Serratia, Bradyrhizobium, Salmonella, Sinomonas and Thiobacillus* (Gong *et al.,* 2022; Tian *et al.,* 2021). The isolates of the PSB *Bacillus megaterium, Bacillus spp*., and *Arthrobacter spp*. have all been isolated from Kenyan soils. They are the microorganisms that are most prevalent and have a wide range of strains in soils. Nevertheless, only five percent of all isolates are effective in terms of their capacity to phosphate-solubilize (Ndung'u-Magiroi *et al*., 2012). The complexes of iron (Fe) and

aluminum (Al) oxides and hydroxides in most Kenyan soils causes P deficiency, which dispossesses plants arising up to 80% of the added P (Ndung'u-Magiroi *et al.*, 2012).

2.5 The Significance of Phosphorus-Solubilizing Bacteria in Agriculture

PSBs capable of converting insoluble P to soluble forms can be used as biofertilizers to better utilize the phosphorus contained in soils. This boosts the amount of soluble phosphorus in the environment (Tahir e*t al.*, 2018). Since it is preferable to use an environmentally sustainable approach (i.e., a model that stresses the use of biological soil amendments rather than chemicals) to solve the problems of infertile soil, the use of P biofertilizers is a promising strategy for speeding up food production by increasing yield (Babalola & Glick, 2012). PSM function as bio fertilizers by making P available to growing plants that would otherwise be inaccessible. Phosphorus-solubilizing bacteria may encourage plant growth by enhancing biological nitrogen fixation efficiency, producing phytohormones, and boosting the bioavailability of essential minerals including zinc and iron (Wani *et al.*, 2007).

In pot experiments and in the field, many studies on PSB inoculation have reported an increased plant yield and P uptake which is a proof that PSB has future potential sustainable agriculture (Gupta *et al.*, 2021; Boubekri *et al*., 2021;; Yu *et al*., 2022; Wang *et al.*, 2022a; Pande *et al.*, 2017). In previous experiments, the PSB establishment rate was 5.6 06 spores g soil in a pot experiment using fungi as a biofertilizers (wheat husks bearing 20% perlite- carrier material) (Wang *et al.*, 2015). Benefits of using microbial rhizosphere management for sustainable agriculture practices include increased phosphate bioavailability to crops, boosted root and shoot biomass, enhanced root length and shoot

length, boosted fresh and dry shoot weights, P-labeled phosphate uptake, and significant grain and dry matter yield enhancements (Fasusi *et al.*, 2021).

Phosphate-solubilizing bacteria have also shown significant synergistic outcome on the joint growth and development of crops (Minaxi *et al*., 2013). Apart from solubilizing P, more of PSB has the potential as biocontrol agents against a diversity of plant pathogens (Mitra *et al*., 2020; Pandit *et al*., 2022). Phosphorus solubilizing microorganism control pathogens by developing such antifungal compounds (phenolic, and flavonoids), antibiotics , siderophores, lytic enzymes and hydrogen cyanide, which all serve to inhibit pathogen proliferation (Vandana *et al.*, 2021).

PSMs technology increases the productiveness and agricultural usage of soils which are saline to alkaline without the environmental or health threats that come from using artificial fertilizers endlessly. *Kushneria sp.* YCWA18, is a bacterium that can solubilize either inorganic and organic phosphorus which has shown modest saline-alkaline based agriculture (Beck. *et al.,* 2014). At various NaCl concentrations, Tricalcium phosphate could be dissolved by the strains of *Pseudomonas aeruginosa* PSBI3-1, *Aerococcus sp.* PSBCRG1-1, *Aspergillus sp.* PSFNRH-2 and *A. terreus* PSFCRG2-1 (Srinivasan e*t al.,* 2012) . In the existence of NaCl concentrations of approximately 5%, the PSM *Burkholderia cepacia* positively affected the development of maize crop (Pande e*t al*., 2020). These bacterial organisms have all shown potential use as biofertilizers in saline agriculture utilizing alkaline soils with other beneficial characteristics. In a series of tests

on bacterial solubilization, the proportion of phosphorus released increased but then decreased as the NaCl concentration was increased up to 0.8 M (Srinivasan *et al.*, 2012).

Table 1 . Effects of some phosphorous solubilizing bacteria on plants

Bacteria strain	**Test crop**	**Result**	**Reference**
Pseudomonas aeruginosa	Chinese cabbage	Increase biomass and plant length	(Wang *et al.*, 2017)
Bacillus sp. and Pseudomonas sp.	Sesame (Sesamum indicum)	Increased seed production	(Jahan *et al.*, 2013)
Bacillus thuringiensis	Rice (*Oryza sativa)*	Improved shoot length	(Rao *et al.*, 2015)
Pseudomonas striata and Glomus fasciculatum	Soybean wheat	Improve rooting and promotes grain yield	(Mahanta & Rai, 2008)
Rhizobium tropici Rhizobium phaseoli	Common beans	Increase the number of nodules, shoot and root biomass	(Bechtaoui *et al.*, 2019; Wekesa *et al.*, 2021)
Burkholderia cepacia ,	Maize	Improved plant growth	(Li *et al.*, 2017)

Paenibacillus sp, Pseudomonas sp			
Paenibacillus beijingensis	Wheat	Improved soil available P and plant P uptake	(Li *et al.*, 2020)
Enterobacter cloacae, Bacillus thuringiensis, Pseudomonas pseudoalcaligenes	Potato	Enhance yield and nutrient uptake	(Pantigoso *et al*., 2022)

2.6 Mechanisms of Phosphorus Solubilizing Bacteria

There have been theories that justify the mechanism of solubilization of inorganic phosphate. Mobilization and immobilization by mineralization are the main bacterial mechanisms in solubilization. To mineralize organic P molecules, microbes, particularly bacteria, produce phosphatase enzymes. The terms "phosphatase activity" relate to the combined but separate functions of the enzymes phosphomonoesterase (PME) and phosphodiesterase (PDE). PDE is known to hydrolyze organic P complexes such as nucleic acids and phospholipids into Phosphomonoesterase (inositol phosphates and mononucleotides). According to Khan *et al., (*2013) and Park *et al*., (2022),PSB like, *Pseudomonas, Enterobacter* and *Pantoea* can enzymatically mineralized soil phosphorous into soluble forms.

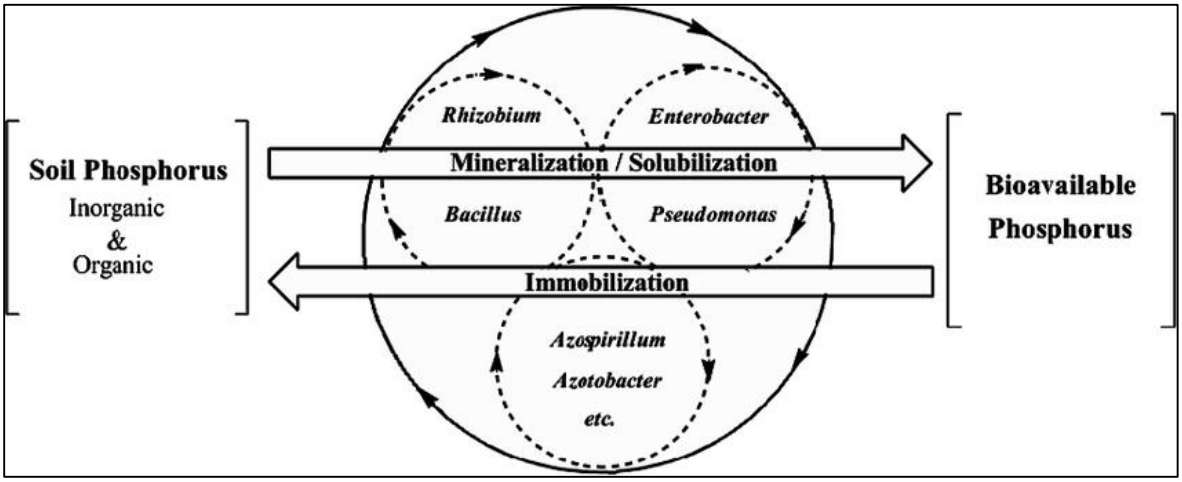


Figure 4. Soil Phosphorous by immobilization and mobilization by bacteria. Source: Mitran *et al.*, (2018).

Other study outcomes have revealed that the most common mechanism is the amalgamation of compounds capable of dissolving mineral encompassing siderophores, organic acids, hydroxyl ions, protons, and carbon iv oxide (Mitran *et al*., 2018; Pecoraro *et al.*, 2021). When synthesized alongside their hydroxyl and carboxyl ions, organic acids are known to chelate cations or reduce the pH, thereby releasing phosphorous (Wei *et al*., 2018). While the direct oxidation pathway is responsible for the release of organic acids which find their way into the periplasmic space (Zhao *et al.,* 2014), their excretion is followed by a decline in ph. This phenomenon leads to acidification of the involved microbial cells and the surroundings, thereby releasing P ions substitution of H^+ for Ca_2^+ (Timofeeva *et al.*, 2022).As a result,(Illmer *et al.*, 1995) suggested the hydrogen ion acidification theory. According to the theory, H^+ released is linked to cation assimilation. Phosphorus is solubilized as a product of NH^+_4 assimilation and H^+ excretion.

The discharge of H+ to the outer surface in exchange for the absorption of cations or with the aid of H^+ translocation. The solubilization of mineral phosphates can be accomplished using ATPase as an alternative to the production of organic acids. (Rodríguez & Fraga, 1999b). Additionally, it was shown that the assimilation of ammonium ions in microbial cells is followed by the release of protons, which results in the solubilization of phosphorus without the production of organic acids (Sharma *et al.,* 2013). Furthermore, of all organic acids, gluconic acid is the most effective solubilizer of mineral phosphate; it chelates the cations attached to soil phosphate to make the phosphate available for plant uptake (Suleman *et al.,* 2018).Gram-negative bacteria are known to solubilize mineral phosphate by converting glucose to gluconic acid via direct oxidation mechanism (Sashidhar & Podile, 2010).

In glucose dehydrogenases (GDH), pyrroloquinoline Quinone (PQQ) serves as a redox cofactor, resulting in phosphate solubilization (An & Moe, 2016).Two more ways that microbes solubilize mineral phosphate include the synthesis of chelating chemicals and the formation of inorganic acids including sulphatic, carbonic, and nitric acid. On the other hand, it has been suggested that organic acids are more effective than inorganic acids at releasing soluble phosphorus from soil. In reality, the formation of organic acids during P solubilization by PSM is not the only factor contributing to an elevated P surge in culture media. Another method of microbial phosphate solubilization is the release of enzymes. Lecithin-acting enzymes, for instance, cause this state to increase in a culture medium containing lecithin and produce choline (Aberathna *et al.*, 2022).

2.7 Factors Influencing Bacterial Phosphate Solubilization

PSB's capacity to transform insoluble phosphorus into soluble forms is attributed to the soil's nutritional richness, the bacteria's physiological ability and the bacteria's growth status. PSB has a stronger tendency to solubilize phosphate in soils from harsh environmental conditions than PSB present in soils from more favorable environments, such as alkaline-rich soils, soils with a high degree of nutrient deficit, or soils from high or low temperature settings (Johan *et al.,* 2021). Studies on the impact of temperature on bacteria in phosphorus solubilization has been unreliable since most reported temperature information differs (Saadouli *et al.,* 2021). Oehl *et al*. (2001) observed that the optimal temperature for phosphorus solubilization at maximum is 20–25°C, whereas Kang *et al.* (2002) and Varsha *et al*. (2002) documented 28°C. Others, including Rosado *et al*. (1998); Kim *et al*. (1997a), and Fasim *et al*. (2002), and Johri *et al.* (1999), have found that the best temperature for Phosphorous solubilization is 30°C. P solubilization in desert soil was observed by Nautiyal *et al.* (2000) and Nahas (1996) at an extreme temperature of 45°C, while Johri *et al.* (1999) observed solubilization at a low temperature of 10°C. Microbial interactions in soil coupled by vegetation cover and ecological conditions, land use, plant types and organic matter, soi pH are all factors influencing the solubilization of P (Heidari *et al.,* 2020; Musarrat & Khan, 2014). Hot humid climates solubilize phosphorus more quickly, while cool dry climates do so more slowly.

In comparison to a saturated wet soil, a well-aerated soil would allow for faster phosphorus solubilization (Bargaz e*t al.,* 2021). Zhang *et al.* (2014) recently noted that adding small quantities of inorganic P to the plant rhizosphere can endorse phytic acid bacterial

mineralization, improving plant phosphorus nutrition. Phosphate solubilizers were often supported by lime and compost, which were used as soil improvers. According to Yu *et al.,* (2021) crop rotation increased population richness and diversity of Phosphorus Solubilizing Bacteria. In terms of pH, phosphorous solubilization bacteria tolerates both acidic and alkaline soils as well as optimal soil pH (Sanchez-Gonzalez *et al.,* 2023).

2.8 Undesirable Effects of Inorganic Phosphates

To alleviate food hunger in Sub-Saharan Africa, chemical fertilizers are routinely employed in excessive and disproportionate amounts to increase agricultural yields. However, chemical fertilizers above a certain threshold level harm the soil and entire ecosystems in addition to being absorbed in agricultural plants (Aktar *et al*., 2009; Khalid *et al.,* 2018). Despite inorganic phosphate (P) playing crucial roles in several biological processes and signaling pathways in plants, continuous application on lands causes deleterious influences on environments majorly water bodies and soil. When excess is applied at inappropriate time, such as right before it rains, most of it is carried away and finds itself in local streams (Guignard e*t al.*, 2017). This type of pollution is considered a nonpoint source of pollution. It extremely causes eutrophication (a decline of dissolved oxygen in water bodies instigated by an upsurge of minerals and organic nutrients) of rivers and lakes. This reduced level of oxygen in water ends up suffocating aquatic animals.

Another negative effect of chemical fertilizers is compaction of the soil. The overuse of fertilizers over extended periods of time and heavy cropping is one of the main causes of compaction. Problems brought on by excessive soil strength, root development restriction, poor aeration, poor drainage, runoff, erosion, and soil deterioration are all brought on by

soil compaction (Shaheb e*t al.*, 2021). Disturbance and destruction of soil microorganisms is another major negative influence of chemical fertilizers. Studies have revealed that countless fertilization treatments across the world have a significant effect on the structure of soil microbial biomass and the community (Bai *et al.*, 2020). In Kenya, and most specifically Western Kenya, excessive use of inorganic chemical fertilizer in the agricultural areas has devastated the microbiota in the rivers and caused heavy siltation of Lake Victoria leading to eutrophication. This has encouraged the growth of large volumes of algae and other biomass such as papyrus, water hyacinth that consumes all the oxygen in the water, causing an ecosystem degeneration. Due to poor agricultural practices and overuse of inorganic fertilizer, soil pH is in most farms below 5.5. At this acidic pH most soils in Western Kenya have been found to be predominantly deficient in nitrogen, phosphorus, and potassium. Phosphorus, one of the key elements for plant growth, precipitates and is rendered unavailable under an acidic pH. In accordance with this, hydrogen and aluminum ions end up being poisonous and may harm the plants. Even with substantial external agricultural input in the form of inorganic fertilizer being employed, soil acidity can limit crop yield and result in poor crop harvest and quality if ignored. Poor soils have unfavorable effects on plant nutrient bioavailability, which makes plants more susceptible to disease and reduces their ability to produce.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The regions of isolation that represented Western Kenya were chosen by means of purposive random systematic sampling from the corresponding counties and sub-counties. The sampling locations (marked on the map in Figure 5) were Chaptais (N 0° 48.36'; E 34° 28.26') in Bungoma County, Teso South (N 0° 33.729'; E 34° 16.21'), Emuhaya (N 0° 5.42'; E 34° 34.65'), and Lurambi (N 0° 0.29'; E 34° 69.71') in Kakamega County. The main source of income of Western Kenya inhabitants is mixed agricultural farming (Ndeda, 2019). Sugarcane, maize, beans, finger millets, bananas, and sweet potatoes are among the main food and cash crops grown in the region (Rao *et al.*, 2015).Western Kenya is typically hot and humid, with year-round rainfall. According to *World Bank Climate Change Knowledge*, (2019) indicates that it received average temperatures of 21.28ºC and average rainfall of 2233.59 mm in the year 2021.All isolates were coded as per the initial of the author followed by the initial of respective county where it was isolated and lastly the digit number.

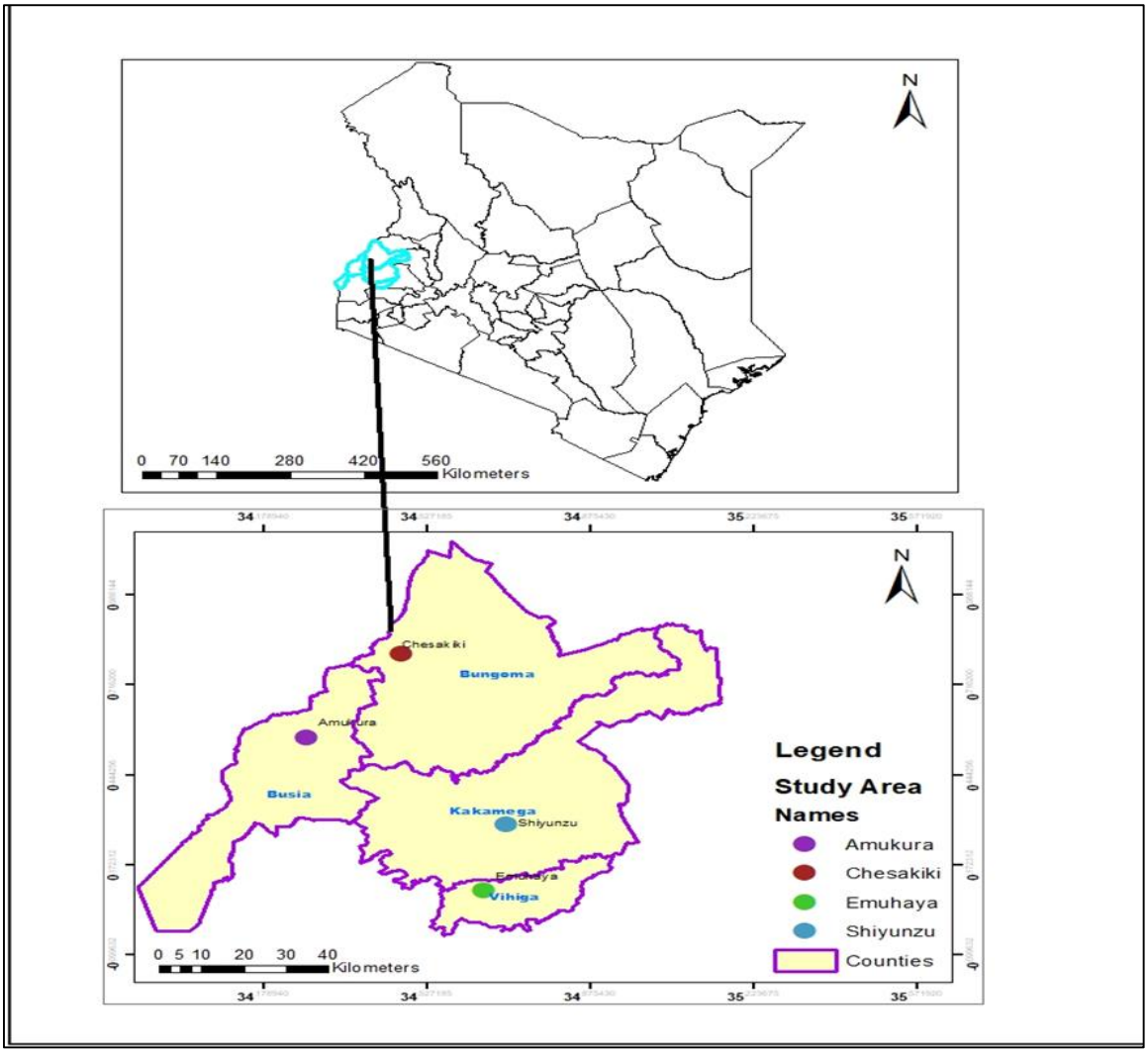


Figure 5. Map of Western Kenya and isolation sites of Phosphorus solubilizing isolates. Source: Author.

3.2 Experimental Design

The study involved an experimental design of screenhouse and laboratory experiments at Science Park Incubation and Innovation Center (SPIIC) and Biotechnology Laboratory at Masinde Muliro University of Science and Technology (MMUST). The study consisted a factorial treatment (6×4×6×2) =288 (Table 2). Two isolates KB5 and KV1 were selected as inoculants considering their high potentiality to solubilize phosphates *in vitro.*

Treatment 1 with KB5 inoculant on a Rosecoco variety, Treatment 2 with KV1 on a Rosecoco variety, Treatment 3 un- inoculated negative control on a Rosecoco, Treatment 4 with KB5 inoculant on a Mwetemania variety, Treatment 5 with KV1 on a Mwetemania variety, Treatment 6 with un- inoculated negative control on a Mwetemania variety. A treatment had n= 6 plants replicated four times to a total of 24 plants per treatment and the experiment was repeated once giving a total of 288 plants (N=288). The Leonard Jars were laid in a randomized blocked design.

Table 2. Screenhouse experimental design.

Treatment	Isolate (Inoculant)	Bean variety
1	KB5	Rosecoco
2	KV1	Rosecoco
3	Un-inoculated Control	Rosecoco
4	KB5	Mwetemania
5	KV1	Mwetemania
6	Un-inoculated Control	Mwetemania

Treatment of common bean varieties with Isolate KB5, KV1 and Negative Control (n= 6, N=288).

3.3 Bacterial Isolation

Root nodules and rhizosphere soil surrounding uprooted common bean were used to isolate bacteria using the method described by Tomer *et al.* (2017). Briefly, flowered bean plants were uprooted with a portion of the soil and the root nodules were collected into sterilized khaki paper bags and taken to the laboratory for morphological identification of

phosphorus solubilizing bacteria within 24 hours. Sampling of experimental plants was done by modifying a protocol by Kawaka *et al.*(2014). Homogenate of root nodules and rhizosphere soil (10% soil in 0.85% saline water) were made using a mortar and pestle followed by serial dilutions which were prepared within 24 hours at room temperature according to Pande *et al.* (2020).A droplet of liquid in diluents in the test tubes were place on the midpoint of sterile NBRIB agar plate and uniformly spread across the surface with the help of a sterilized glass-rod and incubated for five days at 28ºC. Sub culturing was done to obtain the pure isolates (Mohamed *et al.*, 2019).

3.4 Bacterial Identification

Isolates were grown on both solid and liquid nutrient medium of the National Botanical Research Institute's Phosphate Growth Medium (NBRIP) supplemented with Tricalcium phosphate (Nautiyal, 1999). NBRIP contains 10 grams of glucose substrate, 5 grams of $Ca_3(PO_4)_2$, 5 grams of $MgCl_2.6H_2O$, 0.25 grams of $MgSO_4.7H_2O$, 0.2 grams of KCl, 0.1 grams of $(NH_4)_2SO_4$, 15 grams of agar in 1000 milliliters of distilled water. The pH of the media was adjusted to 7.0 before autoclaving. Bacterial strains were introduced into the media by the standard pour plate technique using a sterile dropper (10 µL of aliquots per plate) (Burns, 2005). They were incubated for 7 days at 28°C. At the end of the incubation, PSB were able to grow and were identified through the formation of a halo zone around the colony (Khan e*t al.,* 2013). Colonies that did not form the halo zone were exempted. The colony diameter (C.D) and halo zone diameter (H.D) of each isolate was measured and the Solubilizing Index (SI) was calculated.

3.5 Determination of Solubilization Indexes (SI)

The National Botanical Research Institute's phosphate growth medium (NBRIP) agar medium was sterilely poured into sterile Petri plates that contained insoluble Ca_3 $(PO_4)2$ at a concentration of 5 g/L^{-1} in order to calculate the phosphorous solubilization index (SI). The isolated bacteria were inoculated to the plates after the media had solidified. The plates were then incubated at 28 °C for two weeks before being visually inspected. Employing the subsequent formula by Dipak (2016), the solubilization indices were calculated by measuring the colony diameter and the halo (clear zone) diameter (Figure 6). Three replicas of each experiment were performed.

$$\textit{Solubilizing Index (SI)} = \frac{\text{Isolate's Colony Diameter(C.D)+Isolate's Halo Zone(H.D}}{\text{Isolate's Colony Diameter (C.D)}}$$

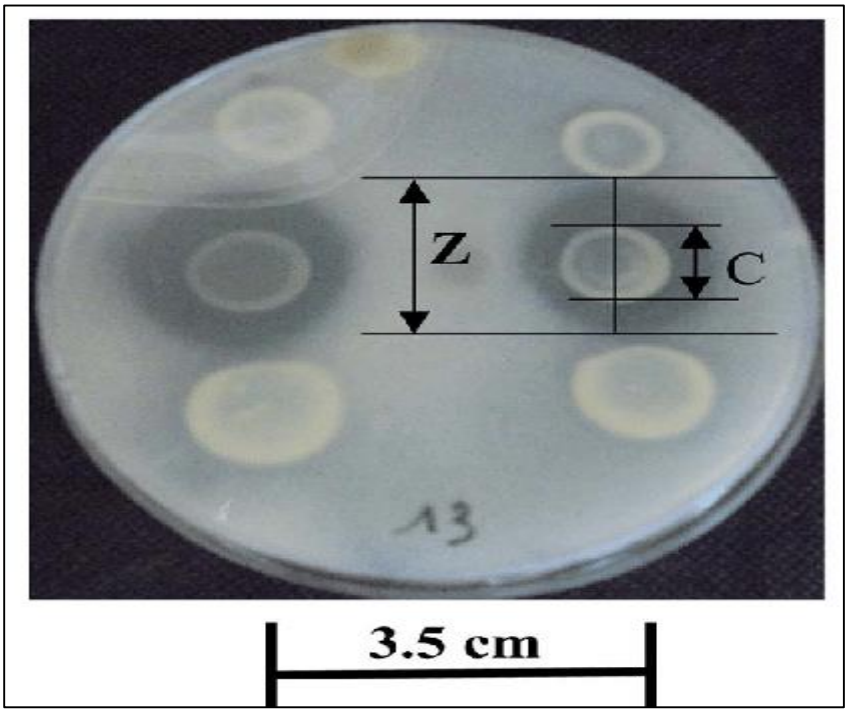


Figure 6. Determining the solubilizing index's C is Colony diameter while Z is Halo zone diameter. Source: Ouattara *et al*.(2019).

3.6 Determination of percentage Phosphate Solubilization of Isolates

Step 1; Determining the LDI (Logarithmic Divergence Index)

Logarithmic Divergence Index

(LDI) = Ln (S.I) – Ln (2)

Where,

Ln (S.I) is Natural logarithm of Solubilizing Index of the isolates

Ln (2) is a natural logarithm of constant when isolate does not solubilize

Step 2; Determination of Corresponding Absolute Number

To find corresponding absolute number

e^{LDI} = C.A.N

Step 3

Finding β

$$\frac{100\%}{C.A.N}$$

Step 4

Finding the corresponding solubilizing index percentage of the isolates

C.S.I = 100% -β

3.7 Determination of Phosphate Solubilizing ability in Liquid Media

A culture of 1 mL of the isolated strains (OD_{600} = 0.5 nm) were inoculated separately into 250 ml Conical Flask containing 150 mL of liquid NBRIP medium supplemented with 0.5 % Tri-calcium phosphate (Thomas Baker, Mumbai India) and incubated at 28°C for 24 hours. Sterile water inoculated into medium was treated as a control. Approximately 1 mL of the supernatant was used after 18000 ×g centrifugation for 5-minute to assess

phosphorus released into the solution. Phosphorus in the supernatant was determine by the molybdenum blue colorimetric method according to Murphy & Riley (1962). The reagents were made up of an ascorbic acid and antimony containing acidified ammonium molybdate solution. This substance combines quickly with the phosphate ion to produce a blue-purple molecule that has an atomic ratio of 1:1 antimony to phosphorus. As long as there is at least 2 g/mL of phosphate in the solution, the complex is extremely stable and follows Beer's law (Figure 9). The absorbance was measured at a wavelength of 800 nm with Ultraviolet and Visible Range Spectrophotometer.

3.8 Determination of Phosphatase Enzyme Activity

The phosphatase activity was calculated using the method described by Behera et al., (2017). A 2.5 ml Eppendorf tube was filled with 1.5 mL of a 24 hour actively growing PSB culture that had been initially inoculated in 250 ml of NBRIP broth. The tube was then centrifuged at 10,000 rpm for 10 min at 4 ˚C. A culture (1 mL) supernatant was combined with 4 mL of Modified Universal Buffer (MUB) (pH 6.5), and then 0.115 M disodium p-nitrophenyl phosphate (tetrahydrate) was added. The mixture was then incubated at 37 ^{o}C for one hour. To stop the growth of the microbial culture, a few drops of toluene were added to the mixture. In order to disrupt and halt the reaction after incubation, 1 mL of 0.5 M calcium chloride solution and 4 mL of 0.5 M sodium hydroxide were added. This was followed by filtration using Whatman filter paper. A UV-Vis spectrophotometer was used to measure the absorbance at 420 nm (Figure 10). A unit of phosphatase enzyme activity was defined as the quantity of enzyme that was able to release 1 nmol of *p*-nitrophenol from disodium *p*-nitrophenyl phosphate in a minute, per one milligram (Rombola *et al*.,

2014). MUB was prepared according to Tabatabai & Bremner, (1969). It consisted of 3.025g Tris-(hydroxymethyl)-aminomethane, 2.9 g maleic acid, 3.5g citric acid, 1.57 g boric acid, 1 M Sodium hydroxide (NaOH) solution (122 mL) and distilled water added to a final volume of 250 mL.

3.9 Determination of PSB Solubilization Potential of the Isolates in Plant System

Phenotypic characteristics of potential selected PSB isolates (KB5 and KV1) were determined by carrying out an experiment in a screenhouse at MMUST Science Park, Incubation and innovation. Two common bean varieties from Kenya Seed Company (Rosecoco and Mwetemania) were used as test crops. Certified bean seeds were surface sterilized with 1% mercuric chloride for 3 minutes followed by rinsing with distilled water thrice and pre-germination in a darkroom using petri dishes. Inoculants were prepared according to Mohamed *et al.*,(2019). The isolates were grown in NBRIB broth for 2 days and cells were harvested by centrifugation at 5000 ×g for 20 min. The cells were re-suspended with sterile distilled water to give a final concentration (10^8 CFU ml^{-1}) in 250 mL conical flask. The seedlings' roots were immersed into the culture for 5 minutes and covered uniformly with 15 mm thick layer of vermiculite in a Leonard's Jars then placed into a completely randomized design alongside negative control (un-inoculated seedlings).

A total of six treatments was replicated four times to obtain 24 experimental units with two trials. Leonard's jars assemblies (Clayton *et al*., 2016) (9 cm diameter, 12 cm height) were filled with the sterile vermiculite (Kenworks, Nairobi, Kenya). Tri-calcium phosphate was provided as soil inorganic phosphorus fertilizer at the rate of 150 mg /kg based on the

nutrient necessities of common bean plants (Abdelmoteleb & Gonzalez-Mendoza, 2020). Depth (5cm) was dug into the Leonard's Jar and two seedlings were placed at equal distances. A modified nutrient solution without phosphorus was supplied to all treatments (Olfati, 2015). After 6 weeks, main shoot and root length and number of leaves per plant were measured and recorded. The same plants were uprooted and oven-dried at 70°C to a constant weight and were grinded after drying to determine total dry weight in grams.

3.10 Bacterial Cell Preparation and Isolation of Genomic DNA.

Culture cells were harvested from a 48 hour (OD_{600} = 0.8) actively growing in nutrient broth of NBRIB. Approximately 1.5 ml (10^8 CFU Ml^{-1}) of bacterial culture were pipetted into 2 mL micro tubes followed by spinning at 20,000 ×g for 5 minutes in a centrifuge. Total DNA of selected PSB isolates was extracted using QIAmp DNA kit (Qiagen, Hilden, Germany) according to manufacturer's protocol. Template DNA (8 µl) was checked for quality by electrophoresis in a 2% agarose gel (pre-stained with ethidium bromide 0.5 µg ml^{-1}), then visualize on a UV trans-illuminator and photographed. The DNA was stored at −20°C for further downstream process analysis. DNA was quantified by Nano drop spectrophotometric analysis.

The following detailed QIAam Protocol of genomic DNA isolation from bacterial suspension cultures was used:

Bacterial culture approximately (1.5 ml) was pipetted into a 2 ml micro centrifuge tube followed by centrifugation for 5 min at 20,000 x g. Addition of 180 µL lysis Buffer ATL (supplied in the QIAamp DNA Mini Kit) followed by addition 20 µL proteinase K and mixing by vortexing, and incubation at 56°C in a water bath until the tissue is completely

lysed. Two hundred microliters of Buffer AL were added to the sample, pulse-vortexing for 15 seconds and incubated at 70°C for 10 mi. Addition of 200 µL ethanol (96–100%) to the sample and the mixture was carefully applied into the QIAamp Mini spin column (in a 2 ml collection tube) and centrifugation was performed at 6000 x g (8000 rpm) for 1 mi. The filtrate was discarded. Five hundred microliters of wash buffer AW1 was added without wetting the rim and centrifugation done at 6000 x g (8000 rpm) for 1 min. Washing for the second time was done using 500 µL wash buffer AW2 followed by high-speed centrifugation for three minutes. When eluting the DNA, QIAamp Mini spin column was in a clean 1.5 ml micro centrifuge tube and 200 µL elution Buffer AE was added followed by incubation for three minutes and lastly final centrifugation at 6000 x g (8000 rpm).

3.11 Quantification of extracted DNA

The Extracted DNA was quantified by Nano drop spectrophotometer and the concentrations of the DNA of each isolate was measured. The concentration was measured alongside the ratios of proteins and other contaminants to check the purity of the DNA before Polymerase chain reactions and DNA Sequencing.

3.12 Polymerase Chain Reactions (PCR)

16S rRNA gene was amplified using the following universal primers shown in table 3.

Table 3. Universal primers for 16S rRNA

Forward Primer	27 f (5’AGAGTTTGATCCTGGCTCAG 3’)
Reverse Primer	1492r (5' TACGGCTACCTTGTTACGACTT 3')

Source :Dos Santos e*t al*., (2019).

Gene amplification was carried out in a 25 µL reaction volumes containing 2.5 µL 10X DreamTaq buffer (100 mM Tris-HCl, pH 8.0, 500 mM KCl and 1.5 µL 25 mM MgCl), 2.0 µL, 2.5 mM, dNTPs, 0.5 µL of 27f primer (200 ng/µL), 0.5 µL of 1492r primer (200 ng/µL), 0.25 µL DreamTaq DNA polymerase (5U/l) and 10 µl of extracted template of Phosphorus Solubilizing Bacterial DNA. The reaction volume was accustomed up to 25 µL with sterile distilled water.The PCR thermal cycling process consisted of an initial DNA denaturation stop at 94°C for 3 minutes, followed by 35 cycles of DNA denaturation (1 min at 94°C), annealing stage for 1 minute at 57°C and extension for 2 minutes at 72°C, followed by a final elongation stay at 72°C for 8 minutes (Lorenz, 2012).

3.13 Molecular Characterization and Sequencing of 16S rRNA gene

The 16S ribosomal RNA gene was partially sequenced in order to undertake molecular identification of the isolates to the genus level of the chosen PSB strain. The sequences collected for this investigation were examined by the BLAST algorithm for comparison of a nucleotide query sequence against a public nucleotide sequence database in order to identify closely related bacteria against the non-redundant nucleotide BLAST database. In order to compare the 16S rRNA gene sequences of the top two solubilizing isolates with sequences that was retrieved from the NCBI database, phylogenetic analysis using the Neighbor-Joining method was conducted. A phylogenetic tree was constructed to show the position of isolated strains with the species of each genus in the NCBI database and the species of the isolates were identified with closely related strains. The forward and reverse nucleotide contigs were merged using BioEdit 7.2 to reconstruct the full 16S rRNA genes and aligned with CLUSTAL *W* (Tamura *et al*., 2021) .

The phylogenetic tree, which contains PSB sequences of 16S rRNA gene and sequences with high similarity scores from the GenBank database, was constructed with MEGA 11.0 (Tamura *et al.*, 2021) with 1000 bootstrap analysis. The sequences were then submitted to NCBI GenBank database and accession numbers allocated as follows: ON931237, ON931235, ON931 236, ON931234, ON931238, ON931233 and ON931239.Evolutionary analyses were conducted in MEGA11 (Tamura *et al*., 2021). Analyses were conducted using the Maximum Composite Likelihood model. This analysis involved seven nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option).

3.14 Statistical Analysis

The solubilizing indices data were recorded and entered into Microsoft excel (MS 2016) for management. ANOVA was used to determine significance difference between means of the replicated isolates on a petri dish. Data were tested for homogeneity using Shapiro-Wilk and Tukey post-hoc was used to differentiate the means of solubilizing potential at p=0.05. IBM SPSS Version 20 software was used for analysis. Data was presented using tables (Table 4 and Table 5). Relationship between amount of phosphate and phosphatase enzyme activity was analyzed using Pearson's correlation (Coefficient *r*) with a stats model package in Python 3 to test significance relationship between mineralization potential of each isolate. Data was presented using tables and graphs (Table 6 and Figure 8). Screenhouse data (Phenotypic/Growth parameters) were analyzed using a two ANOVA to test significantly different at Tukey $p \leq 0.05$. Biomass data were graphically plotted by Matplotlib package in Python 3 (Figure 12) while the rest of data were presented using

tables and figures (Table 11, Table 12 and Figure 12).

Nucleotide sequences of the PSB isolates were compared with references strains from NCBI GenBank database. Raw sequences were cleaned, edited and assembled Using BioEdit 7.2. BLAST algorithm was used to analyze the sequences of the isolates to identify closely related organism. Nucleotide distribution, Nucleotide alignments (CLUSTAL W) and phylogenetic analysis was performed using Maximum Likelihood method in MEGA software version 11 with bootstrap significant value to determine the robustness (Tamura *et al.,* 2021). *Staphylococcus aureus* strain ACTT 12600 was used an out-group. Original phylogenetic tree (Appendix III) was exported to Fig tree for visualization (Figure 12).

CHAPTER FOUR

RESULTS

4.1 Mineralization Potential of Phosphorus Solubilizing Bacteria

4.1.0 Quantitative Screening of Phosphate by PSB Strains in Agar Plates

Formation of clear zones around the colony was an indicator of Tri-calcium phosphate solubilization by the isolates (Figure 7). Seven isolates out of twenty-six were able to solubilize phosphates in agar plates by forming the halo zones. The colony diameter (C.D) and halo zone diameter (H.D) of each isolate was measured and Solubilization Index (SI) was calculated after seven days' incubation at 28 ºC (Table 4). The phosphate solubilization index of tested bacterial strains ranged from 2.34 to 4.17 Isolate B5 displayed a highest solubilizing index of 4.17 followed by strain KV1 with 3.64. Isolate KK3 followed (2.60), KKI (2.54), KB3 (2.52), and KB2 (2.40). The least performed isolate was KBU with SI of 2.34 in the agar plate.

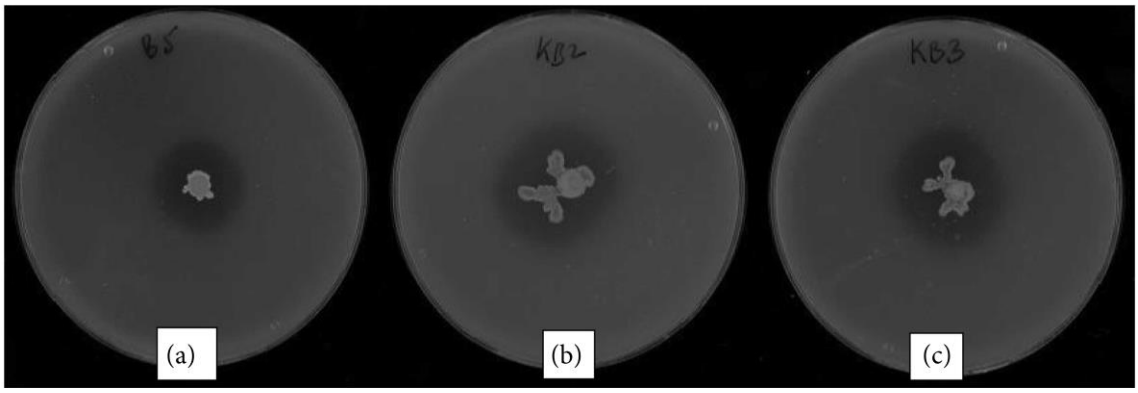


Figure 7.Formation of clear zones of solubilization by isolates. (a) KB5, (b) KB2, (c) KB3 on an agar plate.

Table 4. The mineralization potentials of each isolate.

Isolate	C.D	H. D	S. I
KB5	0.53 ±0.06	1.68 ±0.10	4.17_{a}
KB3	0.77 ±0.15	1.17 ±0.15	2.52_{c}
KB2	0.93 ±0.06	1.30 ±0.10	2.40_{c}
KV1	0.58 ±0.19	1.53 ±0.15	3.64_{ab}
KK1	0.67 ±0.08	1.03 ±0.15	2.54_{c}
KK3	0.47 ±0.15	0.75 ±0.12	2.60_{bc}
KBU	0.88 ±0.12	1.18 ±0.16	2.34_{d}

C.D is Colony diameter ± SD (cm), H.D is halo zone diameter ± SD (cm) and column S.I is Solubilizing Index. S.I values with same superscript letters indicate statistical significance according to turkey test at 5 % ($p \leq 0.05$)

Table 5. The percentage mineralization potentials of each isolate;

Isolate	S. I	L.D. I	C.A. N	β	C.S.I (%)
KB5	4.17	0.73	2.08	47.96	52.04
KB3	2.52	0.25	1.29	79.38	20.62
KB2	2.40	0.15	1.17	83.41	16.59
KV1	3.64	0.61	1.85	54.98	45.02
KK1	2.54	0.24	1.27	78.82	21.18
KK3	2.60	0.30	1.35	77.05	22.95
KBU	2.34	0.16	1.17	85.44	14.56

Percentage Corresponding Solubilizing Index (C.S.I) of each isolate. S.I is Solubilizing Index L.D.I, Logarithmic divergence index, C.A.N, corresponding absolute number.

4.1.1 Quantitative Screening of Phosphates Solubilized by Isolates in Broth Medium

In the current investigation, isolate KV1 yielded more soluble phosphates (1440.92 µg/mL), whereas isolate KB5 showed similar capability for P solubilization at 1370.06 µg/mL (Table 6). Equivalent phosphorus solubilization capacity was shown by the isolates KK1 and KBU, which both solubilized P at concentrations of 1292.88 µg/mL and 1236.65 µg/mL, respectively. Isolates KB2 and KB3 produced phosphate concentrations of 1189.03 µg/mL and 1149.15 µg/mL, respectively, and they both carried out phosphate mineralization on agar plates in a manner that was comparatively similar. In broth media, the Kakamega County KK3 isolate's solubilization potential for phosphorus was the lowest (453.90 µg/mL). The concentration was determined using Beer Lampert standard curve for determining phosphate concentration (Figure 9).

4.1.2 Determination of Phosphatase Enzyme Activity

Isolate KV1 had the highest phosphatase enzyme activity, with a value of 94.92 nmol/min, followed by KB5 (91.49 nmol/min), KK1 (72.24 nmol/min), and KB2 (45.36 nmol/min), while KBU and KB3 had values of 39.59 nmol/min and 32.22 nmol/min respectively. The least effective isolate, KK3, had an activity of 22.55 nmol/min (Table 6). According to a correlation analysis, there is a substantial positive association between the number of phosphates in the medium and the activity of the phosphatase enzyme (Correlation coefficient of r2 = 0.83; Figure 8). The concentration was determined using Beer Lampert standard curve for determining amount of p-Nitrophenol (Figure 10).

Table 6 . Correlation between amount of phosphate in broth and phosphatase activity

Isolate	Amount of phosphate in broth and phosphatase activity	
	P (ug/ml)	Phosphatase activity (nmol min^{-1})
KB5	1370.06 ±39	91.49 ±34
KB3	1149.15 ±4	32.22 ±4.3
KB2	1189.03 ±9	45.36 ±5
KV1	1440.92 ± 92	94.92 ±24
KK1	1292.88 ±6	72.24 ±13
KK3	453.90 ±36	22.55 ±3
KBU	1236.65 ±52	39.59 ±0.8

Mean of phosphatase enzyme activity and amount of phosphate in liquid medium.
Correlation $R^2 = 0.83$

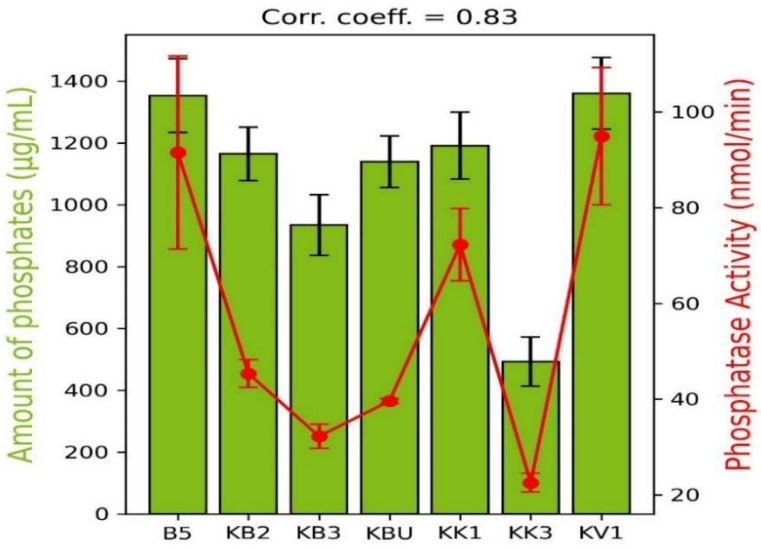


Figure 8. Amount of solubilized phosphorus and Phosphatase enzyme activity by each PSB isolate.

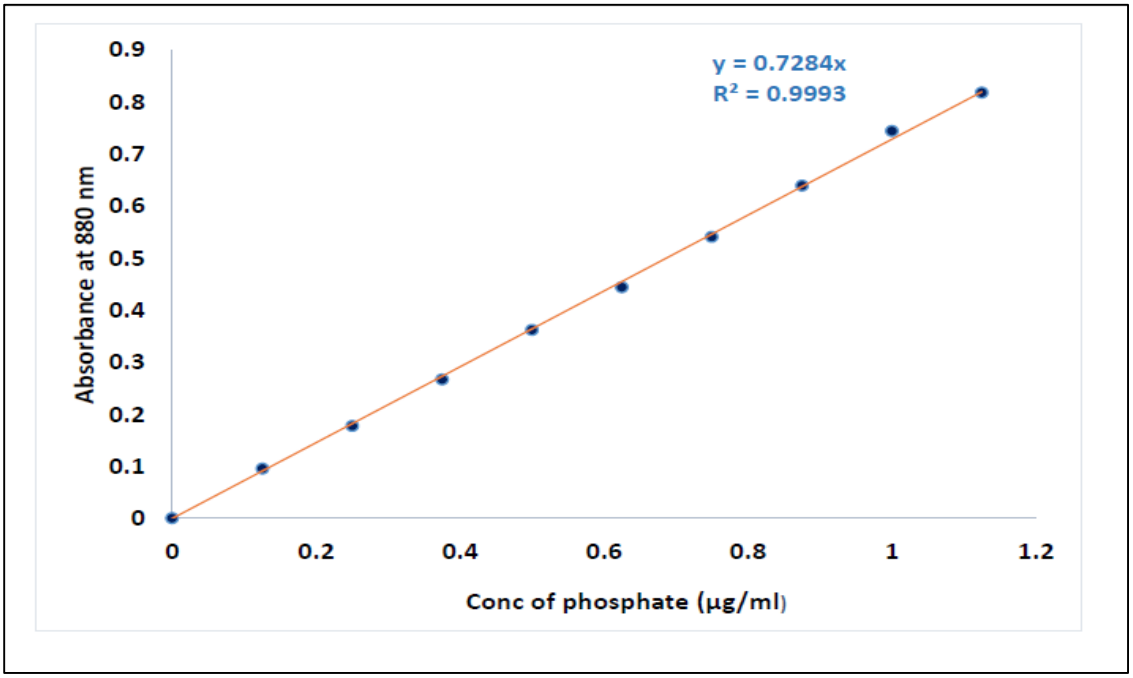


Figure 9. Beer Lampert standard curve for determining phosphate concentration.

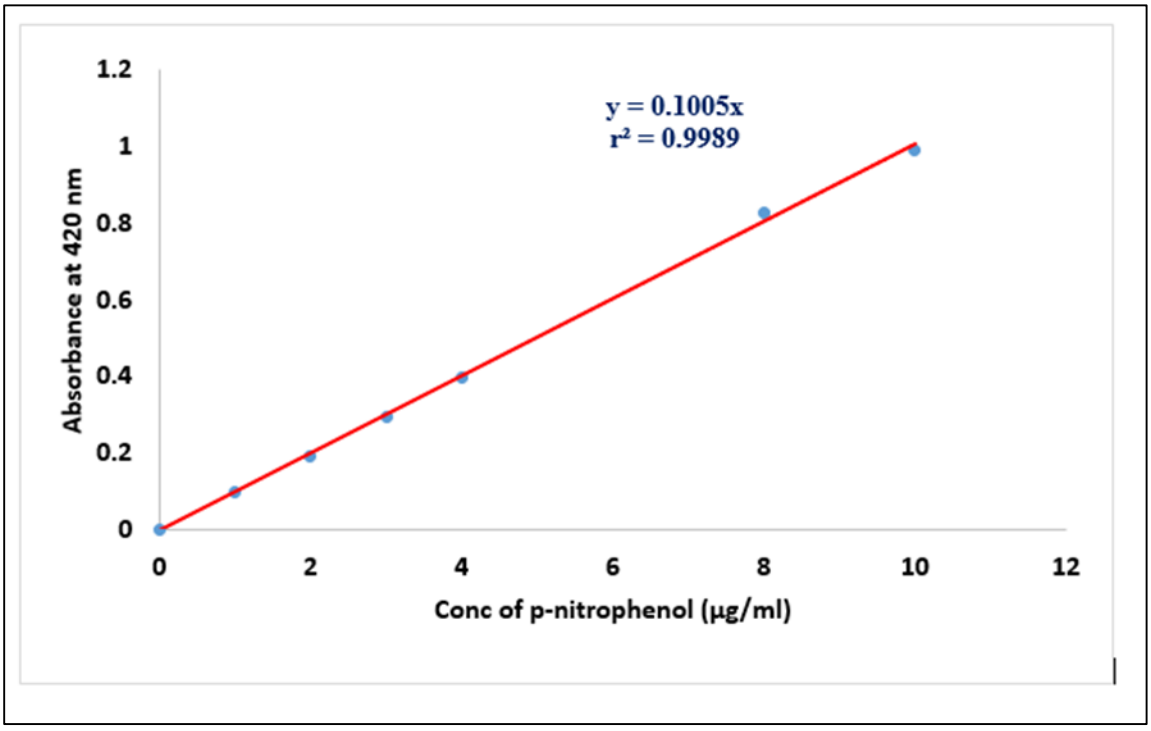


Figure 10. Beer Lampert standard curve for determining amount of p-Nitrophenol.

4.2 Molecular Characterization of Phosphorus Solubilizing Bacteria

4.2.1 Isolate's DNA quantity determination using Nano drop spectrophotometer

The purity of the DNA isolated from all the bacteria isolates was ≥ 1.8 at absorbance ratio 260/280 indicating less contamination with proteins and higher concentration of DNA (Appendix II). At absorbance ratio 260/230, the ratios of the DNA were ≥ 1.8, indicating free contamination from organic compounds.

4.2.2 Determination of Isolate's DNA quality using Gel –Electrophoresis

The DNA was also checked for integrity using Gel electrophoresis (Figure 11). 16S r RNA gene has almost similar size characteristic as evidence by equal bands of template DNA after polymerase chain reaction product of the isolates (Figure 11). Samples were analyzed together with 1 Kb ladder of approximately 1200 base pairs. The results indicated that DNA samples were approximately 1200 base pairs. L (1.2kb DNA Ladder), Lane 2, KBU, Lane 3, KB3, Lane 4, KB5, Lane 5, KBU, Lane 6, KK1, Lane 7, KK3 and Lane 8, KV1.

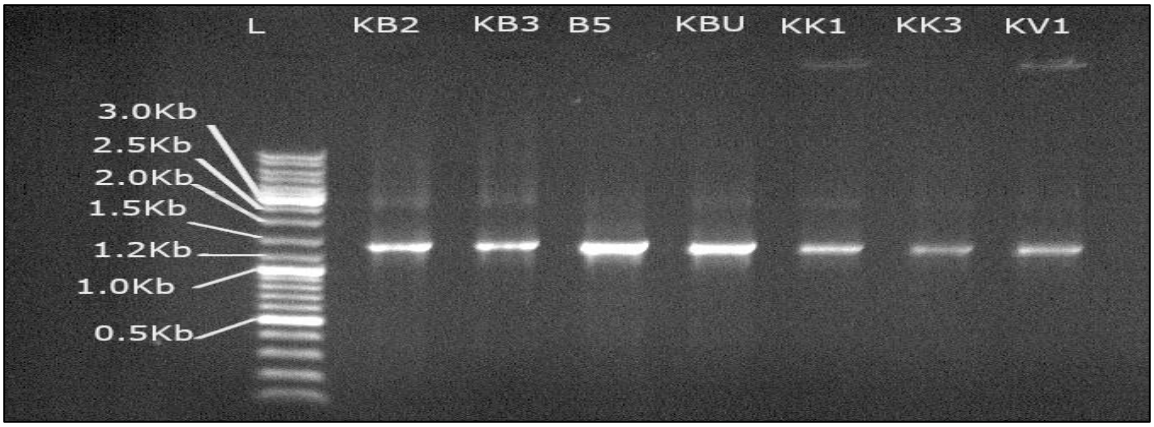


Figure 11. A 16s ribosomal partial gene of the isolates after gel electrophoresis in 1.5% agarose gel.

4.2.3 BLAST and Nucleotide Sequence Characteristics

Upon sequencing the 16S rRNA gene, the nucleotide sequences of the isolates were all approximately 1300 base pairs long and 1.2kb (Figure 11) after amplification by PCR. Table 7 shows the specific identities of the isolates after nucleotide blasting in the NCBI gene bank. The blasting revealed that the isolates belong to two genera; *Enterobacter* and *Pseudomonas.* In a complementary identification, KB5 isolates with 1249 base pairs from Bungoma County was presumably matched to be belonging to *Pseudomonas kribbensis* with 99.60 percentage identity from the gene bank while KB2 from same region with 1104 base pairs was identified with 98.57% as *Enterobacter bugandensis.* KBU isolates which was from Busia County with 1260 base pairs was matched with *Enterobactor tabaci* with 99.28 % identity while KB3 and KK3 from Bungoma and Kakamega counties *were* identified as *Enterobacter mori* with 99.07% (1065 bp) and 98.51% (1059 base pairs) respectively. KVI isolate with 1029 base pairs from Vihiga County was identified as *Enterobactor asburiae* with 98.36% identity while KK1 isolate from Kakamega with 1262 base pairs was identified as *Enterobactor cloacae* with 98.97 % identity. All the 16S Ribosomal gene nucleotides sequences of the phosphorus solubilizing isolates were submitted under submission ID SUB11747981 to GenBank and they were assigned accession numbers indicated in Table 7.

Table 7. Molecular identities of the isolates basing on 16S ribosomal gene

Isolate	Isolation Site	P.I	E.V	Strain Name	Accession Number.
KB5	Bungoma	99.60%	0.0	*Pseudomonas Kribbensis*	ON931237
KB3	Bungoma	99.07%	0.0	*Enterobacter mori*	ON931235
KB2	Bungoma	98.57%	0.0	*Enterobacter bugandensis*	ON931236
KV1	Vihiga	98.36%	0.0	*Enterobacter asburiae*	ON931234
KK1	Kakamega	98.97%	0.0	*Enterobacter cloacae*	ON931238
KK3	Kakamega	98.51%	0.0	*Enterobacter mori*	ON931233
KBU	Busia	99.28%	0.0	*Enterobacter tabaci*	ON931239

NCBI, Blast search analysis; P.I, Percentage identifies, E.V, Expected Value, Sixth Column represents accession numbers from GenBank.

4.2.4 Nucleotide Base Sequence Distribution

Results showed that there was high distribution of Cytosine (C) base with an average percentage of 30.5 (Table 8) amongst all the isolates except isolate KB5 which had 22.1% cytosine base. Distribution of Guanine bases followed with an average percentage of 25.3 and Isolate B5 had highest guanine base distribution of 31.3 % across the gene. This was followed by thymine base with 24.6 % while adenine had the least with 20.6 %.

Table 8. Nucleotide Distribution Frequencies across 16S rRNA gene sequence for each isolate

Isolate	T(U)	C	A	G	Total (kb)
KK3	25.3	31.9	19.7	23.5	1061
KV1	25.4	32.1	19.4	23.1	1029
KB3	25.4	31.9	19.7	22.9	1065
KB2	25.4	31.6	19.8	23.2	1104
KB5	21.1	22.1	25.5	31.3	1249
KK1	24.8	32.4	19.7	23.1	1262
KBU	25.2	32.2	19.7	22.9	1259
Average	24.6	30.5	20.6	25.3	1147

Nucleotide distribution frequencies (MEGA Version 11). All frequencies are given in percentage.

4.3 Phylogenetic analysis of Isolates using Neighbor Joining Analysis

After multiple sequence alignment of the isolate using *Clustal W* (Appendix V.), A phylogenetic relationship was determined with 1000 bootstrap statistical analysis and a construction of a phylogenetic tree with values greater than 60 bootstrap (Appendix III). The three results were visualized using fig tree software (Figure 12). The phylogenetic tree of the PSB isolates using neighbor joining method separated the isolates into two main clusters when compared with other closely related reference organism downloaded from the NCBI database. The *Enterobacter spp*, genus contained six isolates, clustered together forming a related clade while one isolates *Pseudomonas kribbensis* forms its own clade revealing a distant relative to *Enterobacter spp*,. *Staphylococcus aureus* strain ATCC12600 was analyzed as an outgroup organism forming its own branch.

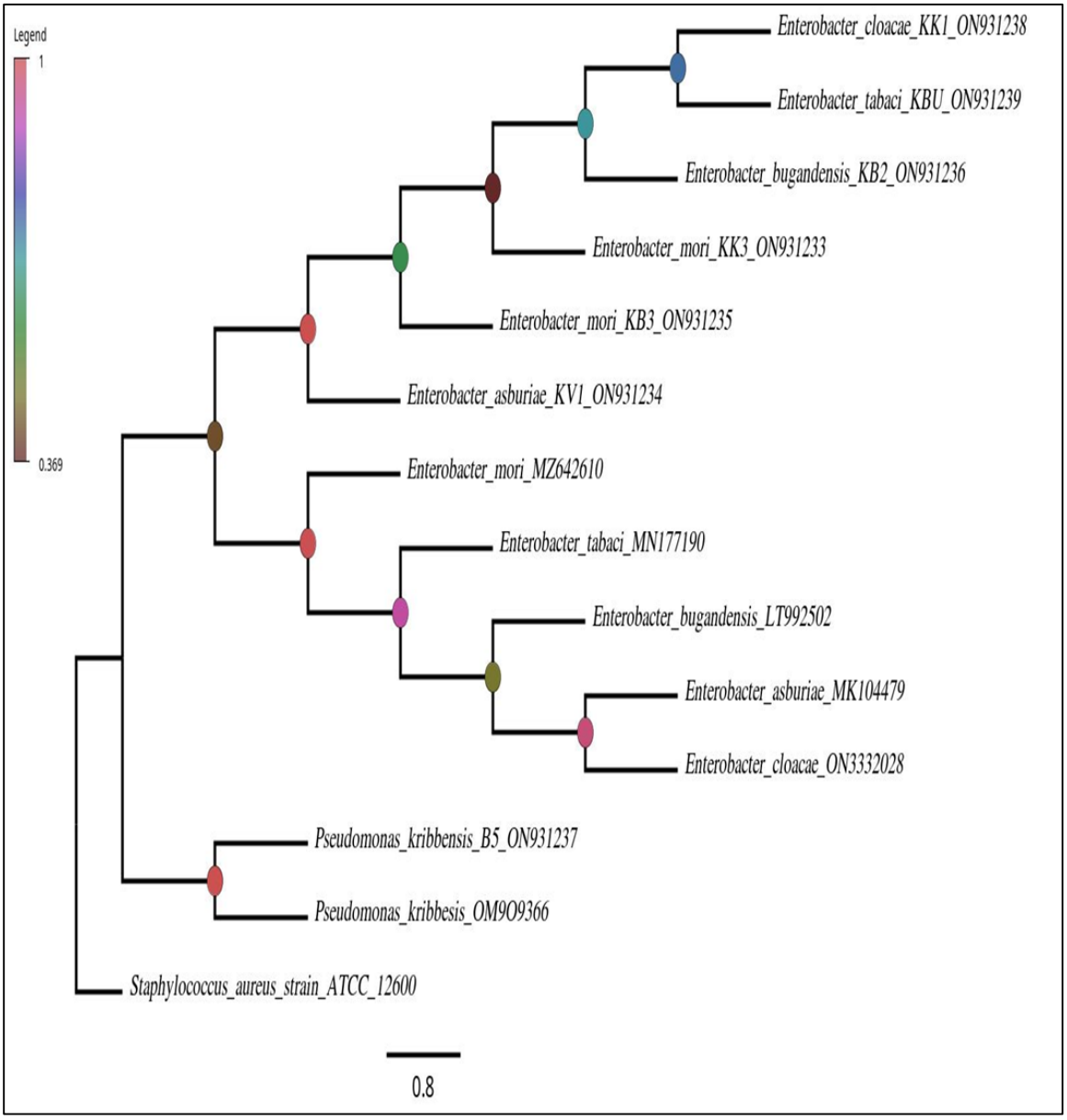


Figure 12. A tree showing phylogenetic relation between isolates with *Staphylococcus aureus* as an out-group. The nodes of the tree are colored as per the legend in which the color corresponds to approximate bootstrap support value.

The number of base substitutions per site from between sequences are shown. There were a total of 1262 positions in the final dataset. Isolate KB5 and KB3 displayed a greater evolutionary divergence index among the isolates while KK3 and KB3 displayed least evolutionary divergence among other isolates (Table 9).

Table 9. Estimates of Evolutionary Divergence between isolates

	KK3	**KV1**	**KB3**	**KB2**	**KB5**	**KK1**	**KBU**
KK3							
KV1	3.022						
KB3	0.369	3.380					
KB2	4.280	3.918	4.366				
KB5	4.900	3.511	5.195	2.648			
KK1	2.827	4.026	1.127	2.838	3.625		
KBU	4.227	2.677	3.625	2.871	4.673	3.521	

4.4 Determination of Plant Growth Characteristics of Potential PSB

In general, all the two isolates (KB5 and KVI) significantly promoted the growth parameters (shoot biomass, root biomass, number of leaves and shoot length) of Mwetemania and Rosecoco bean varieties which are the common legumes grown in Western Kenya for food.

4.4.1 Effects of PSB on Growth Characteristics of Rosecoco Bean Variety

In overall, inoculation with KVI and KB5 strain displayed substantial escalation in number of leaves as compared with controls (Table 10). A Rosecoco variety inoculated with KV1

was able to grow with 27.00 average number of leaves per plant while isolate B5 inoculated into same variety grows to 22.25 average number of leaves per plant while plants that didn't receive any inoculant (Control) grows to 14.75 average number of leaves per plant. KB5 increase the shoot length of Rosecoco at 14.9 cm while KV1 at 16.4 cm while control was able to increase to a length of 11.24 cm.

Table 10. Effects of PSB bacteria inoculation on growth characteristics of the Rosecoco

	Treatment		
Plant Growth Parameter	**B5 Inoculant**	**KV1 Inoculant**	**Control**
Number of leaves per plant	22.25 ± 4.03^{bc}	27.00 ± 4.24^{a}	14.75 ± 2.06^{d}
Shoot length (cm) per plant	14.90 ± 0.37^{b}	16.4 ± 0.51^{a}	11.24 ± 1.27^{c}
Plant dry weight (g) per plant	6.52 ± 1.22^{a}	3.97 ± 0.86^{bc}	2.06 ± 0.78^{c}
Root weight (g) per plant	0.84 ± 0.11^{a}	0.725 ± 0.15^{ab}	0.44 ± 0.18^{c}

Means ± SD values with same statistical letter (s) within rows are not significantly different. (Two Way ANOVA test $p \leq 0.05$ at turkey post hoc).

4.4.2 Effects of PSB on Growth Characteristics of Mwetemania Bean Variety

Mwetemania variety grows significantly after inoculated with KV1 isolate as it was able to yield a number of leaves of 30 per plant (Table 11). KB5 Isolate followed with 24.75 average number of leaves per plant while control plants grow to 18.25 number of leaves

per plant. Inoculation of Isolate KV1 greatly increased the shoot lengths of Mwetemania up to 17.75 cm while the control was the least in shoot length with 10.4 cm long.

Table 11. Effects of PSB bacteria inoculation on growth characteristics of the Mwetemania

	Treatment		
Plant Growth Parameter	**KB5 Inoculant**	**KV1 Inoculant**	**Control**
Number of leaves per plant	24.75 ± 2.87^{abc}	30.5 ± 5.17^{a}	18.25 ± 2.75^{cd}
Shoot length (cm) per plant	13.85 ± 0.90^{b}	17.75 ± 0.79^{a}	10.40 ± 0.53^{c}
Plant dry weight (g) per plant	6.15 ± 1.14^{a}	4.08 ± 0.75^{b}	2.15 ± 0.81^{c}
Root weight (g) per plant	0.69 ± 0.10^{ab}	0.73 ± 0.31^{ab}	0.37 ± 0.15^{b}

Means ± SD values with same statistical letter (s) within rows are not significantly different. (Two Way ANOVA test $p \leq 0.05$ at turkey post hoc).

4.4.3 Effects of PSB on Biomass of the Bean Varieties

In terms of shoot dry weight, KB5 isolate performed better in the two bean varieties as it yielded an average 6.52 grams per plant in Rosecoco and 6.15 grams per plant in Mwetemania.KV1 isolate yielded a shoot dry weight of 4.08 grams in Mwetemania variety and 3.97 grams in Rosecoco variety. The negative controls of Mwetemania and Rosecoco yielded 2.15 grams and 2.06 grams respectively. In root biomass, the performance was consistently similar to shoot biomass as B5 isolate also performed greatly in both Mwetemania and Rosecoco with 0.69 grams and 0.84 grams respectively.KV1 isolate

followed with 0.73 grams in Mwetemania and 0.72 grams in Rosecoco. Negative Controls yielded 0.44 grams in Rosecoco and 0.37 grams in Mwetemania (Figure 13 and 14).

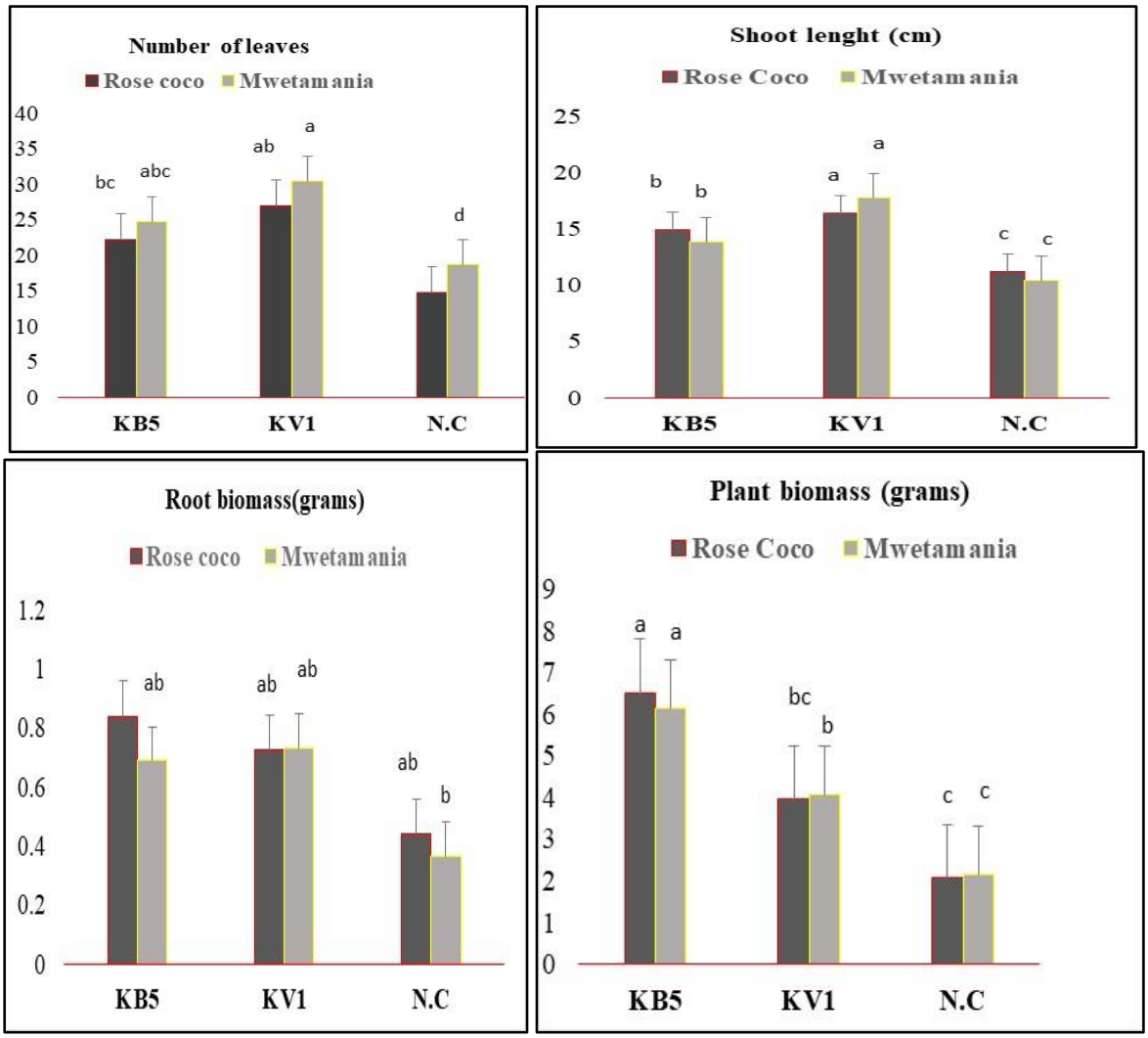


Figure 13. Plant growth parameters of Rosecoco and Mwetemania bean varieties after 42 days of. Inoculation with KB5 and KVI Phosphorus solubilizing bacteria. N.C denotes a negative control. Plant biomass and Root biomass are means of dry weights in grams while shoot length is means in cm. letters at the top of error bars represents significant differences at $p \leq 0.05$.

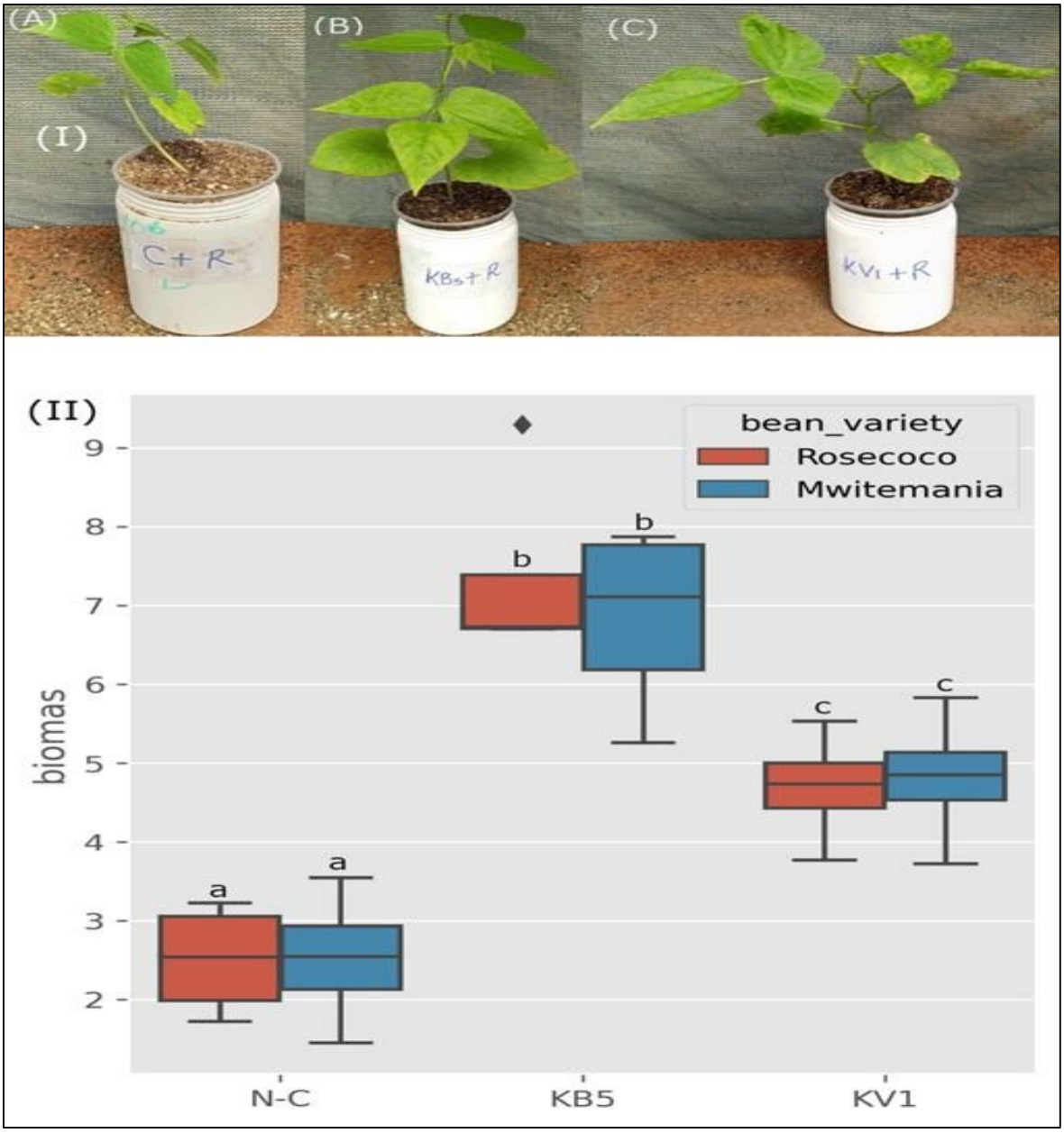


Figure 14.(I) Box plot showing the effects of bacteria strains inoculation on total biomass of a Rosecoco variety under phosphorus free nutrient in a Screenhouse. (a) Non- inoculated Control. (b) Inoculated with KB5 strain. (c)Inoculated with KV1 strain. (II) Effects of bacteria strains on total biomass in grams for both varieties (a) Biomass of negative control (b) Biomass of plants inoculated with KB5 (c) Biomass of plants inoculated with KV1

CHAPTER FIVE

DISCUSSION

5.1 Mineralization Potential of Phosphorus Solubilizing Bacteria Isolates

Common beans' roots and rhizosphere contain nitrogen-fixing and nodulating bacteria, however there are also other helpful rhizobacteria, such as PSB, that successfully colonize bean roots and nodules and support plant growth and development (Bhattacharyya & Jha, 2012; Figueiredo *et al.*, 2008; Wekesa *et al.*, 2021). Phosphorus in the soil, which is recognized as the second most important indicator of soil fertility after nitrogen, is necessary for the early stages of plant development (Razaq *et al.*, 2017). Legumes including common beans demand a lot of P due to their capacity to fix nitrogen through nodulation and their symbiotic relationship with PSB (Mitran *et al*., 2018). In the present study, it was found out that PSB naturally resides in the rhizosphere of common beans and interacts with nitrogen-fixing bacteria to influence plant performance in phosphorus-depleted soil. Amongst the seven investigated isolates, KB5 and KV1 isolates showed the greatest ability for mineralization and phosphate solubilization. These two PSB isolates demonstrated phosphate solubilization in broth test and agar assay at almost same levels, as well as phosphatase enzyme activity. This demonstrates that the PSB isolates' tendency to solubilize phosphate in both agar and broth testing was consistent with other investigations' findings from Rahman *et al.*, (2014) , Tariq *et al.*, (2022) and Z. Wang *et al.*, (2022b). The highest levels of phosphate solubilization, the highest levels of phosphatase enzyme activity, and the highest potential for bio-inoculant creation for sustainable agricultural output were seen in isolates KV1 and KB5, respectively (Alori *et al.*, 2017). Evidence that the phosphatase enzyme contributes to the process of phosphate

solubilization ability in bacteria as previously reported may be found in the high connection between phosphatase activity and the amount of phosphorus solubilized (Anil & Lakshmi, 2010; Behera *et al*., 2017; Cabugao *et al*., 2017).

5.2 Molecular and Phylogenetic Characteristics of Phosphorus Solubilizing Isolates

The phosphorus-solubilizing bacteria isolated from the rhizosphere of common beans in Western Kenya belonged to two generic clusters of *Enterobacter spp.* and *Pseudomonas spp.*, and they have also been previously reported in other host plants according to a molecular analysis of the seven isolates using partial sequencing of a 16S ribosomal gene (Thakur & Putatunda, 2017; Yadav *et al*., 2014). The group of *Enterobacter spp*, dominated the strains of study since out of the seven isolates, six were closely identified to be related to *Enterobacter spp*, The *Enterobacter spp*. have been previously reported in other plant rhizospheres and they have high potential for phosphorus solubilization but very little information is associated with common beans (Kirui e*t al.*, 2022; Mendoza-Arroyo *et al.*, 2020). *Pseudomonas sp*. has been isolated and recognized as one of the most effective phosphorus-solubilizing bacteria in both monocots and dicots in earlier investigations (Blanco-Vargas *et al*., 2020; Waday *et al.*, 2022; Yu *et al*., 2022) which exhibits future use as bio-inoculants . Out of the seven isolated strains from Western Kenya, two strains (KB5 and KV1) were assessed for their efficacy *in vitro* and *in vivo* in mineralization of inorganic phosphates and plant growth characteristics. Among the tested PSB strains from the region, KB5 which was closely related to *Pseudomonas kribbensis* and KV1 which was closely related to *Enterobacter asburiae* displayed maximum phosphate solubilization in both agar and broth medium respectively.

5.3 Influence of Potential PSB Isolates on Plant Growth Promoting Characteristics

KB5 and KVI isolates were chosen for screening in the screen house based on their best results in phosphatase enzyme activity, the number of phosphates converted in agar and broth assays, and their ability to promote the growth of the Rosecoco and Mwetemania bean varieties, which are mostly grown in Western Kenya. The performance of the isolates in terms of total dry weights was significantly different when determining the plant biomass of the two bean varieties. In comparison to the KV1 isolate and the negative control, strain B5 had a considerable impact on the biomass of the plants. In terms of plant morphological features (plant height and number of leaves), isolates KV1 outperformed isolate B5 (Table 10 and 11). This was also supported by the plant variety, as Mwetemania outperformed the Rosecoco type. B5–*Pseudomonas kribbensis* is genetically related to other previously studied *Pseudomonas* sp. including *Pseudomonas fluorescens* (Otieno *et al.*, 2015; Yadav *et al.*, 2014) and *Pseudomonas koreensis* (Srivastava *et al.*, 2019) that have been reported to highly solubilize phosphorus and promoted plant growth characteristics and therefore the isolate may exert a vital impact in common bean nutrition, through the absorption of soluble phosphorus. Given that the KV1 strain (*Enterobacter asburiae*) has been previously reported to boost plant growth parameters under harsh conditions (Mahdi *et al*., 2020), we also report that it can boost the growth and development of leguminous plants in phosphorus-depleted soils in the current study.

CHAPTER SIX

CONCLUSIONS, RECOMMENDATIONS AND SUGGESTIONS FOR FURTHER RESEARCH

6.1 Conclusions

1. PSB extracted from the rhizosphere of common beans are novel and can be found in a variety of microbial communities. In the present study and literature review, we have isolated, identify and characterize phosphorus-solubilizing bacterial strains from the rhizosphere of common beans for the first time in Western Kenya soil. Two possible PSB strains, KVI-*Enterobacter asburiae* and KB5-*Pseudomonas kribbensis,* have been identified among the isolated strains as being promising and very effective strains that can be employed to address the issue of phosphorus deficiency in soil for long-term crop production. In conclusion, the isolates were also able to mineralize high phosphorus concentrations in both agar media and broth medium as well as enzymatic activity.
2. The PSB isolates from this study belonged to *Pseudomonas* and *Enterobacter* genus as characterized using molecular identification through 16S ribosomal RNA partial sequencing and phylogenetic relationship. Most of *Enterobacter* isolated in the study were closely related.
3. Investigating the effects of genetically diverse phosphorus solubilizing bacteria on the phenotypic traits of Rosecoco and Mwetemania bean varieties as well as evaluating their mineralization potential is a way to understand the growth-promoting characteristics of these bacteria as well as a justification for the application of useful bio-inoculants to leguminous crops for sustainable production

in tropical regions (Alori *et al*., 2017). In addition to being able to greatly increase plant development parameters., The study is a contribution to the solution of food insecurity in Kenya and Sub-Saharan Africa is it intends to improve and sustain agriculture, sometimes known as " climate smart agriculture" (Newell *et al.*, 2019). One of the reliable and early-maturing crops that can easily be used to reduce hunger in Africa is common beans (*Common Beans Kenya*, 2020). Mwetemania and Rosecoco are not only important agricultural crop in providing food but also have some health benefits. Mwetemania are known for reducing cholesterol and blood sugar levels due to their high fiber and folate contents (Nchanji & Ageyo, 2021).

6.2 Recommendations

1. The present study did not address some of the parameters including determining P availability in soil and plant system after the PSB Inoculation. This could be a basis for future investigations involving mineralization of PSB colonizing common beans. This study involved use of Tricalcium phosphate as a source of organic phosphate. In the years to come, we advise future researchers using Tricalcium phosphate (TCP) to include either aluminum phosphate ($AIPO_4$) or iron phosphate ($FePO_4$) to test bacteria mineralization. P solubilization using these compounds is highly recommended due to the fact that TCP is a weak phosphate.
2. Future research can examine PSB strains that colonize common bean roots and nodules based on phosphorus activating genes, genome-based characterization, comparisons, and gene identification responsible for the solubilization of phosphate

in these PSB isolates, as well as metagenomics to comprehend the influence of genetic factors on the strains and the diversity of endophytic microbial communities, in order to fully assess the usefulness of these potential strains as microbial fertilizers. Future research should also focus on gaining a better understanding of these bacteria's interactions with nutrients, especially phosphorus, so that compatible organisms can be identified and used as effective inoculants in sustainable plant production systems in specific regions.

3. Lastly, basing on the findings of the study, we highly recommend the use of KV1 and B5 isolates to be used as potential biofertilizers since they displayed maximum efficacy in plant growth promotion.

6.3 Suggestions for Further Research

1. Genetic diversity of PSB using other genetic makers such as *rec A* , *gyrase B* is required in ecological sites of Western Kenya basing on the fact that the present study only employed 16S rRNA genetic maker.
2. To expand the knowledge of the phylogenetic diversity of PSB in plant rhizosphere, an extended analysis of genomes of bacterial families through metagenomics analysis is considered a current field of study that future and present scientist should search on.
3. Screening of diverse PSB colonizing other plants including cereals and leguminous plants is urgently needed for development of biofertilizers suitable for each plant.

REFERENCES

Abdelmoteleb, A., & Gonzalez-Mendoza, D. (2020). Isolation and Identification of Phosphate Solubilizing *Bacillus spp.* From *Tamarix ramosissima* Rhizosphere and Their Effect on Growth of Phaseolus vulgaris Under Salinity Stress. *Geomicrobiology Journal*, *37*(10), 901–908. https://doi.org/10.1080/01490451.2020.1795321

Abebe, T. G., Tamtam, M. R., Abebe, A. A., Abtemariam, K. A., Shigut, T. G., Dejen, Y. A., & Haile, E. G. (2022). Growing Use and Impacts of Chemical Fertilizers and Assessing Alternative Organic Fertilizer Sources in Ethiopia. *Applied and Environmental Soil Science*, *2022*, e4738416. https://doi.org/10.1155/2022/4738416

Aberathna, A. A. A. U., Satharasinghe, D. A., Jayasooriya, A. P., Jinadasa, R. N., Manopriya, S., Jayaweera, B. P. A., Fernando, C. A. N., Weerathilake, W. A. D. V., Prathapasinghe, G. A., Liyanage, J. A., & Premarathne, J. M. K. J. K. (2022). Increasing the Bioavailability of Phosphate by Using Microorganisms. *International Journal of Agronomy*, *2022*, e4305501. https://doi.org/10.1155/2022/4305501

Ahmed, T., Shahid, M., Noman, M., Hussain, S., Khan, M. A., Zubair, M., Ismail, M., Manzoor, N., Shahzad, T., & Mahmood, F. (2019). Plant Growth-Promoting Rhizobacteria as Biological Tools for Nutrient Management and Soil Sustainability. In A. Kumar & V. S. Meena (Eds.), *Plant Growth Promoting Rhizobacteria for Agricultural Sustainability: From Theory to Practices* (pp. 95–110). Springer. https://doi.org/10.1007/978-981-13-7553-8_5

Aktar, Md. W., Sengupta, D., & Chowdhury, A. (2009). Impact of pesticides use in agriculture: Their benefits and hazards. *Interdisciplinary Toxicology*, *2*(1), 1–12. https://doi.org/10.2478/v10102-009-0001-7

Alaylar, B., Egamberdieva, D., Karadayi, M., Arora, N., & Arora, K. (2020). Integration Of Molecular Tools in Microbial Phosphate Solubilization Research in Agriculture Perspective.*World Journal of Microbiology and Biotechnology*, 93. https://doi.org/10.1007/s11274-020-02870-x

Alori, E. T., & Babalola, O. O. (2018). Microbial Inoculants for Improving Crop Quality and Human Health in Africa. *Frontiers in Microbiology*, *9*, 2213. https://doi.org/10.3389/fmicb.2018.02213

Alori, E. T., Glick, B. R., & Babalola, O. O. (2017). Microbial Phosphorus Solubilization and Its Potential for Use in Sustainable Agriculture. *Frontiers in Microbiology*, *8*. https://doi.org/10.3389/fmicb.2017.00971

An, R., & Moe, L. A. (2016). Regulation of Pyrroloquinoline Quinone-Dependent Glucose Dehydrogenase Activity in the Model Rhizosphere-Dwelling Bacterium *Pseudomonas putida* KT2440. *Applied and Environmental Microbiology*, *82*(16), 4955–4964. https://doi.org/10.1128/AEM.00813-16

Anil, K., & Lakshmi, T. (2010). Phosphate Solubilization Potential and Phosphatase Activity Of Rhizospheric *Trichoderma Spp*. *Brazilian Journal of Microbiology*, *41*(3), 787–795. https://doi.org/10.1590/S1517-83822010005000031

Averill, C., Bhatnagar, J. M., Dietze, M. C., Pearse, W. D., & Kivlin, S. N. (2019). Global Imprint of Mycorrhizal Fungi on Whole-Plant Nutrient Economics. *Proceedings of*

the National Academy of Sciences, *116*(46), 23163–23168. https://doi.org/10.1073/pnas.1906655116

Ayyaz, K., Zaheer, A., Rasul, G., & Mirza, M. S. (2016). Isolation and identification by 16S rRNA sequence analysis of plant growth-promoting azospirilla from the rhizosphere of wheat. *Brazilian Journal of Microbiology*, *47*(3), 542–550. https://doi.org/10.1016/j.bjm.2015.11.035

Sankaralingam, S., Duraipandian, P., & Shankar, T. (2014). Screening and characterization of phosphate solubilizing bacterium Enterobacter cancerogenus isolated from rhizosphere soil of local weed plant. *International Journal of Advanced Scientific and Technical Research, 4*, 721-735.

Babalola, O. O., & Glick, B. R. (2012). *The Use of Microbial Inoculants in African Agriculture: Current Practice and Future Prospects*. 10.

Bai, Chang, Y.-Y., Hussain, M., Lu, B., Zhang, J.-P., Song, X.-B., Lei, X.-S., & Pei, D. (2020). Soil Chemical and Microbiological Properties Are Changed by Long-Term Chemical Fertilizers That Limit Ecosystem Functioning. *Microorganisms*, *8*(5), 694. https://doi.org/10.3390/microorganisms8050694

Bargaz, A., Elhaissoufi, W., Khourchi, S., Benmrid, B., Borden, K. A., & Rchiad, Z. (2021). Benefits of Phosphate Solubilizing Bacteria on Belowground Crop Performance for Improved Crop Acquisition Of Phosphorus. *Microbiological Research*, *252*, 126842. https://doi.org/10.1016/j.micres.2021.126842

Bashir, I., Lone, F. A., Bhat, R. A., Mir, S. A., Dar, Z. A., & Dar, S. A. (2020). Concerns and Threats of Contamination on Aquatic Ecosystems. *Bioremediation and Biotechnology*, 1–26. https://doi.org/10.1007/978-3-030-35691-0_1

Bechtaoui, N., Raklami, A., Tahiri, A.-I., Benidire, L., El Alaoui, A., Meddich, A., Göttfert, M., & Oufdou, K. (2019). Characterization Of Plant Growth Promoting Rhizobacteria and Their Benefits on Growth and Phosphate Nutrition of Faba Bean and Wheat. *Biology Open*, *8*(7), bio043968. https://doi.org/10.1242/bio.043968

Behera, B. C., Yadav, H., Singh, S. K., Mishra, R. R., Sethi, B. K., Dutta, S. K., & Thatoi, H. N. (2017). Phosphate solubilization and acid phosphatase activity of *Serratia sp.* Isolated from Mangrove Soil of Mahanadi River Delta, Odisha, India. *Journal, Genetic Engineering & Biotechnology*, *15*(1), 169–178. https://doi.org/10.1016/j.jgeb.2017.01.003

Bhardwaj, D., Ansari, M. W., Sahoo, R. K., & Tuteja, N. (2014). Biofertilizers Function as Key Player in Sustainable Agriculture by Improving Soil Fertility, Plant Tolerance and Crop Productivity. *Microbial Cell Factories*, *13*(1), 66. https://doi.org/10.1186/1475-2859-13-66

Bhattacharyya, P. N., & Jha, D. K. (2012). Plant Growth-Promoting Rhizobacteria (PGPR): Emergence in Agriculture. *World Journal of Microbiology & Biotechnology*, *28*(4), 1327–1350. https://doi.org/10.1007/s11274-011-0979-9

Bisht, N., & Chauhan, P. (2020). *Excessive and Disproportionate Use of Chemicals Cause Soil Contamination and Nutritional Stress*. https://doi.org/10.5772/intechopen.94593

Blanco-Vargas, A., Rodríguez-Gacha, L. M., Sánchez-Castro, N., Garzón-Jaramillo, R., Pedroza-Camacho, L. D., Poutou-Piñales, R. A., Rivera-Hoyos, C. M., Díaz-Ariza, L. A., & Pedroza-Rodríguez, A. M. (2020). Phosphate-solubilizing *Pseudomonas*

sp., and *Serratia sp.*, co-culture for Allium cepa L. growth promotion. *Heliyon*, *6*(10), e05218. https://doi.org/10.1016/j.heliyon.2020.e05218

Boubekri, K., Soumare, A., Mardad, I., Lyamlouli, K., Hafidi, M., Ouhdouch, Y., & Kouisni, L. (2021). The Screening of Potassium- and Phosphate-Solubilizing Actinobacteria and the Assessment of their Ability to Promote Wheat Growth Parameters. *Microorganisms*, *9*(3), Article 3. https://doi.org/10.3390/microorganisms9030470

Burns, R. (2005). *Environmental microbiology: A laboratory manual*.

Cabugao, K. G., Timm, C. M., Carrell, A. A., Childs, J., Lu, T.-Y. S., Pelletier, D. A., Weston, D. J., & Norby, R. J. (2017). Root and Rhizosphere Bacterial Phosphatase Activity Varies with Tree Species and Soil Phosphorus Availability in Puerto Rico Tropical Forest. *Frontiers in Plant Science*, *8*. https://www.frontiersin.org/articles/10.3389/fpls.2017.01834

Chaudhary, P., Singh, S., Chaudhary, A., Sharma, A., & Kumar, G. (2022). Overview of biofertilizers in crop production and stress management for sustainable agriculture. *Frontiers in Plant Science*, *13*, 930340. https://doi.org/10.3389/fpls.2022.930340

Chouhan, G. K., Jaiswal, D. K., Gaurav, A. K., Mukherjee, A., & Verma, J. P. (2021). Chapter 17—PGPM as a potential bioinoculant for enhancing crop productivity under sustainable agriculture. In A. Rakshit, V. S. Meena, M. Parihar, H. B. Singh, & A. K. Singh (Eds.), *Biofertilizers* (pp. 221–237). Woodhead Publishing. https://doi.org/10.1016/B978-0-12-821667-5.00009-9

Clarridge, J. (2004). Impact of 16S rRNA Gene Sequence Analysis for Identification of Bacteria on Clinical Microbiology and Infectious Diseases. *Clinical Microbiology*

Reviews, *17*, 840–862, table of contents. https://doi.org/10.1128/CMR.17.4.840-862.2004

Clayton, A., Lira Junior, M., Fracetto, G., Fernando, J., & Júlia, K. (2016). Evaluation methods used for phosphate-solubilizing bacteria. *African Journal of Biotechnology*, *15*, 1796–1805. https://doi.org/10.5897/AJB2015.15020

Cole, J. C., Smith, M. W., Penn, C. J., Cheary, B. S., & Conaghan, K. J. (2016). Nitrogen, phosphorus, calcium, and magnesium applied individually or as a slow release or controlled release fertilizer increase growth and yield and affect macronutrient and micronutrient concentration and content of field-grown tomato plants. *Scientia Horticulturae*, *211*, 420–430. https://doi.org/10.1016/j.scienta.2016.09.028

Common beans Kenya: Climate risk assessment (2020, March 12). https://ccafs.cgiar.org/resources/publications/common-beans-kenya-climate-risk-assessment

Demi, S. M., & Sicchia, S. R. (2021). Agrochemicals Use Practices and Health Challenges of Smallholder Farmers in Ghana. *Environmental Health Insights*, *15*, 11786302211043032. https://doi.org/10.1177/11786302211043033

dos Santos, H. R. M., Argolo, C. S., Argôlo-Filho, R. C., & Loguercio, L. L. (2019). A 16S rDNA PCR-based theoretical to actual delta approach on culturable mock communities revealed severe losses of diversity information. *BMC Microbiology*, *19*(1), 74. https://doi.org/10.1186/s12866-019-1446-2

Fageria, N. K., & Baligar, V. (2008). The Role of Nutrient Efficient Plants in Improving Crop Yields in the Twenty First Century. *J. Plant Nutr.*, *31*. https://doi.org/10.1080/01904160802116068

Fageria, N. K., Baligar, V. C., & Li, Y. C. (2008). The Role of Nutrient Efficient Plants in Improving Crop Yields in the Twenty First Century. *Journal of Plant Nutrition*, *31*(6), 1121–1157. https://doi.org/10.1080/01904160802116068

Fasusi, O. A., Cruz, C., & Babalola, O. O. (2021). Agricultural Sustainability: Microbial Biofertilizers in Rhizosphere Management. *Agriculture*, *11*(2), Article 2. https://doi.org/10.3390/agriculture11020163

Fernández Bidondo, L., Silvani, V., Colombo, R. P., Pérgola, M., Bompadre, M., & Godeas, A. (2011). Pre-symbiotic and symbiotic interactions between *Glomus intraradices* and two *Paenibacillus species* isolated from AM propagules. In vitro and in vivo assays with soybean (AG043RG) as plant host. *Soil Biology & Biochemistry - SOIL BIOL BIOCHEM*, *43*, 1866–1872. https://doi.org/10.1016/j.soilbio.2011.05.004

Figueiredo, M., Martinez, C., Burity, H., & Chanway, C. (2008). Plant growth-promoting rhizobacteria for improving nodulation and nitrogen fixation in the common bean (Phaseolus vulgaris L.). *World Journal of Microbiology and Biotechnology*, *24*, 1187–1193. https://doi.org/10.1007/s11274-007-9591-4

Franco-Duarte, R., Černáková, L., Kadam, S., S. Kaushik, K., Salehi, B., Bevilacqua, A., Corbo, M. R., Antolak, H., Dybka-Stępień, K., Leszczewicz, M., Relison Tintino, S., Alexandrino de Souza, V. C., Sharifi-Rad, J., Melo Coutinho, H. D., Martins, N., & Rodrigues, C. F. (2019). Advances in Chemical and Biological Methods to Identify Microorganisms—From Past to Present. *Microorganisms*, *7*(5), Article 5. https://doi.org/10.3390/microorganisms7050130

Fróna, D., Szenderák, J., & Harangi-Rákos, M. (2019). The Challenge of Feeding the World. *Sustainability*, *11*(20), Article 20. https://doi.org/10.3390/su11205816

Fukuda, K., OGAWA, M., TANIGUCHI, H., & Saito, M. (2016). Molecular Approaches to Studying Microbial Communities: Targeting the 16S Ribosomal RNA Gene. *Journal of UOEH*, *38*, 223–232. https://doi.org/10.7888/juoeh.38.223

Gao, Y., Yu, G., Luo, C., & Zhou, P. (2012). Groundwater Nitrogen Pollution and Assessment of Its Health Risks: A Case Study of a Typical Village in Rural-Urban Continuum, China. *PLoS ONE*, *7*(4), e33982. https://doi.org/10.1371/journal.pone.0033982

García-Fraile, P., Menéndez, E., Rivas, R., García-Fraile, P., Menéndez, E., & Rivas, R. (2015). Role Of Bacterial Biofertilizers in Agriculture and Forestry. *AIMS Bioengineering*, *2*(3), 183–205. https://doi.org/10.3934/bioeng.2015.3.183

Gong, A., Wang, G., Sun, Y., Song, M., Dimuna, C., Gao, Z., Wang, H., & Yang, P. (2022). Dual Activity of *Serratia Marcescens* Pt-3 In Phosphate-Solubilizing and Production of Antifungal Volatiles. *BMC Microbiology*, *22*(1), 26. https://doi.org/10.1186/s12866-021-02434-5

Goswami, S., Dubey, A., Singh, N., Correspondence, S., Goswami, & Maurya, B. (2019). *Role of phosphorus solubilizing microorganisms and dissolution of insoluble phosphorus in soil*. 3905–3913.

Guignard, M. S., Leitch, A. R., Acquisti, C., Eizaguirre, C., Elser, J. J., Hessen, D. O., Jeyasingh, P. D., Neiman, M., Richardson, A. E., Soltis, P. S., Soltis, D. E., Stevens, C. J., Trimmer, M., Weider, L. J., Woodward, G., & Leitch, I. J. (2017). Impacts of Nitrogen and Phosphorus: From Genomes to Natural Ecosystems and Agriculture.

Frontiers in Ecology and Evolution, *5*. https://www.frontiersin.org/articles/10.3389/fevo.2017.00070

Gupta, R., Anshu, Noureldeen, A., & Darwish, H. (2021). Rhizosphere Mediated Growth Enhancement Using Phosphate Solubilizing Rhizobacteria and Their Tri-Calcium Phosphate Solubilization Activity Under Pot Culture Assays In Rice (*Oryza sativa.*). *Saudi Journal of Biological Sciences*, *28*(7), 3692–3700. https://doi.org/10.1016/j.sjbs.2021.05.052

Heidari, E., Mohammadi, K., Pasari, B., Rokhzadi, A., & Sohrabi, Y. (2020). Combining The Phosphate Solubilizing Microorganisms with Biochar Types in Order to Improve Safflower Yield and Soil Enzyme Activity. *Soil Science and Plant Nutrition*, *66*(2), 255–267. https://doi.org/10.1080/00380768.2019.1704180

Illmer, P., Barbato, A., & Schinner, F. (1995). Solubilization of hardly-soluble AlPO4 with P-solubilizing microorganisms. *Soil Biology and Biochemistry*, *27*(3), 265–270. https://doi.org/10.1016/0038-0717(94)00205-F

Jahan, M., Nassiri Mahallati, M., Amiri, M. B., & Ehyayi, H. R. (2013). Radiation Absorption and Use Efficiency of Sesame as Affected by Biofertilizers Inoculation in A Low Input Cropping System. *Industrial Crops and Products*, *43*, 606–611. https://doi.org/10.1016/j.indcrop.2012.08.012

Javadi Nobandegani, M. B., Saud, H. M., & Yun, W. M. (2015). Phylogenetic Relationship of Phosphate Solubilizing Bacteria According to 16S rRNA Genes. *BioMed Research International*, *2015*, 201379. https://doi.org/10.1155/2015/201379

Johan, P. D., Ahmed, O. H., Omar, L., & Hasbullah, N. A. (2021). Phosphorus Transformation in Soils Following Co-Application of Charcoal and Wood Ash. *Agronomy*, *11*(10), Article 10. https://doi.org/10.3390/agronomy11102010

Kalayu, G. (2019). Phosphate Solubilizing Microorganisms: Promising Approach as Biofertilizers. *International Journal of Agronomy*, *2019*, e4917256. https://doi.org/10.1155/2019/4917256

Kawaka, F., Dida, M. M., Opala, P. A., Ombori, O., Maingi, J., Osoro, N., Muthini, M., Amoding, A., Mukaminega, D., & Muoma, J. (2014). Symbiotic Efficiency of Native Rhizobia Nodulating Common Bean (*Phaseolus vulgaris L.*) in Soils of Western Kenya. *International Scholarly Research Notices*, *2014*, e258497. https://doi.org/10.1155/2014/258497

Khalid, S., Shahid, M., Natasha, Bibi, I., Sarwar, T., Shah, A. H., & Niazi, N. K. (2018). A Review of Environmental Contamination and Health Risk Assessment of Wastewater Use for Crop Irrigation with a Focus on Low and High-Income Countries. *International Journal of Environmental Research and Public Health*, *15*(5), 895. https://doi.org/10.3390/ijerph15050895

Khan, M., Ahmad, E., Zaidi, A., & M., O. (2013). Functional Aspect of Phosphate-Solubilizing Bacteria: Importance in Crop Production. In *Bacteria in Agrobiology: Crop Productivity*. https://doi.org/10.1007/978-3-642-37241-4_10

Kirui, C. K., Njeru, E. M., & Runo, S. (2022). Diversity and Phosphate Solubilization Efficiency of Phosphate Solubilizing Bacteria Isolated from Semi-Arid Agroecosystems of Eastern Kenya. *Microbiology Insights*, *15*, 11786361221088992. https://doi.org/10.1177/11786361221088991

Krasilnikov, P., Taboada, M. A., & Amanullah. (2022). Fertilizer Use, Soil Health and Agricultural Sustainability. *Agriculture*, *12*(4),4. https://doi.org/10.3390/agriculture12040462

Kumar, A., Sharma, S., & Mishra, S. (2016). Evaluating Effect of Arbuscular Mycorrhizal Fungal Consortia and *Azotobacter Chroococcum* in Improving Biomass Yield of *Jatropha curcas*. *Plant Biosystems - An International Journal Dealing with All Aspects of Plant Biology*, *150*(5), 1056–1064. https://doi.org/10.1080/11263504.2014.1001001

Kumar, S., Diksha, Sindhu, S. S., & Kumar, R. (2022). Biofertilizers: An ecofriendly technology for nutrient recycling and environmental sustainability. *Current Research in Microbial Sciences*, *3*, 100094. https://doi.org/10.1016/j.crmicr.2021.100094

Kumar, S., Kumar, S., & Mohapatra, T. (2021). Interaction Between Macro- and Micro-Nutrients in Plants. *Frontiers in Plant Science*, *12*, 665583. https://doi.org/10.3389/fpls.2021.665583

Li, Y., Li, Q., Guan, G., & Chen, S. (2020). Phosphate Solubilizing Bacteria Stimulate Wheat Rhizosphere and Endosphere Biological Nitrogen Fixation by Improving Phosphorus Content. *PeerJ*, *8*, e9062. https://doi.org/10.7717/peerj.9062

Li, Y., Liu, X., Hao, T., & Chen, S. (2017). Colonization and Maize Growth Promotion Induced by Phosphate Solubilizing Bacterial Isolates. *International Journal of Molecular Sciences*, *18*(7), E1253. https://doi.org/10.3390/ijms18071253

Lorenz, T. C. (2012). Polymerase Chain Reaction: Basic Protocol Plus Troubleshooting and Optimization Strategies. *Journal of Visualized Experiments : JoVE*, *63*, 3998. https://doi.org/10.3791/3998

Mącik, M., Gryta, A., & Frąc, M. (2020). Chapter Two - Biofertilizers in agriculture: An overview on concepts, strategies and effects on soil microorganisms. In D. L. Sparks (Ed.), *Advances in Agronomy* (Vol. 162, pp. 31–87). Academic Press. https://doi.org/10.1016/bs.agron.2020.02.001

Mahanta, D., & Rai, R. (2008). *Effects of sources of phosphorus and biofertilizers on productivity and profitability of soybean (Glycine max)–wheat (Triticum aestivum) system*. *53*, 279–284.

Mahanty, T., Bhattacharjee, S., Goswami, M., Bhattacharyya, P., Das, B., Ghosh, A., & Tribedi, P. (2017). Biofertilizers: A potential approach for sustainable agriculture development. *Environmental Science and Pollution Research*, *24*(4), 3315–3335. https://doi.org/10.1007/s11356-016-8104-0

Mahdi, I., Fahsi, N., Hafidi, M., Allaoui, A., & Biskri, L. (2020). Plant Growth Enhancement using Rhizospheric Halotolerant Phosphate Solubilizing Bacterium Bacillus licheniformis QA1 and Enterobacter asburiae QF11 Isolated from Chenopodium quinoa Willd. *Microorganisms*, *8*(6), Article 6. https://doi.org/10.3390/microorganisms8060948

Maisonet-Guzman, O. E. (2011, July 18). Food Security and Population Growth in the 21st Century. *E-International Relations*. https://www.e-ir.info/2011/07/18/food-security-and-population-growth-in-the-21st-century/

Manzoor, M., Abbasi, M. K., & Sultan, T. (2017). Isolation of Phosphate Solubilizing Bacteria from Maize Rhizosphere and their Potential for Rock Phosphate Solubilization–Mineralization and Plant Growth Promotion. *Geomicrobiology Journal*, *34*(1), 81–95. https://doi.org/10.1080/01490451.2016.1146373

Medici, A., Szponarski, W., Dangeville, P., Safi, A., Dissanayake, I. M., Saenchai, C., Emanuel, A., Rubio, V., Lacombe, B., Ruffel, S., Tanurdzic, M., Rouached, H., & Krouk, G. (2019). Identification of Molecular Integrators Shows that Nitrogen Actively Controls the Phosphate Starvation Response in Plants. *The Plant Cell*, *31*(5), 1171–1184. https://doi.org/10.1105/tpc.18.00656

Mendoza-Arroyo, G., Chan, M., Aguila Ramírez, R., Morales, O., Efraín, R., Solís, C., Chab-Ruiz, A., Cob-Rivera, K., Dzib-Castillo, B., Tun Che, R., & Camacho-Chab, J. (2020). Inorganic Phosphate Solubilization by a Novel Isolated Bacterial Strain Enterobacter sp. ITCB-09 and Its Application Potential as Biofertilizer. *Agriculture*, *10*. https://doi.org/10.3390/agriculture10090383

Meng, X., Chen, W.-W., Wang, Y.-Y., Huang, Z.-R., Ye, X., Chen, L.-S., & Yang, L.-T. (2021). Effects of phosphorus deficiency on the absorption of mineral nutrients, photosynthetic system performance and antioxidant metabolism in Citrus grandis. *PLoS ONE*, *16*(2), e0246944. https://doi.org/10.1371/journal.pone.0246944

Minaxi, Saxena, J., Chandra, S., & Nain, L. (2013). Synergistic effect of phosphate solubilizing rhizobacteria and arbuscular mycorrhiza on growth and yield of wheat plants. *Journal of Soil Science and Plant Nutrition*, *13*(2), 511–525. https://doi.org/10.4067/S0718-95162013005000040

Mitra, D., Anđelković, S., Panneerselvam, P., Senapati, A., Vasić, T., Ganeshamurthy, A. N., Chauhan, M., Uniyal, N., Mahakur, B., & Radha, T. K. (2020). Phosphate-Solubilizing Microbes and Biocontrol Agent for Plant Nutrition and Protection: Current Perspective. *Communications in Soil Science and Plant Analysis*, *51*(5), 645–657. https://doi.org/10.1080/00103624.2020.1729379

Mitran, T., Meena, R. S., Lal, R., Layek, J., Kumar, S., & Datta, R. (2018). Role of Soil Phosphorus on Legume Production. In R. S. Meena, A. Das, G. S. Yadav, & R. Lal (Eds.), *Legumes for Soil Health and Sustainable Management* (pp. 487–510). Springer. https://doi.org/10.1007/978-981-13-0253-4_15

Mitter, E. K., Tosi, M., Obregón, D., Dunfield, K. E., & Germida, J. J. (2021). Rethinking Crop Nutrition in Times of Modern Microbiology: Innovative Biofertilizer Technologies. *Frontiers in Sustainable Food Systems*, *5*. https://www.frontiersin.org/articles/10.3389/fsufs.2021.606815

Mohamed, A. E., Nessim, M. G., Abou-el-seoud, I. I., Darwish, K. M., & Shamseldin, A. (2019). Isolation and selection of highly effective phosphate solubilizing bacterial strains to promote wheat growth in Egyptian calcareous soils. *Bulletin of the National Research Centre*, *43*(1), 203. https://doi.org/10.1186/s42269-019-0212-9

Murphy, J., & Riley, J. P. (1962). A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta*, *27*, 31–36. https://doi.org/10.1016/S0003-2670(00)88444-5

Musarrat, J., & Khan, M. (2014). Factors Affecting Phosphate-Solubilizing Activity of Microbes: Current Status. *Phosphate Solubilizing Microorganisms: Principles and*

Application of Microphos Technology, 63–85. https://doi.org/10.1007/978-3-319-08216-5_3

Nautiyal, C. S. (1999). An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. *FEMS Microbiology Letters*, *170*(1), 265–270. https://doi.org/10.1111/j.1574-6968.1999.tb13383.x

Nchanji, E. B., & Ageyo, O. C. (2021). Do Common Beans (*Phaseolus vulgaris L.*) Promote Good Health in Humans? A Systematic Review and Meta-Analysis of Clinical and Randomized Controlled Trials. *Nutrients*, *13*(11), 3701. https://doi.org/10.3390/nu13113701

Ndeda, M. A. J. (2019). Population movement, settlement and the construction of society to the east of Lake Victoria in precolonial times: The western Kenyan case. *Les Cahiers d'Afrique de l'Est / The East African Review*, *52*, Article 52. https://doi.org/10.4000/eastafrica.473

Ndung'u-Magiroi, K. W., Herrmann, L., Okalebo, J. R., Othieno, C. O., Pypers, P., & Lesueur, D. (2012). Occurrence and genetic diversity of phosphate-solubilizing bacteria in soils of differing chemical characteristics in Kenya. *Annals of Microbiology*, *62*(3), 897–904. https://doi.org/10.1007/s13213-011-0326-2

Newell, P., Taylor, O., Naess, L. O., Thompson, J., Mahmoud, H., Ndaki, P., Rurangwa, R., & Teshome, A. (2019). Climate Smart Agriculture? Governing the Sustainable Development Goals in sub-Saharan Africa. *Frontiers in Sustainable Food Systems*, *3*. https://www.frontiersin.org/articles/10.3389/fsufs.2019.00055

Odelade, K. A., & Babalola, O. O. (2019). Bacteria, Fungi and Archaea Domains in Rhizospheric Soil and Their Effects in Enhancing Agricultural Productivity.

International Journal of Environmental Research and Public Health, *16*(20), 3873. https://doi.org/10.3390/ijerph16203873

Oehl, F., Oberson, A., Probst, M., Fliessbach, A., Roth, H.-R., & Frossard, E. (2001). Kinetics of Microbial Phosphorus Uptake in Cultivated Soils. *Biology and Fertility of Soils*, *34*(1), 31–41. https://doi.org/10.1007/s003740100362

Olfati, J. (2015). *Design and Preparation of Nutrient Solution Used for Soilless Culture of Horticultural Crops*. https://doi.org/10.5772/59478

Otieno, N., Lally, R., Kiwanuka, S., Lloyd, A., Ryan, D., Germaine, K., & Dowling, D. (2015). Plant growth promotion induced by phosphate solubilizing endophytic *Pseudomonas* isolates. *Frontiers in Microbiology*, *6*. https://www.frontiersin.org/articles/10.3389/fmicb.2015.00745

Ouattara, A., Coulibaly, K., Konate, I., Isma&euml, B., Kebe, L., Tidou, A. S., & Filali-Maltouf, A. (2019). Selection of Cocoa Tree (*Theobroma cacao Linn*) Endophytic Bacteria Solubilizing Tri-Calcium Phosphate, Isolated from Seedlings Grown on Soils of Six Producing Regions of Côte d'Ivoire. *Advances in Microbiology*, *9*(9), Article 9. https://doi.org/10.4236/aim.2019.99051

Pahalvi, H., Majeed, L., Nisar, B., & Kamili, A. (2021). *Chemical Fertilizers and Their Impact on Soil Health* (pp. 1–20). https://doi.org/10.1007/978-3-030-61010-4_1

Pande, A., Kaushik, S., Pandey, P., & Negi, A. (2020). Isolation, characterization, and identification of phosphate-solubilizing Burkholderia cepacia from the sweet corn cv. Golden Bantam rhizosphere soil and effect on growth-promoting activities. *International Journal of Vegetable Science*, *26*(6), 591–607. https://doi.org/10.1080/19315260.2019.1692121

Pande, A., Pandey, P., Mehra, S., Singh, M., & Kaushik, S. (2017). Phenotypic and genotypic characterization of phosphate solubilizing bacteria and their efficiency on the growth of maize. *Journal, Genetic Engineering & Biotechnology*, *15*(2), 379–391. https://doi.org/10.1016/j.jgeb.2017.06.005

Pandit, M. A., Kumar, J., Gulati, S., Bhandari, N., Mehta, P., Katyal, R., Rawat, C. D., Mishra, V., & Kaur, J. (2022). Major Biological Control Strategies for Plant Pathogens. *Pathogens*, *11*(2), Article 2. https://doi.org/10.3390/pathogens11020273

Panneerselvam, P., Senapati, A., Kumar, U., Sharma, L., Lepcha, P., Prabhukarthikeyan, S. R., Jahan, A., Parameshwaran, C., Govindharaj, G. P. P., Lenka, S., Nayak, P. K., Mitra, D., Sagarika, M. S., Thangappan, S., & Sivakumar, U. (2019). Antagonistic and plant-growth promoting novel Bacillus species from long-term organic farming soils from Sikkim, India. *3 Biotech*, *9*(11), 416. https://doi.org/10.1007/s13205-019-1938-7

Pantigoso, H. A., He, Y., Manter, D. K., Fonte, S. J., & Vivanco, J. M. (2022). Phosphorus-solubilizing bacteria isolated from the rhizosphere of wild potato *Solanum bulbocastanu*m enhance growth of modern potato varieties. *Bulletin of the National Research Centre*, *46*(1), 224. https://doi.org/10.1186/s42269-022-00913-x

Park, Y., Solhtalab, M., Thongsomboon, W., & Aristilde, L. (2022). Strategies of organic phosphorus recycling by soil bacteria: Acquisition, metabolism, and regulation. *Environmental Microbiology Reports*, *14*(1), 3–24. https://doi.org/10.1111/1758-2229.13040

Pecoraro, L., Wang, X., Shah, D., Song, X., Kumar, V., Shakoor, A., Tripathi, K., Ramteke, P. W., & Rani, R. (2021). Biosynthesis Pathways, Transport Mechanisms and Biotechnological Applications of Fungal Siderophores. *Journal of Fungi*, *8*(1), 21. https://doi.org/10.3390/jof8010021

Prabhu, N., Borkar, S., & Garg, S. (2018). *Phosphate solubilization mechanisms in alkaliphilic bacterium Bacillus marisflavi FA7*. http://irgu.unigoa.ac.in/drs/handle/unigoa/5143

Rahman, M., Quadir, Q., Rahman, A., Nahar, M., Abul, M., & Chowdhury, K. (2014). Screening and characterization of Phosphorus solubilizing Bacteria and their effect on Rice seedlings. *Research in Agriculture Livestock and Fisheries*, *1*, 27–35. https://doi.org/10.3329/ralf.v1i1.22353

Rao, E. J. O., Midega, C., Atieno, F., Auma, J., Cadilhon, J. J., Mango, N., Odhiambo, G. D., Oduor, F. N., Okeyo, I., Okeyo, T., & Wesonga, M. (2015). *A situational analysis of agricultural production and marketing, and natural resources management systems in West Kenya*. https://repository.maseno.ac.ke/handle/123456789/307

Rao, W.-H., Li, L., Wu, W., Chen, J., & Xiao-Hong, P. (2015). Effects of *Bacillus thuringiensis* on the Growth Parameters of Rice (*Oryza sativa*) Seedlings Under the Cadmium Stress. *Chinese Journal of Agricultural Biotechnology*, *23*, 1458–1464. https://doi.org/10.3969/j.issn.1674-7968.2015.11.008

Razaq, M., Zhang, P., Shen, H., & Salahuddin. (2017). Influence of nitrogen and phosphorous on the growth and root morphology of Acer mono. *PLoS ONE*, *12*(2), e0171321. https://doi.org/10.1371/journal.pone.0171321

Rodríguez, H., & Fraga, R. (1999a). Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnology Advances*, *17*(4), 319–339. https://doi.org/10.1016/S0734-9750(99)00014-2

Rodríguez, H., & Fraga, R. (1999b). Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnology Advances*, *17*(4), 319–339. https://doi.org/10.1016/S0734-9750(99)00014-2

Rombola, T. H., Pedrinho, E. A. N., de Macedo Lemos, E. G., Gonçalves, A. M., dos Santos, L. F. J., & Pizauro, J. M. (2014). Identification and enzymatic characterization of acid phosphatase from *Burkholderia gladioli*. *BMC Research Notes*, *7*, 221. https://doi.org/10.1186/1756-0500-7-221

Saadouli, I., Mosbah, A., Ferjani, R., Stathopoulou, P., Galiatsatos, I., Asimakis, E., Marasco, R., Daffonchio, D., Tsiamis, G., & Ouzari, H.-I. (2021). The Impact of the Inoculation of Phosphate-Solubilizing Bacteria *Pantoea agglomerans* on Phosphorus Availability and Bacterial Community Dynamics of a Semi-Arid Soil. *Microorganisms*, *9*(8), 1661. https://doi.org/10.3390/microorganisms9081661

Sanchez-Gonzalez, M. E., Mora-Herrera, M. E., Wong-Villarreal, A., De La Portilla-López, N., Sanchez-Paz, L., Lugo, J., Vaca-Paulín, R., Del Aguila, P., & Yañez-Ocampo, G. (2023). Effect of pH and Carbon Source on Phosphate Solubilization by Bacterial Strains in Pikovskaya Medium. *Microorganisms*, *11*(1), Article 1. https://doi.org/10.3390/microorganisms11010049

Sashidhar, B., & Podile, A. R. (2010). Mineral phosphate solubilization by rhizosphere bacteria and scope for manipulation of the direct oxidation pathway involving

glucose dehydrogenase. *Journal of Applied Microbiology*, *109*(1), 1–12. https://doi.org/10.1111/j.1365-2672.2009.04654.x

Sasson, A. (2012). Food security for Africa: An urgent global challenge. *Agriculture & Food Security*, *1*(1), 2. https://doi.org/10.1186/2048-7010-1-2

Satyaprakash, M., Sadhana, E. U. B., & Vani, S. (2017). Phosphorous and Phosphate Solubilising Bacteria and their Role in Plant Nutrition. *International Journal of Current Microbiology and Applied Sciences*, *6*, 2133–2144. https://doi.org/10.20546/ijcmas.2017.604.251

Shaheb, M. R., Venkatesh, R., & Shearer, S. A. (2021). A Review on the Effect of Soil Compaction and its Management for Sustainable Crop Production. *Journal of Biosystems Engineering*, *46*(4), 417–439. https://doi.org/10.1007/s42853-021-00117-7

Sharma, S. B., Sayyed, R. Z., Trivedi, M. H., & Gobi, T. A. (2013). Phosphate Solubilizing Microbes: Sustainable Approach for Managing Phosphorus Deficiency in Agricultural Soils. *SpringerPlus*, *2*, 587. https://doi.org/10.1186/2193-1801-2-587

Sharma, U., Paliyal, S. S., Sharma, S. P., & Sharma, G. D. (2014). Effects of continuous use of chemical fertilizers and manure on soil fertility and productivity of maize-wheat under rainfed conditions of the Western Himalayas. *Communications in Soil Science and Plant Analysis*, *45*(20), 2647–2659. https://www.cabdirect.org/cabdirect/abstract/20143384572

Shen, J., Yuan, L., Zhang, J., Li, H., Bai, Z., Chen, X., Zhang, W., & Zhang, F. (2011). Phosphorus Dynamics: From Soil to Plant1. *Plant Physiology*, *156*(3), 997–1005. https://doi.org/10.1104/pp.111.175232

Silva, L. I. da, Pereira, M. C., Carvalho, A. M. X. de, Buttrós, V. H., Pasqual, M., & Dória, J. (2023). Phosphorus-Solubilizing Microorganisms: A Key to Sustainable Agriculture. *Agriculture*, *13*(2), Article 2. https://doi.org/10.3390/agriculture13020462

Srinivasan, R., Karaoz, U., Volegova, M., MacKichan, J., Kato-Maeda, M., Miller, S., Nadarajan, R., Brodie, E. L., & Lynch, S. V. (2015). Use of 16S rRNA Gene for Identification of a Broad Range of Clinically Relevant Bacterial Pathogens. *PLOS ONE*, *10*(2), e0117617. https://doi.org/10.1371/journal.pone.0117617

Srinivasan, R., Yandigeri, M. S., Kashyap, S., & Alagawadi, A. R. (2012). Effect of salt on survival and P-solubilization potential of phosphate solubilizing microorganisms from salt affected soils. *Saudi Journal of Biological Sciences*, *19*(4), 427–434. https://doi.org/10.1016/j.sjbs.2012.05.004

Srivastava, A. K., Saxena, P., Sharma, A., Srivastava, R., Jamali, H., Bharati, A. P., Yadav, J., Srivastava, A. K., Kumar, M., Chakdar, H., Kashyap, P. L., & Saxena, A. K. (2019). Draft Genome Sequence of a Cold-Adapted Phosphorous-Solubilizing *Pseudomonas koreensis* P2 isolated from Sela Lake, India. *3 Biotech*, *9*(7), 256. https://doi.org/10.1007/s13205-019-1784-7

Suleman, M., Yasmin, S., Rasul, M., Yahya, M., Atta, B. M., & Mirza, M. S. (2018). Phosphate Solubilizing Bacteria with Glucose Dehydrogenase Gene for Phosphorus Uptake and Beneficial Effects on Wheat. *PLoS ONE*, *13*(9), e0204408. https://doi.org/10.1371/journal.pone.0204408

Tabatabai, M. A., & Bremner, J. M. (1969). Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biology and Biochemistry*, *1*(4), 301–307. https://doi.org/10.1016/0038-0717(69)90012-1

Tahir, M., Khalid, U., Ijaz, M., Shah, G. M., Naeem, M. A., Shahid, M., Mahmood, K., Ahmad, N., & Kareem, F. (2018). Combined application of bio-organic phosphate and phosphorus solubilizing bacteria (*Bacillus* strain MWT 14) improve the performance of bread wheat with low fertilizer input under an arid climate. *Brazilian Journal of Microbiology*, *49*, 15–24. https://doi.org/10.1016/j.bjm.2017.11.005

Tajini, F., Trabelsi, M., & Drevon, J.-J. (2011). Co-inoculation with *Glomus intraradices* and *Rhizobium tropici* CIAT899 increases P use efficiency for N 2 fixation in the common bean (*Phaseolus vulgaris L.*) under P deficiency in hydroaeroponic culture. *Symbiosis*, *53*, 123–129. https://doi.org/10.1007/s13199-011-0117-3

Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution*, *38*(7), 3022–3027. https://doi.org/10.1093/molbev/msab120

Tariq, M. R., Shaheen, F., Mustafa, S., ALI, S., Fatima, A., Shafiq, M., Safdar, W., Sheas, M. N., Hameed, A., & Nasir, M. A. (2022). Phosphate solubilizing microorganisms isolated from medicinal plants improve growth of mint. *PeerJ*, *10*, e13782. https://doi.org/10.7717/peerj.13782

Thakur, I., & Putatunda, C. (2017). In vitro Phosphate Solubilization by Enterobacter spp. Isolated from Wheat Rhizosphere. *Journal of Pure and Applied Microbiology*, *11*, 2007–2015. https://doi.org/10.22207/JPAM.11.4.43

Tian, J., Ge, F., Zhang, D., Deng, S., & Liu, X. (2021). Roles of Phosphate Solubilizing Microorganisms from Managing Soil Phosphorus Deficiency to Mediating Biogeochemical P Cycle. *Biology*, *10*(2), 158. https://doi.org/10.3390/biology10020158

Timofeeva, A., Galyamova, M., & Sedykh, S. (2022). Prospects for Using Phosphate-Solubilizing Microorganisms as Natural Fertilizers in Agriculture. *Plants*, *11*(16), Article 16. https://doi.org/10.3390/plants11162119

Tomer, S., Suyal, D. C., Shukla, A., Rajwar, J., Yadav, A., Shouche, Y., & Goel, R. (2017). Isolation and Characterization of Phosphate Solubilizing Bacteria from Western Indian Himalayan soils. *3 Biotech*, *7*(2), 1–5.

Toro, M. (2007). Phosphate Solubilizing Microorganisms in The Rhizosphere of Native Plants from Tropical Savannas: An Adaptive Strategy to Acid Soils. In E. Velázquez & C. Rodríguez-Barrueco (Eds.), *First International Meeting on Microbial Phosphate Solubilization* (pp. 249–252). Springer Netherlands. https://doi.org/10.1007/978-1-4020-5765-6_37

Vandana, U. K., Rajkumari, J., Singha, L. P., Satish, L., Alavilli, H., Sudheer, P. D. V. N., Chauhan, S., Ratnala, R., Satturu, V., Mazumder, P. B., & Pandey, P. (2021). The Endophytic Microbiome as a Hotspot of Synergistic Interactions, with Prospects of Plant Growth Promotion. *Biology*, *10*(2), Article 2. https://doi.org/10.3390/biology10020101

Waday, Y. A., Girma Aklilu, E., Bultum, M. S., Ramayya Ancha, V., & Beyene, D. (2022). Isolation and Characterization of Plant Growth-Promoting Rhizobacteria from Coffee Plantation Soils and Its Influence on Maize Growth. *Applied and*

Environmental Soil Science, *2022*, e5115875. https://doi.org/10.1155/2022/5115875

Wang, H., Liu, S., Zhai, L., Zhang, J., Ren, T., Fan, B., & Liu, H. (2015). Preparation and utilization of phosphate biofertilizers using agricultural waste. *Journal of Integrative Agriculture*, *14*(1), 158–167. https://doi.org/10.1016/S2095-3119(14)60760-7

Wang, Z., Xu, G., Ma, P., Lin, Y., Yang, X., & Cao, C. (2017). Isolation and Characterization of a Phosphorus-Solubilizing Bacterium from Rhizosphere Soils and Its Colonization of Chinese Cabbage (Brassica campestris ssp. Chinensis). *Frontiers in Microbiology*, *8*, 1270. https://doi.org/10.3389/fmicb.2017.01270

Wang, Z., Zhang, H., Liu, L., Li, S., Xie, J., Xue, X., & Jiang, Y. (2022a). Screening Of Phosphate-Solubilizing Bacteria and Their Abilities of Phosphorus Solubilization and Wheat Growth Promotion. *BMC Microbiology*, *22*(1), 296. https://doi.org/10.1186/s12866-022-02715-7

Wang, Z., Zhang, H., Liu, L., Li, S., Xie, J., Xue, X., & Jiang, Y. (2022b). Screening Of Phosphate-Solubilizing Bacteria and Their Abilities of Phosphorus Solubilization and Wheat Growth Promotion. *BMC Microbiology*, *22*(1), 296. https://doi.org/10.1186/s12866-022-02715-7

Wani, P. A., Khan, M. S., & Zaidi, A. (2007). Synergistic effects of the inoculation with nitrogen-fixing and phosphate-solubilizing rhizobacteria on the performance of field-grown chickpea. *Journal of Plant Nutrition and Soil Science*, *170*(2), 283–287. https://doi.org/10.1002/jpln.200620602

Wei, Y., Zhao, Y., Shi, M., Cao, Z., Lu, Q., Yang, T., Fan, Y., & Wei, Z. (2018). Effect of organic acids production and bacterial community on the possible mechanism of phosphorus solubilization during composting with enriched phosphate-solubilizing bacteria inoculation. *Bioresource Technology*, *247*, 190–199. https://doi.org/10.1016/j.biortech.2017.09.092

Wekesa, C. S., Furch, A. C. U., & Oelmüller, R. (2021). Isolation and Characterization of High-Efficiency Rhizobia From Western Kenya Nodulating With Common Bean. *Frontiers in Microbiology*, *12*, 697567. https://doi.org/10.3389/fmicb.2021.697567

White, P. J., & Brown, P. H. (2010). Plant nutrition for sustainable development and global health. *Annals of Botany*, *105*(7), 1073–1080. https://doi.org/10.1093/aob/mcq085

World Bank Climate Change Knowledge Portal. (n.d.). Retrieved 25 October 2022, from https://climateknowledgeportal.worldbank.org/

Yadav, K., Kumar, C., Archana, G., & Kumar, G. N. (2014). *Pseudomonas fluorescens* ATCC 13525 Containing an Artificial Oxalate Operon and Vitreoscilla Hemoglobin Secretes Oxalic Acid and Solubilizes Rock Phosphate in Acidic Alfisols. *PLOS ONE*, *9*(4), e92400. https://doi.org/10.1371/journal.pone.0092400

Yu, H., Wang, F., Shao, M., Huang, L., Xie, Y., Xu, Y., & Kong, L. (2021). Effects of Rotations With Legume on Soil Functional Microbial Communities Involved in Phosphorus Transformation. *Frontiers in Microbiology*, *12*. https://www.frontiersin.org/articles/10.3389/fmicb.2021.661100

Yu, H., Wu, X., Zhang, G., Zhou, F., Harvey, P. R., Wang, L., Fan, S., Xie, X., Li, F., Zhou, H., Zhao, X., & Zhang, X. (2022). Identification of the Phosphorus-Solubilizing Bacteria Strain JP233 and Its Effects on Soil Phosphorus Leaching

Loss and Crop Growth. *Frontiers in Microbiology*, *13*, 892533. https://doi.org/10.3389/fmicb.2022.892533

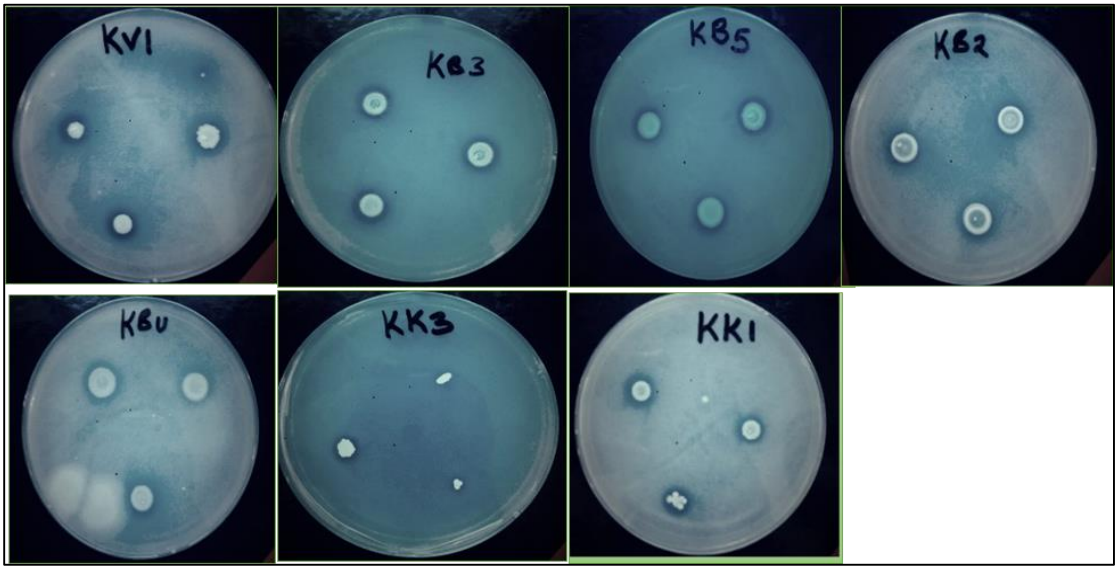
Zhang, L., Ding, X., Chen, S., He, X., Zhang, F., & Feng, G. (2014). Reducing carbon: Phosphorus ratio can enhance microbial phytin mineralization and lessen competition with maize for phosphorus. *Journal of Plant Interactions*, *9*(1), 850–856. https://doi.org/10.1080/17429145.2014.977831

Zhao, K., Penttinen, P., Zhang, X., Ao, X., Liu, M., Yu, X., & Chen, Q. (2014). Maize rhizosphere in Sichuan, China, Hosts Plant Growth Promoting *Burkholderia Cepacia* with Phosphate Solubilizing And Antifungal Abilities. *Microbiological Research*, *169*(1), 76–82. https://doi.org/10.1016/j.micres.2013.07.003

Zhu, F., Qu, L., Hong, X., & Sun, X. (2011). Isolation and Characterization of a Phosphate-Solubilizing Halophilic *Bacterium kushneria sp*. YCWA18 from Daqiao Saltern on the Coast of Yellow Sea of China. *Evidence-Based Complementary and Alternative Medicine: ECAM*, *2011*, 615032. https://doi.org/10.1155/2011/615032

APPENDICES

Appendix I. Isolate's growth on plates

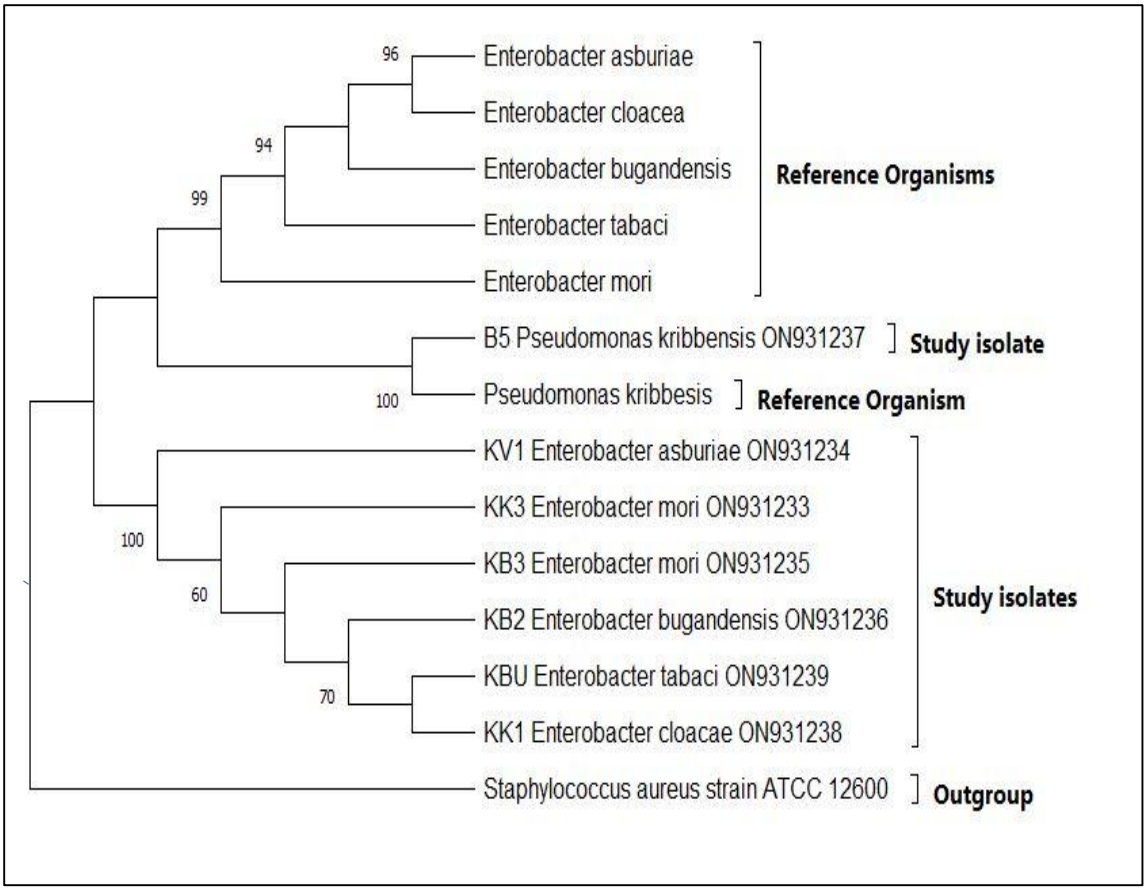


Appendix II. DNA Quantification analysis of the isolates using spectrophotometer

Isolates	Concentrations (ng/µl)	$A_{260/280}$	$A_{260/230}$
KB2	409.9	2.15	2.42
KB3	1292.2	2.21	2.48
KB5	1412.6	2.24	2.47
KBU	1287.5	2.19	2.49
KK1	1330.5	2.2	2.42
KK3	1354.4	2.22	2.46
KV1	1356.56	2.23	2.43

U.V Spectroscopy.

Appendix III. A Phylogenetic tree of the isolated PSB strains with reference organism and an outgroup organism



Appendix IV. NCBI GenBank information for KB5 isolate

GenBank ▾ Send to: ▾

Pseudomonas kribbensis strain B5 16S ribosomal RNA gene, partial sequence

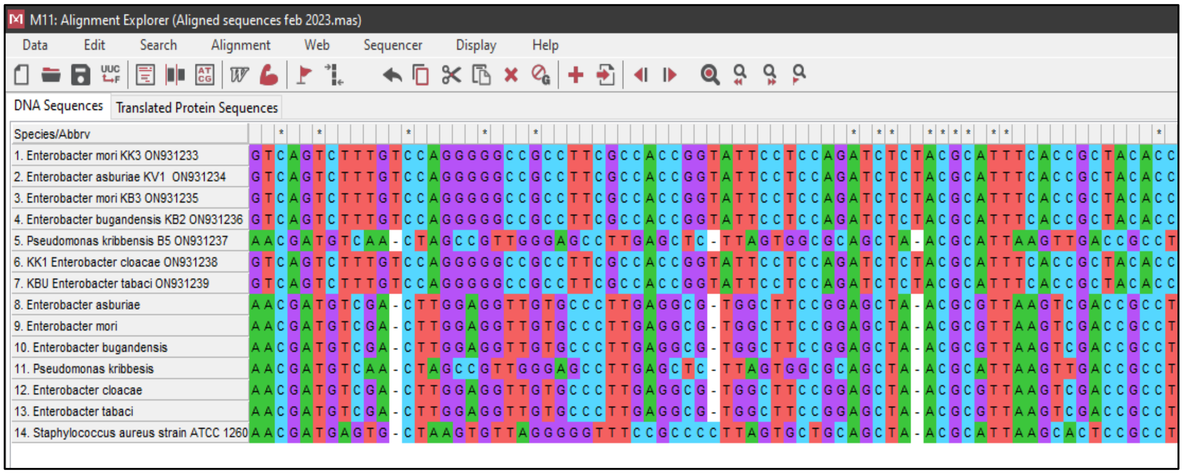
GenBank: ON931237.1

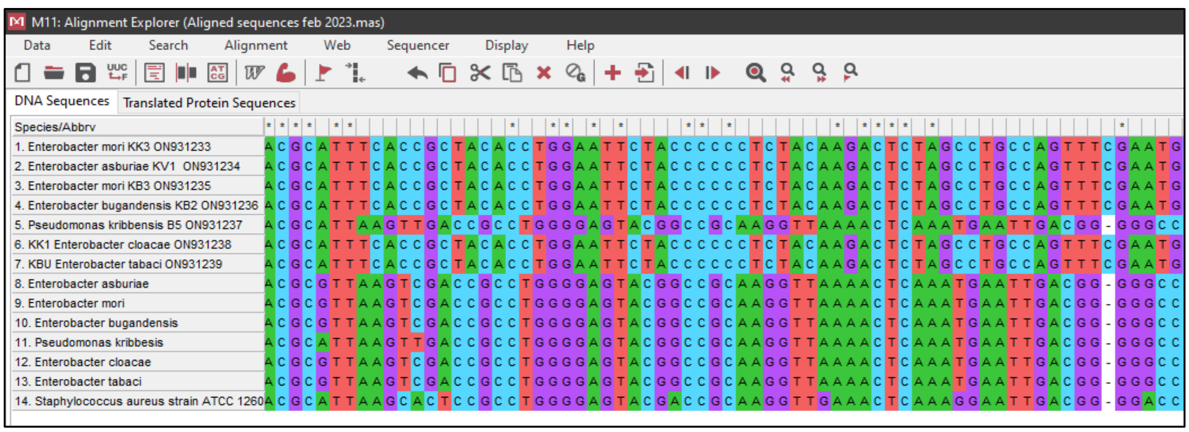
FASTA Graphics

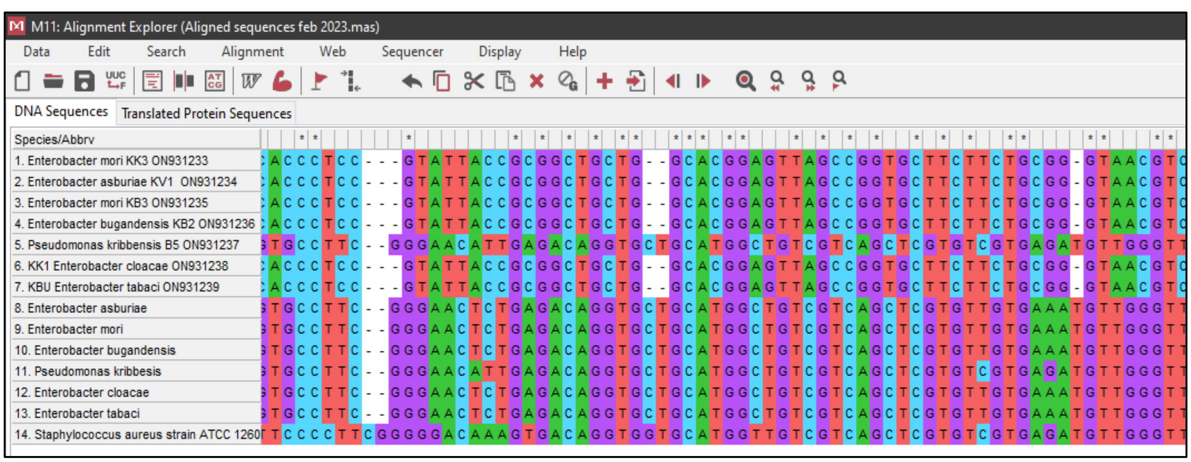
Go to:

```
LOCUS       ON931237               1249 bp    DNA     linear   BCT 12-JUL-2022
DEFINITION  Pseudomonas kribbensis strain B5 16S ribosomal RNA gene, partial
            sequence.
ACCESSION   ON931237
VERSION     ON931237.1
KEYWORDS    .
SOURCE      Pseudomonas kribbensis
  ORGANISM  Pseudomonas kribbensis
            Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales;
            Pseudomonadaceae; Pseudomonas.
REFERENCE   1  (bases 1 to 1249)
  AUTHORS   Kiprotich,K., Muoma,J., Omayio,D., Ndombi,S. and Wekesa,C.
  TITLE     Direct Submission
  JOURNAL   Submitted (06-JUL-2022) Biological, Masinde Muliro University of
            Science and Technology, 50100, Kakamega 00100, Kenya
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
FEATURES             Location/Qualifiers
     source          1..1249
                     /organism="Pseudomonas kribbensis"
```

Appendix V. Multiple sequence alignment (*Clustal W*) of 16S rRNA Gene







Appendix VI. FASTA file sequences for PSB isolates

```
>B5
CCGGCGGGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGGACAACGTTTCGAAAGG
AACGCTAATACCGCATACGTCCTACGGGAGAAAGCAGGGGACCTTCGGGCCTTGCGCTAT
CAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGACGATC
CGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTA
CGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCG
TGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGTTGTAGATT
AATACTCTGCAATTTTGACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAG
CCGCGGTAATACAGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAG
GTGGTTCGTTAAGTTGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCCAAAACT
GGCGAGCTAGAGTATGGTAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGA
TATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGC
GAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCA
ACTAGCCGTTGGGAGCCTTGAGCTCTTAGTGGCGCAGCTAACGCATTAAGTTGACCGCCT
GGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTG
GAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGCCTTGACATCCAATGA
ACTTTCCAGAGATGGATTGGTGCCTTCGGGAACATTGAGACAGGTGCTGCATGGCTGTCG
TCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTAACGAGCGCAACCCTTGTCCTTAG
TTACCAGCACGTTATGGTGGGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGT
GGGGATGACGTCAAGTCATCATGGCCCTTACGGCCTGGGCTACACACGTGCTACAATGGT
CGGTACAAAGGGTTGCCAAGCCGCGAGGTGGAGCTAATCCCATAAAACCGATCGTAGTCC
GGATCGCAGTCTGCAACTCGACTGCGTGAAGTCGGAATCGCTTAATCCG
```

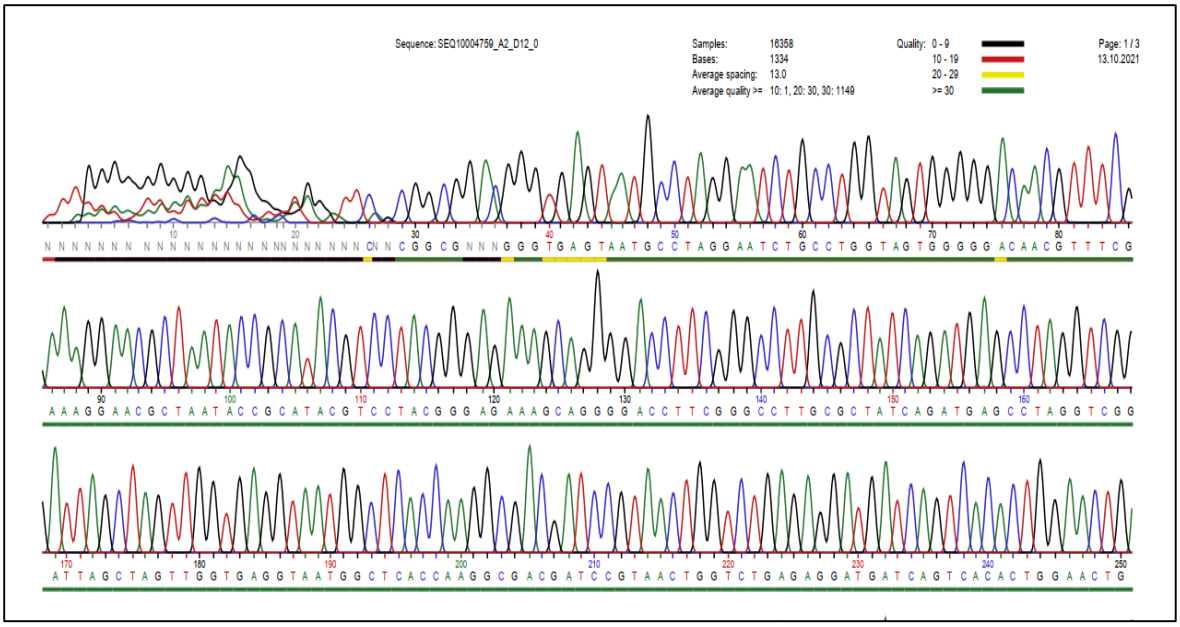
>KK3
CTGATTACGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCGGACTACGACG
CACTTTATGAGGTCCGCTTGCTCTCGCGAGGTCGCTTCTCTTTGTATGCGCCATTGTAGC
ACGTGTGTAGCCCTACTCGTAAGGGCCATGATGACTTGACGTCATCCCCACCTTCCTCCA
GTTTATCACTGGCAGTCTCCTTTGAGTTCCCGGCCACCGCTGGCAACAAAGGATAAGGGT
TGCGCTCGTTGCGGGACTTAACCCAACATTTCACAACACGAGCTGACGACAGCCATGCAG
CACCTGTCTCAGAGTTCCCGAAGGCACCAATCCATCTCTGGAAAGTTCTCTGGATGTCAA
GAGTAGGTAAGGTTCTTCGCGTTGCATCGAATTAAACCACATGCTCCACCGCTTGTGCGG
GCCCCCGTCAATTCATTTGAGTTTTAACCTTGCGGCCGTACTCCCCAGGCGGTCGACTTA
ACGCGTTAGCTCCGGAAGCCACGCCTCAAGGGCACAACCTCCAAGTCGACATCGTTTACG
GCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTGAGCGTCA
GTCTTTGTCCAGGGGGCCGCCTTCGCCACCGGTATTCCTCCAGATCTCTACGCATTTCAC
CGCTACACCTGGAATTCTACCCCCCTCTACAAGACTCTAGCCTGCCAGTTTCGAATGCAG
TTCCCAGGTTGAGCCCGGGGATTTCACATCCGACTTGACAGACCGCCTGCGTGCGCTTTA
CGCCCAGTAATTCCGATTAACGCTTGCACCCTCCGTATTACCGCGGCTGCTGGCACGGAG
TTAGCCGGTGCTTCTTCTGCGGGTAACGTCAAGGGTTATTAACCTATCCCTTCCTCCCCG
CTGAAAGTACTTTACAACCCGAAGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCT
TGCGCCCATTGTGCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCA
GTTCCAGTGCTGGTCATCCTCTCAACCAGCTAGGGATCGTCA
>KV1
TAAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCGGACTACGACGCACTT
TATGAGGTCCGCTTGCTCTCGCGAGGTCGCTTCTCTTTGTATGCGCCATTGTAGCACGTG
TGTAGCCCTACTCGTAAGGGCCATGATGACTTGACGTCATCCCCACCTTCCTCCAGTTTA
TCACTGGCAGTCTCCTTTGAGTTCCCGGCACCGCTGGCAACAAAGGATAAGGGTTGCGCT
CGTTGCGGGACTTAACCCAACATTTCACAACACGAGCTGACGACAGCCATGCAGCACCTG
TCTCAGAGTTCCCGAAGGCACCAATCCATCTCTGGAAAGTTCTCTGGATGTCAAGAGTAG
GTAAGGTTCTTCGCGTTGCATCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCC
GTCAATTCATTTGAGTTTTAACCTTGCGGCCGTACTCCCCAGGCGGTCGACTTAACGCGT
TAGCTCCGGAAGCCACGCCTCAAGGGCACAACCTCCAAGTCGACATCGTTTACGGCGTGG
ACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTGAGCGTCAGTCTTT
GTCCAGGGGGCCGCCTTCGCCACCGGTATTCCTCCAGATCTCTACGCATTTCACCGCTAC
ACCTGGAATTCTACCCCCCTCTACAAGACTCTAGCCTGCCAGTTTCGAATGCAGTTCCCA
GGTTGAGCCCGGGGATTTCACATCCGACTTGACAGACCGCCTGCGTGCGCTTTACGCCCA
GTAATTCCGATTAACGCTTGCACCCTCCGTATTACCGCGGCTGCTGGCACGGAGTTAGCC
GGTGCTTCTTCTGCGGGTAACGTCAAGGGTTATTAACCTTCCCTTCCTCCCCGCTGAAAG
TACTTTACAACCCGAAGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCC
ATTGTGCATATTCCCCACTGCTGCCTCCCGTAGGCTGGACCGTGTCTCAGTTCCAGTGCT
GGCATCCTC

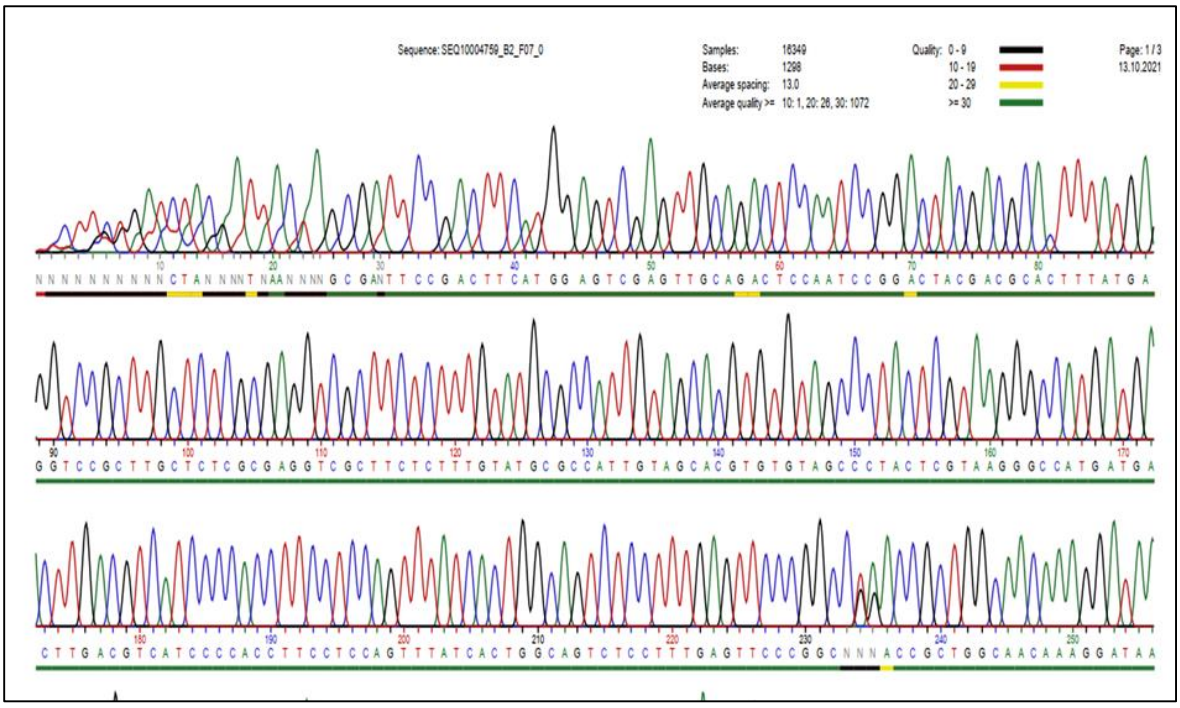
>KB3
CTTACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCGGACTACGACGC
ACTTTATGAGGTCCGCTTGCTCTCGCGAGGTCGCTTCTCTTTGTATGCGCCATTGTAGCA
CGTGTGTAGCCCTACTCGTAAGGGCCATGATGACTTGACGTCATCCCCACCTTCCTCCAG
TTTATCACTGGCAGTCTCCTTTGAGTTCCCGCCGAACCGCTGGCAACAAAGGATAAGGGT
TGCGCTCGTTGCGGGACTTAACCCAACATTTCACAACACGAGCTGACGACAGCCATGCAG
CACCTGTCTCAGAGTTCCCGAAGGCACCAATCCATCTCTGGAAAGTTCTCTGGATGTCAA
GAGTAGGTAAGGTTCTTCGCGTTGCATCGAATTAAACCACATGCTCCACCGCTTGTGCGG
GCCCCCGTCAATTCATTTGAGTTTTAACCTTGCGGCCGTACTCCCCAGGCGGTCGACTTA
ACGCGTTAGCTCCGGAAGCCACGCCTCAAGGGCACAACCTCCAAGTCGACATCGTTTACG
GCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTGAGCGTCA
GTCTTTGTCCAGGGGGCCGCCTTCGCCACCGGTATTCCTCCAGATCTCTACGCATTTCAC
CGCTACACCTGGAATTCTACCCCCCTCTACAAGACTCTAGCCTGCCAGTTTCGAATGCAG
TTCCCAGGTTGAGCCCGGGGATTTCACATCCGACTTGACAGACCGCCTGCGTGCGCTTTA
CGCCCAGTAATTCCGATTAACGCTTGCACCCTCCGTATTACCGCGGCTGCTGGCACGGAG
TTAGCCGGTGCTTCTTCTGCGGGTAACGTCAATTGCTGAGGTTATTAACCTCAACACCTT
CCTCCCCGCTGAAAGTACTTTACAACCCGAAGGCCTTCTTCATACACGCGGCATGGCTGC
ATCAGGCTTGCGCCCATTGTGCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACC
GTGTCTCAGTTCCAGTGCTGCATCCTCTCCAGCTAGGGATCGTCT
>KB2
GTTACAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCGGACTACGACGCA
CTTTATGAGGTCCGCTTGCTCTCGCGAGGTCGCTTCTCTTTGTATGCGCCATTGTAGCAC
GTGTGTAGCCCTACTCGTAAGGGCCATGATGACTTGACGTCATCCCCACCTTCCTCCAGT
TTATCACTGGCAGTCTCCTTTGAGTTCCCGCCGAACGCTGGCAACAAAGGATAAGGGTTG
CGCTCGTTGCGGGACTTAACCCAACATTTCACAACACGAGCTGACGACAGCCATGCAGCA
CCTGTCTCAGAGTTCCCGAAGGCACCAATCCATCTCTGGAAAGTTCTCTGGATGTCAAGA
GTAGGTAAGGTTCTTCGCGTTGCATCGAATTAAACCACATGCTCCACCGCTTGTGCGGGC
CCCCGTCAATTCATTTGAGTTTTAACCTTGCGGCCGTACTCCCCAGGCGGTCGACTTAAC
GCGTTAGCTCCGGAAGCCACGCCTCAAGGGCACAACCTCCAAGTCGACATCGTTTACGGC
GTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTGAGCGTCAGT
CTTTGTCCAGGGGGCCGCCTTCGCCACCGGTATTCCTCCAGATCTCTACGCATTTCACCG
CTACACCTGGAATTCTACCCCCCTCTACAAGACTCTAGCCTGCCAGTTTCGAATGCAGTT
CCCAGGTTGAGCCCGGGGATTTCACATCCGACTTGACAGACCGCCTGCGTGCGCTTTACG
CCCAGTAATTCCGATTAACGCTTGCACCCTCCGTATTACCGCGGCTGCTGGCACGGAGTT
AGCCGGTGCTTCTTCTGCGGGTAACGTCAATTGCTGAGGTTATTAACCTCAACACCTTCC
TCCCCGCTGAAAGTACTTTACAACCCGAAGGCCTTCTTCATACACGCGGCATGGCTGCAT
CAGGCTTGCGCCCATTGTGCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGT
GTCTCAGTTCCAGTGGCTGGTCATCCTCTACCAGCTAGGGATCGTCTAGGTGAGCCGTTA
CCCCTACTAGCTAATCCATCTGGG

>KK1
GTTACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCGGACTACGACGC
ACTTTATGAGGTCCGCTTGCTCTCGCGAGGTCGCTTCTCTTTGTATGCGCCATTGTAGCA
CGTGTGTAGCCCTACTCGTAAGGGCCATGATGACTTGACGTCATCCCCACCTTCCTCCAG
TTTATCACTGGCAGTCTCCTTTGAGTTCCCGGCCACCGCTGGCAACAAAGGATAAGGGTT
GCGCTCGTTGCGGGACTTAACCCAACATTTCACAACACGAGCTGACGACAGCCATGCAGC
ACCTGTCTCAGAGTTCCCGAAGGCACCAAAGCATCTCTGCTAAGTTCTCTGGATGTCAAG
AGTAGGTAAGGTTCTTCGCGTTGCATCGAATTAAACCACATGCTCCACCGCTTGTGCGGG
CCCCCGTCAATTCATTTGAGTTTTAACCTTGCGGCCGTACTCCCCAGGCGGTCGACTTAA
CGCGTTAGCTCCGGAAGCCACGCCTCAAGGGCACAACCTCCAAGTCGACATCGTTTACGG
CGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTGAGCGTCAG
TCTTTGTCCAGGGGGCCGCCTTCGCCACCGGTATTCCTCCAGATCTCTACGCATTTCACC
GCTACACCTGGAATTCTACCCCCCTCTACAAGACTCTAGCCTGCCAGTTTCGAATGCAGT
TCCCAGGTTGAGCCCGGGGATTTCACATCCGACTTGACAGACCGCCTGCGTGCGCTTTAC
GCCCAGTAATTCCGATTAACGCTTGCACCCTCCGTATTACCGCGGCTGCTGGCACGGAGT
TAGCCGGTGCTTCTTCTGCGGGTAACGTCAATCGACAGGGTTATTAACCCTGTCGCCTTC
CTCCCCGCTGAAAGTACTTTACAACCCGAAGGCCTTCTTCATACACGCGGCATGGCTGCA
TCAGGCTTGCGCCCATTGTGCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCG
TGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGT
GAGCCGTTACCCCACCTACTAGCTAATCCCATCTGGGCACATCCGATGGCAAGAGGCCCG
AAGGTCCCCCTCTTTGGTCTTGCGACGTTATGCGGTATTAGCTACCGTTTCCAGTAGTTA
TCCCCCTCCATCAGGCACCCAGACATTACTCACCCCCGCCACTCCAGCGAAGCAGCAAGT
CT

>KBU
ATACGATTACAGCGATTCCGACTTCATGGGAGTCGAGTTGCAGACTCCAATCCGGACTAC
GACGCACTTTATGAGGTCCGCTTGCTCTCGCGAGGTCGCTTCTCTTTGTATGCGCCATTG
TAGCACGTGTGTAGCCCTACTCGTAAGGGCCATGATGACTTGACGTCATCCCCACCTTCC
TCCAGTTTATCACTGGCAGTCTCCTTTGAGTTCCCGGCCTAACCGCTGGCAACAAAGGAT
AAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATTTCACAACAMGAGCTGACGACAGCC
ATGCAGCACCTGTCTCAGAGTTCCCGAAGGCACCAATCCATCTCTGGAAAGTTCTCTGGA
TGTCAAGAGTAGGTAAGGTTCTTCGCGTTGCATCGAATTAAACCACATGCTCCACCGCTT
GTGCGGGCCCCCGTCAATTCATTTGAGTTTTAACCTTGCGGCCGTACTCCCCAGGCGGTC
GACTTAACGCGTTAGCTCCGGAAGCCACGCCTCAAGGGCACAACCTCCCAAGTCGACATC
GTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTG
AGCGTCAGTCTTTGTCCAGGGGGCCGCCTTCGCCACCGGTATTCCTCCAGATCTCTACGC
ATTTCACCGCTACACCTGGAATTCTACCCCCCTCTACAAGACTCTAGCCTGCCAGTTTCG
AATGCAGTTCCCAGGTTGAGCCCGGGGATTTCACATCCGACTTGACAGACCGCCTGCGTG
CGCTTTACGCCCAGTAATTCCGATTAACGCTTGCACCCTCCGTATTACCGCGGCTGCTGG
CACGGAGTTAGCCGGTGCTTCTTCTGCGGGTAACGTCAATTGCTGAGGTTATTAACCTCA
GCACCTTCCTCCCCGCTGAAAGTACTTTACAACCCGAAGGCCTTCTTCATACACGCGGCA
TGGCTGCATCAGGCTTGCGCCCATTGTGCAATATTCCCCACTGCTGCCTCCCGTAGGAGT
CTGGACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTC
GCCTAGGTGAGCCGTTACCCCACCTACTAGCTAATCCCATCTGGGCACATCTGATGGCAA
GAGGCCCGAAGGTCCCCCTCTTTGGTCTTGCGACGTTATGCGGTATTAGCTACCGTTTCC
AGTAGTTATCCCCCTCCATCAGGCAGTTCCCAGACATTACTCACCCTCCGCCGCTCCACC

Appendix VII. Sequence of the isolate KV1 produce by 16S rRNA partial sequencing





Appendix VIII. Screening of plants treated with inoculants

Appendix IX. Spectrophotometric values

Concentration of P	OD 880
0	0
0.125	0.09525
0.25	0.17725
0.375	0.2665
0.5	0.362
0.625	0.44425
0.75	0.541
0.875	0.63875
1	0.74375
1.125	0.81825

Concentration of p-nitrophenyl	OD 420
0	0
1	0.09975
2	0.195
3	0.2935
4	0.39725
8	0.82875
10	0.99075

Appendix X. Research Proposal Approval Letter

MASINDE MULIRO UNIVERSITY OF SCIENCE AND TECHNOLOGY (MMUST)
Tel: 056-30870
Fax: 056-30153
E-mail: directordps@mmust.ac.ke
Website: www.mmust.ac.ke
P.O Box 190
Kakamega – 50100
Kenya

Directorate of Postgraduate Studies

Ref: MMU/COR: 509099
Kiprotich Kelvin,
SBB/G/01-53357/2018
P.O. Box 190-50100
KAKAMEGA

18th March 2022

Dear Mr. Kiprotich,

RE: APPROVAL OF PROPOSAL

I am pleased to inform you that the Directorate of Postgraduate Studies has considered and approved your Masters proposal entitled: ***''Molecular Characterization of Phosphorous Solubilizing Bacteria Colonizing Common Bean RHIZOSHERES in Western Kenya.''*** and appointed the following as supervisors:

1. Prof. John O. Muoma - MMUST
2. Dr. Dennis Omayio - MMUST

You are required to submit through your supervisor(s) progress reports every three months to the Director of Postgraduate Studies. Such reports should be copied to the following: Chairman, School of Natural Sciences Graduate Studies Committee; Chairman, Department of Biological Sciences & Departmental Graduate Studies Committee. Kindly adhere to research ethics consideration in conducting research.

It is the policy and regulations of the University that you observe a deadline of two years from the date of registration to complete your Master's thesis. Do not hesitate to consult this office in case of any problem encountered in the course of your work.

We wish you the best in your research and hope the study will make original contribution to knowledge.

Yours Sincerely,

MASINDE MULIRO UNIVERSITY OF SCIENCE AND TECHNOLOGY DIRECTORATE OF POSTGRADUATE STUDIES P.O. Box 190 - 50100, KAKAMEGA (K) Sign:..... Date:.....

Prof. Stephen O. Odebero, PhD, FIEEP
DIRECTOR, DIRECTORATE OF POSTGRADUATE STUDIES

Appendix XI. NACOSTI Research Permit

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **458505**

Date of Issue: **12/January/2023**

RESEARCH LICENSE

This is to Certify that Mr.. Kelvin Kiprotich of Masinde Muliro University of Science and Technology, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2013 (Rev.2014) in Bungoma, Busia, Kakamega, Vihiga on the topic: Molecular Characterization of Phosphorus Solubilizing Bacteria colonizing common bean (Phaseolus vulgaris.L) rhizosphere in Western Kenya. for the period ending : 12/January/2024.

License No: **NACOSTI/P/23/22012**

458505

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.

See overleaf for conditions

Appendix XII. Research Article

Hindawi
International Journal of Microbiology
Volume 2023, Article ID 6668097, 10 pages
https://doi.org/10.1155/2023/6668097

Research Article

Molecular Characterization and Mineralizing Potential of Phosphorus Solubilizing Bacteria Colonizing Common Bean (*Phaseolus vulgaris* L.) Rhizosphere in Western Kenya

Kelvin Kiprotich, John Muoma, Dennis O. Omayio, Tavasi S. Ndombi, and Clabe Wekesa

Department of Biological Sciences, School of Natural Sciences, Masinde Muliro University of Science and Technology, P.O. Box 190, Kakamega 50100, Kenya

Correspondence should be addressed to Kelvin Kiprotich; kiprotichkelvin29@gmail.com

Received 25 November 2022; Revised 6 February 2023; Accepted 14 February 2023; Published 1 March 2023

Academic Editor: Zhun Li

Phosphorus solubilizing bacteria (PSB) are a category of microbes that transform insoluble phosphates in soil into soluble forms that crops can utilize. Phosphorus in natural soils is abundant but poorly soluble. Hence, introducing PSB is a safer way of improving its solubility. The aim of this study was to genetically characterize and determine the mineralization capability of selected PSB colonizing rhizospheres of common beans in Western Kenya. Seven potential phosphorus solubilizing bacteria (PSB) were isolated from various subregions of Western Kenya. 16S ribosomal RNA gene sequencing and National Center for Biotechnology Information (NCBI), Basic Local Alignment Search Tool (BLAST) identified the isolates. The phosphate solubilization potential of the isolates was evaluated under agar and broth medium of National Botanical Research Institute's phosphate (NBRIP) supplemented with tricalcium calcium phosphate (TCP). Identified isolates were as follows: KK3 as *Enterobacter mori*, B5 (KB5) as *Pseudomonas kribbensis*, KV1 as *Enterobacter asburiae*, KB3 as *Enterobacter mori,* KK1 as *Enterobacter cloacae*, KBU as *Enterobacter tabaci*, and KB2 as *Enterobacter bugandensis*. The strains B5 and KV1 were the most effective phosphorus solubilizers with 4.16 and 3.64 indices, respectively. The microbes converted total soluble phosphate concentration in broth medium which was 1395 and 1471 P μg/mL, respectively. The least performing isolate was KBU with a 2.34 solubility index. Significant ($p \leq 0.05$) differences in plant biomass for Rose coco and Mwitemania bean varieties were observed under inoculation with isolates B5 and KV1. PSB isolates found in common bean rhizospheres exhibited molecular variations and isolates B5 and KV1 are the potential in solving the insufficiency of phosphorus for sustainable crop production.

1. Introduction

Phosphorus (P) is the second most important nutrient for plant growth and development. It plays a significant role in key metabolic pathways such as nutrient uptake, biological oxidation, and energy metabolism [1]. Crops need significant nutrients in order to grow and produce substantial yields in any production system [2, 3]. The urgent need to feed the world's ever-growing population is putting immense strain on arable land around the world [4]. The quality of food-producing habitats have depreciated overtime due to land overuse and excessive application of destructive inorganic fertilizers [5]. Nitrogen, phosphorus, and potassium (NPK) fertilizers have been widely used in agricultural practice around the world to provide macronutrients that promote plant growth and, as an outcome, increase crop productivity [6]. Chemical fertilizers have undoubtedly provided benefits to modern cropping systems, but their overuse has harmed the health of agricultural soils and disrupted the important plant growth-promoting rhizobacteria (PGPR), resulting in lower production [7]. Due to environmental and health concerns brought up by the pervasive usage of chemical fertilizers to deliver nutrients in agriculture [8], current studies are focusing on developing