Genetic Diversity of Legume Nodulating Bacteria and the Effect of Nitrogen Sources on the Yield of Common Bean in Western Kenya

By

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DECLARATION

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

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DEDICATION

I dedicate this work to all my family members: Mrs. M. Jairo, Mrs. Molly Kawaka, Donnell Kawaka, Edrian Kawaka and little Eileen Kawaka for their support, encouragement and understanding during the entire study period.

ABSTRACT

Declining soil fertility in general and nitrogen (N) in particular limit crop production on many smallholder farms in western Kenya. Soil N deficiency is usually ameliorated with inorganic N fertilizers but their high costs preclude their use by resource poor smallholder farmers. In addition there are concerns that increased use of inorganic fertilizers could lead to environmental degradation. Organic inputs offer sustainable alternatives to expensive inorganic N fertilizers but the common organic materials on smallholder farms are not available in adequate amounts. The use of none traditional organic materials such as water hyacinth has been reported to supply plant nutrients but has not been adequately tested. Integration of legumes such as the common bean in cropping systems is another option that has the potential to contribute soil N through symbiotic nitrogen fixation. However there is paucity of information on the abundance, symbiotic effectiveness and identity of native bacterial strains from soils in western Kenya thus making it difficult to make informed decisions on the inoculation need. This study was conducted to compare the response of common bean to water hyacinth compost, inorganic N fertilizer and determine the abundance, symbiotic efficiency (SE) and identity of the native bacteria nodulating legumes. Soil sampling was carried out in three farms that had been used for growing common bean for at least two seasons and one fallow land with no known history of growing common bean or inoculation in Kisumu and Kakamega. The abundance of soil bacteria and symbiotic efficiency (SE) were determined in a greenhouse experiment. Deoxyribonucleic acid (DNA) was extracted from the bacteria and the 16S rRNA gene sequenced to establish their identity. Field experiments were laid out in a randomized complete block design (RCBD) replicated four times at Masinde Muliro University of Science and Technology and Kisumu for two consecutive seasons during the short rains of October to December, 2013 and the long rains of April to August, 2014. The treatments consisted of two bean varieties, rhizobium inoculation and non inoculation, with and without phosphorus (P), urea and water hyacinth compost. The population of bacteria ranged from 3.2×10^1 to 3.5×10^4 cells per gram of soil. Cluster analysis of the 16S rRNA gene grouped the native bacterial strains into five genera: *Rhizobium*, *Bacillus*, *Pantoea*, *Enterobacter* and *Klebsiella*. The bacterial strains had high SE compared to the first commercial the inoculant, CIAT 899 and ranged from *Rhizobium* sp (74%) to *Klebsiella variicola* (170%). The two species of *Klebsiella variicola* (*MUST005* and *KSM005*) and *Enterobacter hormaechei* recorded higher SE than strain 446, a second inoculant. These native species should be exploited for the development of inoculation programmes to enhance N fixation in the region. Low levels of P were recorded in fallow soils at the two sites while Aluminium levels were high in Kakamega. Urea significantly improved yields of common bean in the short rain season compared to the other treatments at the two sites. However, in the long rains season water hyacinth compost gave higher yields than urea and control. This study reveals that the use of water hyacinth compost can serve as a substitute for inorganic N source.

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LIST OF ABBREVIATIONS AND ACRONYMS

Amplified Fragment Length Polymorphism	AFLP
Basic Local Alignment Search Tool	BLAST
Biological Nitrogen Fixation	BNF
Bromothymol blue	BTB
Cetyl Trimethylammonium Bromide	CTAB
Deoxyribonucleic Acid	DNA
DNA-DNA Hybridization	DDH
Ethylene DiamineTetraacetic Acid	EDTA
Exopolysaccharides	EPS
Farm Yard Manure	FYM
Government of Kenya	GoK
Integrated Natural Resource Management	INRM
Integrated Nutrient Management	INM
Integrated Soil Fertility Management	ISFM
Internal Transcribed Spacer	ITS
International Center for Tropical Agriculture	CIAT
Kenya Seed Company	KSC
Legume Nodulating Bacteria	LNB
Long Rains	LR
Low-Input Sustainable Agriculture	LISA
Microbial Resources Centre Laboratory	MIRCEN
Ministry of Agriculture	MoA

Molecualr Evolutionary Genetics Analysis	MEGA
Multiple Sequence Alignment Program	MAFFT
National Center for Biotechnology Information	NCBI
Neighbour Joining	NJ
Organic Carbon	OC
Organic Materials	OMs
Polymerase Chain Reaction	PCR
Randomized Complete Block Design	RCBD
Restriction Fragment Length Polymorphism	RFLP
Ribonucleic Acid	RNA
Ribosomal Ribonucleic Acid	rRNA
Short Rains	SR
Tris-EDTA buffer	TE
Unweighted Pair Group Method with Arithmetic Mean	UPGMA
Yeast Extract Agar	YMA
Yeast Extract Mannitol Agar	YEMA

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CHAPTER ONE

INTRODUCTION

1.1 Background Information

Many countries in sub-Saharan Africa, including Kenya, are characterized by increased population growth and food insecurity (Seck et al., 2013). In addition, there are increasing environmental and economic concerns associated with conventional farming such as low soil productivity, pollution and high cost of inorganic fertilizers. These problems have necessitated the development and adoption of alternative food production practices. Sustainable soil fertility management has been reported to be key to food production without compromising environmental stability (Lal, 2009).

Historically, legume crops such as common beans (*Phaseolus vulgaris L.*), have played an important role in soil fertility, particularly through addition of biologically fixed nitrogen, enhancement of soil organic matter and prevention of nutrient leaching (Mothapo, 2011). The common bean is a grain legume grown on more than four million hectares annually in Africa (Buruchara et al., 2011). The crop is also important in a range of other developing countries of Central America and Andean region of South America (Singh and Schwartz, 2010). In Kenya, common bean is ranked among the major grain food crops (GOK, 2006). The beans are grown for subsistence and also for regional markets where they play an important role in food security and income generation. In parts of western Kenya, per capita consumption depends on consumer preferences and can be as high as 66 kg/capita/year (Broughton et al., 2003).

This quantity of beans can provide substantial amounts of both protein and calories in the diet. In nutritional terms, beans are often called the "poor man's meat" because they are a cheap source of proteins and are rich in minerals and vitamins (Beebe et al., 2000). The bean products are consumed at various stages of plant development, and thus, offer a staggered and prolonged food supply in the form of leaves, green pods, fresh grain, as well as dry grains. Integration of common bean into existing cropping systems has therefore the potential to mitigate food insecurity in Kenya, if challenges associated with its production are addressed.

The national annual dry bean production in Kenya is about 380,000 metric tons (t) which is far below the pulse demand of 749,000 metric tons (Bationo et al., 2008; Ministry of National Planning and Development, 2002). Dry bean production is predominantly by small-scale farmers and has been on the decline in recent years. For example, production per hectare declined from 0.8 t ha^{-1} in 1990 to less than 0.5 t ha^{-1} against a potential yield of 1.5 t ha^{-1} (MoA, 2005). The low bean yields have been attributed to various constraints such as diseases, insect pests and poor agronomic practices (Otsyula and Ajang, 1995). In addition, soil fertility depletion is a major constraint to crop production among most small-holder farmers in Kenya and is considered the fundamental biophysical root cause of declining per capita food production in the region (Sanchez et al., 1997). In particular, deficiency of nitrogen (N), the nutrient taken up by beans in the largest amounts among the essential plant nutrients (Vance, 2001), is a major constraint to its productivity on many smallholder farming systems (CIAT, 1989).

Unfortunately, small-holder farmers, who are the major dry bean producers in Kenya rarely apply nitrogenous fertilizers in bean production, relying mainly on the presumed ability of the bean to fix its own nitrogen. Beans are however known to be poor nitrogen-fixers (Hardarson, 1993). There are several options that are available to manage nitrogen deficiency on smallholder farms. Chemical fertilizers are often considered to offer immediate solution to nutrient deficiencies in soil (Chaia et al., 2010; Gentili et al., 2006), but these chemical fertilizers are expensive and most small-holder famers cannot therefore afford them. The other options that are used to replenish N include use of organic materials such as crop residues, animal manures and agroforestry tree prunings (Mathu et al., 2012). Application of these organic materials to soils has multiple roles such as increasing the soil organic carbon content, soil microbial activity, and improves the soil structure and the nutrient status (Sanni and Adesina, 2012). However, most of the commonly available organic materials on smallholder farms are often of inadequate quantity and of poor quality to meet the crop nutrient demand (Opala, 2011).

The use of non-traditional, largely unexploited, organic resources to augment common organic inputs in crop production has therefore received considerable research attention in the recent past (Opala et al., 2012). One such organic material is the water hyacinth (*Eichhornia crassipes*), a water weed that is abundant in Lake Victoria. The effect of the water hyacinth infestation has been observed to have a negative impact on the economic status of the local fishing community as the weed kills fish due to oxygen depletion.

Despite the problems associated with water hyacinth, it has been reported to be rich in nitrogen which could be as high as 3.2 % of its dry matter (Gunnarsson and Petersen, 2007). It also has other macronutrients that are essential for plant nutrition (Center et al., 2002; Sannigrahi et al., 2002). Water hyacinth compost has been reported to enhance productivity in several crops such as tomatoes (Kayum et al., 2008), rice (Amitava et al., 2008) and *Zea mays* (Chukwuka and Omotayo, 2009). It therefore has the potential to be a cheaper source of N compared to inorganic N fertilizers on smallholder farms.

Biological nitrogen fixation represents the major source of N input in agricultural soils including those in arid regions (Zahran, 1999). The major N fixing systems are the symbiotic systems, which can play a significant role in improving the fertility and productivity of soils low N. The symbioses between *Rhizobium* and legumes are a cheaper and usually more effective agronomic practice for ensuring an adequate supply of N for legume-based crop and pasture production than the application of N fertilizer (Zahran, 1999).

Common bean establishes symbiotic associations with a wide range of root-nodule nitrogen-fixing bacteria called rhizobia (Girvan et al., 2003). Initially, based on the cross-inoculation-group concept, all bean nodulating rhizobia were classified as *Rhizobium leguminosarium* symbiovar (sv.) *phaseoli* (Aserse et al., 2012). Later, due to the advancement of new molecular biological techniques and the isolation of different strains from various areas of the world, different common bean nodulating bacteria have been isolated and described (Laguerre et al., 2001).

Environmental factors influence all aspects of nodulation and symbiotic N_2 fixation, in some cases reducing rhizobial survival and diversity in soil; in others affecting nodulation or nitrogen fixation and even growth of the host. Most leguminous plants require a neutral or slightly acidic soil for growth, especially when they depend on symbiotic N_2 fixation (Bordeleau and Prevost, 1994).

Soil acidity constrains symbiotic N_2 fixation by limiting *Rhizobium* survival and persistence in soils and reducing nodulation (Ibekwe et al., 1997). Strong soil acidity is associated with Al, H, iron (Fe) and manganese (Mn) toxicities to plant roots in the soil solution and corresponding deficiencies of the available P, molybdenum (Mo), calcium (Ca), magnesium (Mg) and potassium (K) (Gathumbi et al., 2002; Jorge and Arruda, 1997). One of the possible ways to ameliorate soil acidity is through the use of organic materials. The mechanism of ameliorating soil acidity by organic compost may be due to the exchange of proton (H^+) between soil and the added compost (Mokolobate and Haynes, 2002; Wong et al., 1998).

During the initial decomposition of the compost, some formation of phenolic, humic-like material may occur and these organic anions consume protons from the soil, thus tending to raise the equilibrium pH (Haynes and Mokolobate, 2001). The other mechanism that has been proposed to explain the increase in soil pH by organic materials is the specific adsorption of humic material and/or organic acids onto hydrous surfaces of Al and Fe oxides by ligand exchange with corresponding release of OH^- (Swarnam and Murugan, 2014).

However the use of water hyacinth as organic compost in western Kenya to reduce soil acidity, enhance biological nitrogen fixation and improve common bean yield has not been investigated.

Apart from soil acidity other multiple factors may compromise the efficiency of introduced inoculant strains in symbiosis with common bean (Rufini et al., 2014). One of these factors is the ability of common bean to nodulate with different species of rhizobia (Sánchez-Juanes et al., 2013), many of which demonstrate low efficiency in N_2 fixation. Nitrogen-fixing strains of rhizobia that are adapted to diverse edaphic conditions, climates and level of competition for infection sites are required for optimal for inoculant production (Barret et al., 2011).

Despite BNF being considered as one of the most important processes in nature, information on native legume nodulating bacteria in western Kenya is scarce (Odee et al., 1997). It is therefore important to enhance common bean production among the small holder farmers through the use of inoculation in order to optimize the contribution of biological nitrogen fixation in a variety of edaphic and climatic conditions. In addition the information will be helpful to plant breeders in identifying niche based elite and native strains of nodule bacteria that are compatible with different newly bred common bean lines of to optimize BNF. Plant breeders could improve common bean productivity through identification of superior genotypes with greatest capacity for BNF and nitrogen assimilation.

Research efforts using the newly developed lines to improve symbiotic nitrogen fixation have been directed towards developing breeding lines capable of nodulation and nitrogen fixation at high soil nitrate or ammonium concentrations (Herridge and Rose, 2000). Currently many laboratories are selecting legumes for enhanced N fixation. However most techniques for studying or estimating symbiotic N fixation are not useful for breeders because they are expensive, time consuming or inconvenient to field conditions (Herridge and Rose, 2000).

1.2 Problem Statement

The decline in soil fertility particularly N and the high cost of inorganic fertilizer is one of the main constraints to crop production among smallholder farmers in western Kenya. The decline primarily results from continuous cultivation without adequate addition of external nutrient inputs. Intensive farming practices that accomplish high yields require inorganic N sources which are not only expensive and out of reach of most small-scale farmers, but have also been reported to create environmental problems (Tilman et al., 2002). The extensive use of inorganic fertilizers in agriculture is currently under debate due to environmental concern and fear for consumer health (Soren et al., 2013). Sustainable, environmentally friendly and cheaper sources of N such as organic inputs are always of inadequate quantity and of poor quality to meet the crop nutrient demand (Opala, 2011). Smallholder farmers in western Kenya rely mainly on legume crops such as common bean to replenish soil N through biological nitrogen fixation, despite the fact that beans are known to be poor nitrogen-fixers (Hardarson, 1993). Inoculation of legumes is necessary in the absence of compatible rhizobia and when rhizobial populations are low or inefficient in fixing N (Catroux et al., 2001). However, commercial inoculants used in western Kenya still contain exotic cultures from United States of America which may not be well adapted to local conditions (Waswa, 2013). Despite the natural internal resources presented by rhizobia-legume associations in N fixation, limited information is available on the regional abundance, symbiotic efficiency (SE) and diversity of native common bean nodulating bacteria in western Kenya.

1.3 Justification

Low soil N is usually ameliorated with inorganic N fertilizers but their high costs preclude their use by resource poor smallholder farmers. Therefore, mitigation of N deficiencies in soil could be expanded to include non-traditional and largely unexploited organic resources such as water hyacinth. The weed is abundant in L. Victoria and can help mitigate soil nutrient deficiencies on small holder farms along this region. Although the weed has caused problems to the fishing industry along the lake basin region, it could be turned into an asset by converting it into a source of N nutrient for crops because of its high N content (Gunnarsson and Petersen, 2007). The use of organic materials also reduces Al toxicity through production of organic acids that form complex with Al^{3+} leading to high crop yields in acid soils (Haynes and Mokolobate, 2001). Water hyacinth compost is therefore likely to alleviate soil constraints that limit legume nodule initiation, render P and Mo more available to crops and increase pH for optimum BNF (Cristina et al., 2012). Exploitation of the legume-rhizobia symbiosis in agricultural systems requires knowledge on the identity of legume nodulating bacteria in different agro-ecological zones as foreign strains introduced as inoculants often fail to adapt well (Cheng et al., 2009). Currently commercial inoculants used in Kenya still contain exotic cultures from other regions which may not be well adapted to local conditions (Waswa, 2013). In addition, there is limited information on previous studies that have quantified the abundance, symbiotic efficiency and genetic diversity of native legume nodulating bacteria. Therefore identification of new and elite strains offers the opportunity to improve BNF within different geographical locations (Appunu and Dhar, 2006).

1.4 Objectives

1.4.1 General Objective

To determine the diversity and symbiotic efficiency of native bacteria nodulating legumes and evaluate the effects of organic and inorganic nitrogen sources on the yield of common bean varieties

1.4.2 Specific Objectives

1. To assess the abundance and genetic diversity of comoon bean nodulating bacteria in soils in western Kenya.
2. To determine the symbiotic efficiency of native bacterial strains nodulating common bean in western Kenya.
3. To compare the effects of water hyacinth compost and inorganic N sources (urea) on yield and yield components of common bean in western Kenya.

1.5 Hypothesis

1. The abundance and diversity of native bacteria nodulating common bean in western Kenya does not depend on soil properties and land use settings.
2. Native bacteria nodulating common bean in western Kenya do not have different symbiotic efficiency (SE).
3. The application of water hyacinth compost and inorganic (urea) N source does not affect yield components of common bean

CHAPTER TWO

LITERATURE REVIEW

2.1 Distribution of common bean in Kenya

Common bean (*Phaseolus vulgaris L.*) is one of the most important legume crops in Kenya and is cultivated on an estimated 700,000 ha (Karanja et al., 2002). A low average yield of 750 kg/ha is realized, against a potential of 1500 kg/ha (Ministry of National Planning and Development, 2002). The major constraints to bean production in western Kenya are diseases, soil infertility and insect pests (Monda et al., 2003). The distribution of common bean production in Kenya is mainly in the highlands and medium altitude areas. As shown in Figure 1 (Katungi et al., 2009), approximately 75 % of the annual cultivation of beans take place in three regions namely; Rift valley, Nyanza, and Eastern regions (Gichangi et al., 2012). The rift valley contributes the biggest share, accounting for 33% of the national output followed by Nyanza and Western regions each accounting for 22 % (Katungi et al., 2009). The output from Eastern parts of the country and the coast is constrained by lack of improved varieties and inadequate use of fertilisers (Katungi et al., 2009).

Although Kenya has two seasons growing for crop production, a significant number of farmers grow the crop once a year because of adverse climatic conditions (Katungi et al., 2009). The Rift valley and the Western regions collectively produce 55% of the national outputs and allocate land to common beans once a year, during March to May season commonly known as the long rains while farmers in the Central and Eastern regions grow the beans twice a year but only 70 percent of the farmers in the Eastern region grow it in the long rains (Gichangi et al., 2012).

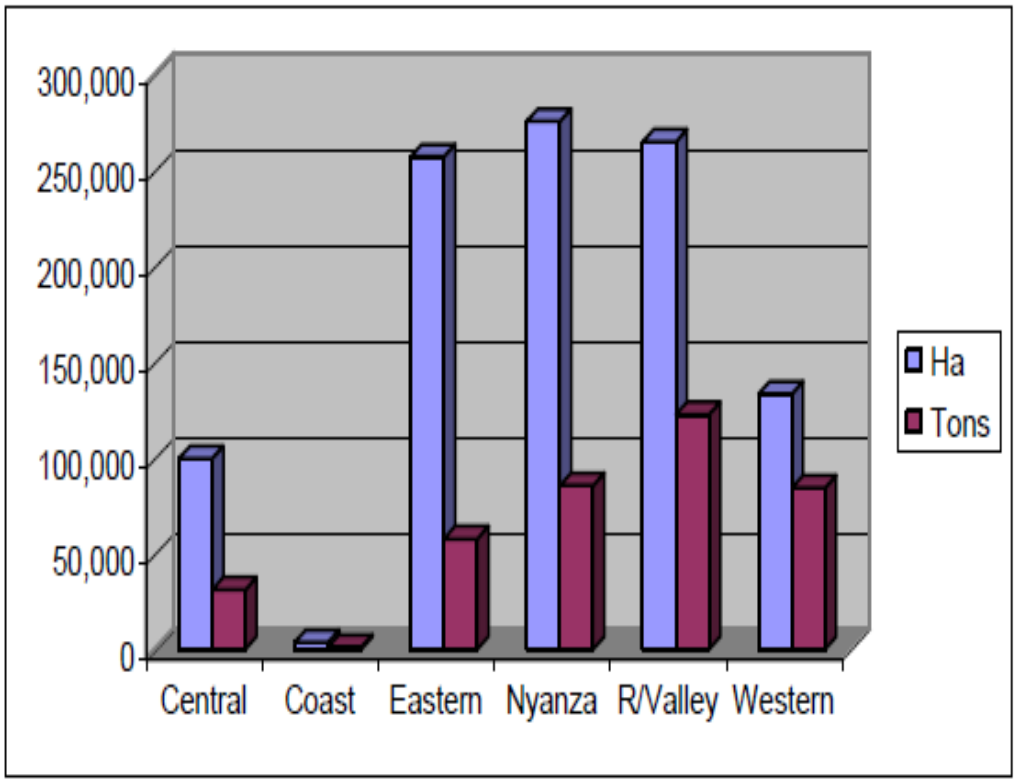


Figure 1: Distribution of common bean acreage and production in Kenya, 2009

2.2 Rhizobia-legume root association

Nitrogen is an essential nutrient for plant growth and development. Intensive farming practices that accomplish high yields require inorganic fertilizers. These inorganic inputs are expensive and may also create environmental problems (Mutegi et al., 2012). Their use is therefore currently under debate due to environmental concern and fear for consumer health (Halima et al., 2013). Currently, there is a growing level of interest in environmental friendly sustainable agricultural practices and organic farming systems (Lee and Song, 2007; Tu et al., 2006).

Increasing and extending the role of biofertilizers such as those presented by rhizobia would decrease the need for chemical fertilizers and reduce adverse environmental effects. The development and implementation of sustainable agriculture technique like biofertilization is of major importance in alleviating environmental pollution and the deterioration of nature (Öğütcü and Algur, 2014).

One such sustainable agricultural strategy is presented by the association of *Rhizobium* and legumes. Rhizobia are nitrogen-fixing soil bacteria capable of inducing the formation of root or stem nodules on leguminous plants. The bacteria reduce atmospheric nitrogen to ammonia for the benefit of such plants. In many agricultural systems, rhizobia have therefore been used to enhance nitrogen fixation in legumes (Ilyas et al., 2012; Teaumroong and Boonkerd, 1998). The association produces 50% of 175 million tonnes of total biological nitrogen fixation, annually providing nearly half of all N used in agriculture (Adiguzel et al., 2010).

2.3 Biological Nitrogen Fixation

The amount of nitrogen gas (N_2) in the atmosphere is approximately 78-80 % which unusable by most living organisms (Hill et al., 2013). Nitrogen is one of the prime elements required essentially for the synthesis of enzymes, proteins, chlorophyll, DNA and RNA (Ahmad et al., 2012). Nitrogen therefore plays a critical role in determining the health of living organisms including microbes and plants.

In nodulating legumes, the N demand is fulfilled through symbiotic N_2 fixation wherein atmospheric N_2 is converted to usable N (NH_3) by nitrogenase of Rhizobia (Zaidi et al., 2012). Some plants benefit from nitrogen-fixing bacteria when the bacteria die and release nitrogen to the environment or when the bacteria live in close association with the plant. In legumes and a few other plants, the bacteria live in small growths on the roots called nodules where N fixation takes place.

2.3.1 Benefits of Biological Nitrogen Fixation

Nitrogen gas is the most abundant in the atmosphere and accounts for approximately 78-80 % (Garrison, 2006). The process of BNF involves conversion of atmospheric nitrogen (N) to ammonia, a form of N that can be utilized by plants and involves the enzyme dinitrogenase that splits the triple-bond inert atmospheric nitrogen (N_2) into organic ammonia molecule (Cheng, 2008; Vessey et al., 2005). The process is regarded as a renewable resource for sustainable agriculture as it helps to reduce N fertilizer requirements and thus increases economic returns to producers (Walley et al., 2007).

The N fixation has the potential to contribute greatly to more economically viable and environmentally friendly agriculture (Alberton et al., 2006). It is estimated that the 80–90% of the N available to plants in natural ecosystems originates from BNF (Rascio and Rocca, 2008). BNF contributes to the replenishment of soil N, and reduces the need for industrial N fertilizers (Larnier et al., 2005).

The process offers an economically attractive and ecologically sound means of reducing external N input (Yadvinder-Singh et al., 2004).

2.3.2 Mechanisms of Biological Nitrogen Fixation

Biological nitrogen fixation is a process that is catalyzed by a two-component nitrogenase complex (Yan et al., 2010). The enzyme nitrogenase catalyzes the simultaneous reduction of one N_2 and 2 H^+ to ammonia and a molecule of hydrogen gas. The enzyme consists of two proteins, an iron protein and a molybdenum-iron protein. The process utilizes 16 moles of ATP and a supply of electrons and protons and is known to occur optimally between legumes and rhizobia (de Carvalho et al., 2011).

$$N_2 + 8H^+ + 8e^- + 16\ ATP \longrightarrow 2\ NH_3 + H_2 + 16\ ADP + 16\ Pi$$

The symbiotic relationship between the roots of legumes and certain soil bacteria accounts for the development of a specific organ, the symbiotic root-nodule, whose primary function is nitrogen fixation (Shvaleva et al., 2010). Depending on the type of microorganism, the energy required for the reduction during N fixation is generated by photosynthesis, respiration or fermentation (Hardarson et al., 2003). The maintenance of nitrogenase activity in the nodule requires a delicate balance. The process requires oxygen for respiration needed to supply the energy demands of the N reduction process. However oxygen irreversibly inactivates the nitrogenase enzyme complex (Sánchez et al., 2011).

These conflicting demands are reconciled by control of oxygen flux through a diffusion barrier in the nodule cortex and by the plant oxygen carrier, legheamoglobin, which is present exclusively in the nodule (Minchin et al., 2008).

However there are some rhizobia species that are able to grow under low oxygen conditions using nitrate as electron acceptor to support respiration in a process known as denitrification by which bacteria reduce sequentially nitrate (NO_3-) or nitrite (NO_2-) to Nitrogen (N_2) (Sánchez et al., 2010). Nitrate is reduced to nitrite by either a membrane-bound or a periplasmic nitrate reductase, and nitrite reductase catalyzes the reduction of nitrite to nitric oxide (NO). Nitric oxide is further reduced to nitrous oxide (N_2O) by nitric oxide reductases and, finally, N_2O is converted to N_2 by the nitrous oxide reductase enzyme (van Spanning et al., 2007). The significance of denitrification in rhizobia-legume symbiosis can be appreciated when oxygen concentration in soils decreases during environmental stress such as flooding of the roots, which causes hypoxia (Sánchez et al., 2011). The denitrifying activity therefore works as a mechanism to generate ATP for survival of rhizobia in the soil (Sánchez et al., 2011).

2.4 Factors affecting biological nitrogen fixation

There are several factors that affect nitrogen fixation in legumes. These factors influence all aspects of nodulation and symbiotic N_2 fixation, in some cases reducing rhizobial survival and diversity in soil while in others affecting nodulation or nitrogen fixation and even growth of the host.

2.4.1 Bacteria

Free-living soil bacteria that are beneficial to plant growth are usually referred to as plant growth promoting rhizobacteria (Hayat et al., 2010). These bacteria are also termed as plant health promoting rhizobacteria or nodule promoting rhizobacteria and are associated with the rhizosphere which is an important soil ecological environment for plant–microbe interactions (Hayat et al., 2010). They promote plant growth by colonizing the plant root (Kokalis-Burelle et al., 2006).

According to their relationship with the plants, they are divided into two groups: symbiotic bacteria and free-living rhizobacteria (Khan, 2005). The bacteria are further grouped according to their sites of residence: symbiotic bacteria, which live inside the plant cells, produce nodules, and are localized inside the specialized structures; and free-living rhizobacteria, which live outside the plant cells and do not produce nodules, but still prompt plant growth (Gray and Smith, 2005). The best-known symbiotic bacteria are *Rhizobia*, which produce nodules in leguminous plants.

One of the main pre-requisites for effective nodulation and nitrogen fixation is the presence of an effective and competitive *Rhizobium* strain in the soil (Fening and Danso, 2002; Mathu et al., 2012). The survival and persistence of an adequate number of effective rhizobia in soils is essential to ensure nodulation (Ben Romdhane et al., 2008; Girvan et al., 2003).

In spite of *Rhizobium* being a natural inhabitant of the soil, many soils lack effective strains, especially those strains effective for legumes species not previously grown on a particular soil and therefore necassitates the need for inoculation with effective strains in most soils (Ben Romdhane et al., 2008).

A variety of bacteria have been used as soil inoculants intended to improve the supply of nutrients to crop plants. Species of *Rhizobium* (*Rhizobium, Mesorhizobium, Bradyrhizobium, Azorhizobium, Allorhizobium* and *Sinorhizobium)* have been successfully used to permit an effective establishment of the nitrogen-fixing symbiosis with leguminous crop plants (Hayat et al., 2010). On the other hand, non-symbiotic nitrogen fixing bacteria such as *Azotobacter, Azospirillum, Bacillus,* and *Klebsiella* sp. are also used to inoculate arable land with the aim of enhancing plant productivity (Rey and Schornack, 2013).

2.4.2 Host Plant

The host plant controls rhizobia rhizosphere stimulation and is responsible for initial infection (Driouech, 2010). The specific site and potential number of infections as well as nodulation, potential number of nodules, size and patterns of distribution of the nodules on the root system depends on the host plant (Zahran, 2001). It has also been reported that some leguminous plants do not nodulate and therefore are not able to effectively fix nitrogen (Mathu et al., 2012). Recent studies have shown that the bacterial genes involved in nodulation are activated by phenolic compounds exuded by the host plant (Arora, 2013).

Initiation of nodule formation on compatible host plants results from a molecular dialogue between the host and the bacteria (Perret et al., 2000). The host plants produce flavonoids and related secondary metabolites in the rhizosphere.

These signals can be perceived by a specific bacterial receptor called NodD, which acts as a transcriptional activator of other nodulation genes (Franche et al., 2009). The core of the Nod factor molecule is encoded by canonical nodA, nodB and nodC whereas, for example, nodFE are involved in poly-unsaturation of the fatty acyl group attached to the core molecule (Moulin et al., 2004). Other nodulation genes encode enzymes which add a variety of substituents to the core, as in the case of Nod factors produced by *Azorhizobium caulinodans* (Gibson et al., 2008). The Nod factor acts as an elicitor of root nodule formation by the plant by triggering a developmental program leading to construction of the root nodule and entry of rhizobia into the nodule (Gage, 2004).

Nod factor is an important host specificity determinant (Giraud et al., 2007; Spaink, 2000). Recently however, the Nod factor paradigm was challenged by Giraud et al. (2007), who reported that certain photosynthetic, stem- and root-nodulating bradyrhizobia do not possess canonical nodABC genes but use other mechanisms for signalling to the plant. Their experiments led them to hypothesize that a purine derivative might play a role in triggering nodule formation instead of the Nod factor. This points to the complexity of the symbiotic system and shows that bacteria employ diverse strategies to gain entry into the roots.

2.4.3 Longevity of Nodules

The amount of N fixed by a leguminous crop depends much on the longevity of the nodules on its roots (Zahran, 1991). In all nodule types, the N fixation period is optimal between 4 and 5 weeks after infection. Beyond this period, fast reductions of N fixing bacteroid capacity are detectable and a senescence process occurs in the N fixing nodule zone. This phenomenon is related to the onset of pod filling in grain legumes like soybean, pea and common bean (Puppo et al., 2005). The lifespan of nodule is largely determined by four factors: the physiological condition of the legume, the moisture content of the soil, the presence of any parasites, and the strain of rhizobia forming the nodule (Driouech, 2010).

During maturity, legumes fill developing seeds with nutrients and storage compounds. As plants put more energy into seed production, the N fixing activity of the bacteroids eventually decreases (Zahran, 1999). Eventually the nodules stop functioning and disintegrate, releasing bacteroids into the soil. In favorable soil conditions, these rhizobia may survive and infect new plants during the next cropping season (Prévost and Antoun, 2007). However, in intensive agricultural systems it is usually necessary to add rhizobial inoculant with every crop (Lucy et al., 2004). Plants may also shed their nodules early if affected by severe drought (Gil-Quintana et al., 2012).

2.4.4 Temperature

Temperature is considered as one of the most important physical factors affecting BNF. In the tropics, the maximum soil temperatures have been reported to exceed 40 ˚C at 5 cm and 50 ˚C at 1 cm depth and limit nodulation (Grange et al., 2007; Hungria et al., 2000). However some *Rhizobium* strains have been reported to survive at temperatures around 70ºC in dry soil (Abbott and Murphy, 2007).

The temperature requirement for BNF in tropical legumes vary between 27 ºC and 40 ºC while rhizobia growth requires temperature range between 32 ºC and 47 ºC, though tolerance varies among species and strains (Zahran, 1999). High temperatures can prevent nodulation and inhibit the activity of N fixation in legumes even though root nodules may be insulated from the highest temperatures by the soil (Giller, 2001). Conversely, cool temperatures lead to delayed development of plants including delays in the formation of nodules, and so decreased rates of N_2 fixation (Giller, 2001). Survival of rhizobia in soils at high temperatures appears to be improved by the presence of clay particles and soil organic matter, but many of the soils where high temperatures are experienced are sandy (Giller, 2001).

The plant root infection process is probably the component most affected by high temperatures, with sensitivity located at the nodulation sites (Aranjuelo et al., 2014). High temperatures also inhibit root hair penetration, infection thread formation, nodule initiation, rhizobial release from the infection thread, and bacteriod development (Makarova and Vadim, 2005; Stacey et al., 2006).

Indirect effects of high temperature on the metabolism of the host plant and direct effects on nitrogen fixation have been recognized for a long time (Ahemad and Kibret, 2014). However the overall balance between photosynthesis and respiration determines levels of N fixation (Werner and Newton, 2006).

2.4.5 Moisture availability

Stress resulting from water generally affects rhizobial survival, growth, longevity of nodules, synthesis of leghaemoglobin and nodule function (Zahran, 2001). Severe stress may lead to irreversible cessation of nitrogen fixation. However different rhizobial strains and plant species vary in relation to drought tolerance (Gibson et al., 2008). Low water supply might affect N_2 fixation by lowering plant carbohydrate supply, carbohydrate transportation to the nodules, or direct impairment of nodule development and activity (Maheshwari, 2008). In general, it has been noted that, the numbers of rhizobia in the soil decline drastically as soil dries (Zahran, 2001). A comparative study on the survival of *Rhizobium* and *Bradyrhizobium* in dry soil indicated that, *Bradyrhizobium* strains are more tolerant to desiccation than strains of *Rhizobium* over short periods (Werner and Newton, 2006).

Other studies however found no simple relationship between the desiccation tolerance of fast or slow growing rhizobia but they did find that specific strains of each survived in much greater numbers than others (Giller, 2001).

Rhizobia generally survive poorly on drying in soils which contain only small amounts of clay or organic matter while those strains which survive under greater water stress are those which retain less water within their cells (Giller, 2001). Drought contributes to crop yield loss, including common bean where symbiotic fixation of atmospheric nitrogen (N_2) is sensitive to even modest soil water deficits (Sinclair et al., 2007). In soybean, lines with high nitrogen fixation at pod filling stage were found to have higher yield under water stress than those having low nitrogen fixation (Puangbut et al., 2010).

Three major factors are thought to be involved in drought effects on BNF: oxygen limitation, carbon shortage, and regulation by nitrogen metabolism (Marino et al., 2006). Decline of N_2 fixation with soil drying causes yield reductions due to inadequate N for protein production, which is a critical seed product (Sinclair et al., 2007).

2.4.6 Water logging

Rhizobia are normally aerobic organisms but some strains of *Bradyrhizobium* and *R. meliloti* possess a dissmilatory nitrate reductase which can function as an electron acceptor, and thus enable rhizobia to survive under anaerobic conditions (Jenkins, 2003). Due to the aerobic characteristic of rhizobia, the survival of rhizobia during long periods of flooding is of particular importance in cropping systems in which legumes are grown in rotation with rice.
It has been reported that the size of the population of rhizobia sampled from the field is generally larger when the soil is moist or fully water logged as compared to when the soil is dry (Burgers, 2012).

In contrast, large reductions in the numbers of fast growing rhizobia nodulating chickpea have been reported when grown after paddy rice (Burgers, 2012). Lack of oxygen is also a major problem for root respiration and can rapidly result in loss of nitrogenase activity (Giller, 2001; Tobisa et al., 2014). Water logging can result in the rapid release into the soil certain heavy metals, like iron and manganese, which are highly toxic to both rhizobia and plants in high concentration (Carranca et al., 2015).

Many studies have demonstrated the sensitivity of N fixation to flooding (Bacanamwo and Purcell, 1999; Sparks, 2012; Yamauchi et al., 2013). Legumes grown on nitrate have shown less sensitivity to flooding stress than plants relying on N fixation, indicating the differential sensitivity to flooding between N fixation and nitrate uptake and assimilation (Khatoon et al., 2012). The detrimental effect of water logging is usually attributed to inadequate oxygen supply to sustain various root metabolisms (Ferner et al., 2012).

Water logging-induced soil anaerobosis is also harmful to nodule formation and function in several legume species (Simms and Taylor, 2002; Tobisa et al., 2014). Decreased O_2 concentration in the rhizosphere during flooding offer at least two reasons that nitrate could ameliorate flooding stress relative to N fixation (Thomas and Sodek, 2005; Yamauchi et al., 2013). Firstly, nitrate could be used as an alternative to O_2 as an electron acceptor in hypoxic roots (Brandão and Sodek, 2009; Thomas et al., 2005). Secondly, respiratory energy demands for N fixation and assimilation are higher than those for nitrate uptake and assimilation (Guo et al., 2013; Irigoyen et al., 2014).

Consequently, hypoxic roots of plants supplying plants with nitrogen have less damage than hypoxic roots of plants dependent upon N fixation. Studies have shown that water logging reduces nitrogenase activity and irreversibly alter ultra-structure of cells in many legume root nodules (Tobisa et al., 2014). Decreases in nitrogen accumulation by bean plants under waterlogged conditions might be due in part to reduced nodulation and decreased nitrogenase activity (Jong-Tag et al., 2008; Zahran, 2001). The restriction of assimilatory nitrate reduction is mainly because the conversion of nitrate to ammonia in leaf tissue is coupled to the light reactions of photosynthesis which are inhibited by water logging (Forde, 2000; Jong-Tag et al., 2008).

Interest in N transport in the xylem has centered mainly on its relationship with metabolic processes occurring in other parts of the plant, particularly in the root system (Jong-Tag et al., 2008). While asparagine is the main organic form of transport of plants grown on nitrate, ureides predominate in the transport of N in the xylem of symbiotic plants like soybean. Moreover, nitrate applied to symbiotic plants led to the inhibition of N fixation and a severe reduction in the transport of ureides (Mancuso and Shabala, 2010). Amino acids are less affected, but the most clearly established change is the higher asparagine/glutamine ration for plants grown on nitrate (Jong-Tag et al., 2008; Mancuso and Shabala, 2010).

2.4.7 Light

The effects of light on nitrogen fixation can be ascribed to its effects on photosynthesis and to photoperiodic effects (Zackrisson et al., 2004). A certain light intensity is necessary for maximum nodulation of seedlings and further nodule development of established plants (Gundale et al., 2012). Decreased day length, effects of shading or excessive cloud cover, all have adverse effects on nodulation. Studies have shown that transferring legumes from a low to high light intensity results in a rapid increase of both nodule size and number (Lichtfouse et al., 2011).

Previous studies have indicated that light can have significant, non photosynthetic effects on the establishment of the symbiosis (Ramos et al., 2003; Schmidt et al., 1999). Exposure of excised bean roots to light prior to inoculation stimulated Rhizobium infection, whereas exposure after inoculation inhibited nodule development (Ferguson and Mathesius, 2003). It has also been reported that nodulation is enhanced in various leguminous plants if they are grown under long days as compared to short days and that nodulation is inhibited upon exposure of roots to far-red light (Ladha et al., 1992; Narasimhan et al., 2013). In legumes such as soybean, light stimulates the production of one or more regulatory substances which inhibit the formation of infection threads and which may also inhibit the formation of nodule meristems.

2.4.8 Soil acidity

Acidic soils pose serious problems for the plant, the bacteria and the symbiosis as low pH is often associated with increased Al and Mn toxicity and reduced Ca supply (Giller, 2001). In addition to Al toxicity, acidity may also cause phosphorus (P) and molybdenum (Mo) deficiencies (Hungria et al., 2000; Vistoso et al., 2012). These stresses affect the growth of rhizobia on host legume and symbiosis (Campo and Wood, 2001). The optimal pH for rhizobial growth is considered to be between 6.0 and 7.0 and relatively few rhizobia grow well at pH less than 5.0 (Soares et al., 2014). However, some rhizobia can tolerate acidity better than others, and tolerance may vary among strains within species (Kannaiyan, 2002). Acidity affects early steps in the infection process, including the exchange of molecular signals between symbiotic partners and attachment to the roots (Zahran, 1999). Other stages of nodule establishment and function are also impacted by acidity, as is the growth of the host plant (Martínez-Romero, 2003).

Liming is widely known as the most effective means of correcting soil acidity (The et al., 2006). Application of agricultural lime containing Ca and/or Mg compounds to acid soils increase Ca^{2+} and/or Mg^{2+} ions and reduces Al^{3+}, H^{+}, Mn^{4+}, and Fe^{3+} in the soil solution (Kisinyo et al., 2014). This leads to increase in soil pH and available P due to reduction in P sorption (Sato and Comerford, 2005). In addition to neutralization of soil acidity, lime enhances root development, water and nutrient uptakes, necessary for healthy plant growth (Kisinyo et al., 2014; The et al., 2006).

Other studies have also shown that lime reduces Al toxicity, increases soil pH, available P, Ca, Mg, uptake of N and P thus improving crop productivity in Kenya acid soils (Opala et al., 2010a; Opala et al., 2010b). Apart from the use of lime, organic materials such as farmyard manure have also been shown to increase pH and reduce acidity and could serve as alternative in places where lime is expensive (Opala et al., 2010a). Other organic materials such as water hyacinth could also be tested to assess their potential in managing soil acidity in western Kenya.

2.4.9 Salinity

Some legumes are relatively salt tolerant but high concentrations can damage the nodules indirectly by withdrawing water osmotically from nodules or in situations of high evaporation rates, nodules may be damaged as a result of deposition of salts directly on nodule and roots (Lichtfouse et al., 2011). Among the most common effect of soil salinity include growth inhibition by Na^+ and Cl^-. Elevated Na^+ in soil solution inhibits the uptake of other nutrients such as P, K, Fe, Cu, and Zn directly by interfering with various transporters in the root plasma membrane (Giri et al., 2007).

As with most cultivated crops, the salinity response of legumes varies greatly and depends on such factors as climatic conditions, soil properties, and the stage of growth (Manchanda and Garg, 2008; Swaraj and Bishnoi, 1999). Variability in salt tolerance among crop legumes has been reported with others e.g., *Vicia faba*, *Phaseolus vulgaris*, and *Glycine max*, being more salt tolerant than others, e.g., *Pisum sativum* (Zahran, 1991; Zahran, 2001).

Many studies have shown that some *V. faba* tolerant lines sustained nitrogen fixation under saline conditions (Bouhmouch et al., 2005; Zahran, 2001). Other legumes, such as *Prosopis* (Fagg and Stewart, 1994), *Acacia* (Lafay and Burdon, 2007), and *Medicago sativa* (Polcyn and Garnczarska, 2009), are salt tolerant, but these legume hosts are less tolerant to salt than are their rhizobia. The legume-*Rhizobium* symbioses and nodule formation on legumes are more sensitive to salt or osmotic stress than are the rhizobia (Nogales et al., 2002; Zahran, 1991). Salt stress inhibits the initial steps of *Rhizobium*-legume symbioses.

In a previous study, soybean root hairs showed little curling or deformation when inoculated with *Bradyrhizobium japonicum* in the presence of 170 mM NaCl, and nodulation was completely suppressed by 210 mM NaCl (Katerji et al., 2003). The reduction of N fixing activity by salt stress is usually attributed to a reduction in respiration of the nodules (Moradi et al., 2011) and a reduction in cytosolic protein production, specifically legheamoglobin, by nodules (Garg and Singla, 2004). The depressive effect of salt stress on N fixation by legumes is directly related to the salt-induced decline in dry weight and N content in the shoot (Figueira, 2009). The salt-induced distortions in nodule structure could also be reasons for the decline in the N fixation rate by legumes subject to salt stress (Arora, 2013). Reduction in photosynthetic activity might also affect N fixation by legumes under salt stress (Garg and Singla, 2004). Although the root nodule-colonizing bacteria of the genera *Rhizobium* and *Bradyrhizobium* are more salt tolerant than their legume hosts, they show marked variation in salt tolerance.

Strains of *Rhizobium leguminosarum* have been reported to be tolerant to NaCl concentrations up to 350 mM NaCl in broth culture (Abdelmoumen et al., 1999; Zahran, 1991). Reports have shown that soybean and chickpea rhizobia are tolerant to 340 mM NaCl, with fast-growing strains being more tolerant than slow growing strains (Elsheikh and Wood, 1990). It has been found recently that the slow growing peanut rhizobia are less tolerant than fast-growing rhizobia (Kang et al., 2011).

2.4.10 Nitrogen

Nitrogen added to the soil through fertilization can be a limiting factor that affects the symbiotic relationship between rhizobia and their hosts (Sparks, 2002). The capacity for N fixation by a nodulating legume is influenced at least in two ways by mineral nitrogen in the soil where it is grown. Firstly, the process of nodulation may be promoted by relatively low level of available nitrate or ammonia, higher concentrations of which almost always depress it (Maheshwari, 2008). Secondly, the rate of N_2 fixation by an active, growing and well nodulated legume is always suppressed by nitrate ions (Tow and Lazenby, 2001). Nitrogen fertilizers added to soil may therefore delay the symbiotic process through decreased multiplication of free-living rhizobia (Gage, 2004). Studies have shown that N fertilizers inhibit root hair infection, nodule initiation, growth development, nitrogenase activity and promote premature nodule senescence (Kiers et al., 2006; Liu et al., 2010; Watanabe et al., 2014). Nodulation is reduced or eliminated when the soils have high supplies of ammonium and nitrate (Nahed-Toral et al., 2013).

2.4.11 Phosphorus

Phosphorus (P) is essential macronutrient for plant growth and function. P deficiency is a major constraint of effective N fixation because phosphorus is an important nutrient in the process of nodulation and nitrogen fixation (World Bank, 2006). The high requirement for P in legumes is consistent with the involvement of P in the high rates of energy transfer that must take place in the nodule (White et al., 2007). The more the supply of phosphorus, the more abundant are the nodules (Gowariker et al., 2009). The requirements of host plants for optimal growth and symbiotic dinitrogen fixation processes for P have been assessed by determination of nodule development and functioning (Maheshwari, 2010). The influence of P on symbiotic nitrogen fixation in leguminous plants has received considerable attention, but its role in the process remains unclear (Kouas et al., 2005).

Other studies have shown that P nutrition increases symbiotic dinitrogen fixation in subterranean clover by stimulating host plant growth rather than by exerting specific effects on rhizobial growth or on nodule formation (Robson et al., 1981). The increase of whole plant growth and plant nitrogen concentration in response to increased soil P supply has been noted for several leguminous species including soybean (Bargaz et al., 2011; Israel, 1993; Kouas et al., 2005). Decreased specific nitrogenase activity in nodules of P deficient soybean plants has been associated with decreased energy status of host plant cells of nodules (Cabeza et al., 2014).

2.5 Density of rhizobial population in soils

Population size of indigenous rhizobia determines success of inoculation of legumes which is critical when compatible rhizobia are absent, population densities are low, or when native rhizobia are not effective (Ben Rebah et al., 2007; Brockwell and Bottomley, 1995; Catroux et al., 2001; Giller and Cadisch, 1995). The main source of rhizobia inocula is the soil with great variations in the population size from soil to soil and across regions (Thrall et al., 2011).

The main factors that determine the presence of rhizobia include the presence of appropriate host, soil acidity, seasonal changes and soil texture (Abaidoo et al., 2007; Rupela et al., 1987; Thies et al., 1991). It has been reported that rhizobia survive in soil between symbiotic phases as saprophytes, and their populations depend on many physical and chemical properties of the soil environment and on the frequency of planting legumes in a given area or field (Jarecki and Bobrecka-Jamro, 2012; Martinez-Romero, 2006). A study on 305 soil samples from 17 countries by the International Centre for Nitrogen Fixation by Tropical Legumes showed that while rhizobia were common at a large number of sites, their populations were extremely variable and ranged from zero to >1000 cells g^{-1} soil (Galloway et al., 2004; Peoples et al., 1995). These authors further stated that more than 50% of the soils had fewer than 100 rhizobia g^{-1} soil. These findings suggest that tropical soils do not have sufficient numbers of resident rhizobia to meet legume N demand.

In Zimbabwe population levels of rhizobia ranged between zero and 100 cells of cowpea rhizobia per gram of soil (Zapata and Hera, 1996). Similar low population sizes have been reported for cowpea and groundnut in Egypt (Mpepereki and Wollum, 1991; Van Berkum et al., 2012).

Soils lacking in rhizobia have been reported to occur in areas where indigenous related legumes are absent or where levels of pH, osmotic stress, temperatures and heavy metals are detrimental to rhizobial populations (Catroux et al., 2001). Different studies have pegged the response of indigenous soil rhizobia at varying population levels (Giller, 2001; Thies et al., 1991). However it is common to observe negative response in the presence of high population densities of rhizobia and high nodulation.

Since high specificity exists between *Rhizobium* strains and their host plants, for successful nodulation and fixation, it is essential that the two be compatible. Host legumes can enrich their immediate soil environment with rhizobia through rhizosphere effects (Graham, 2007; Thies et al., 1995). Failure of inoculation to elicit response in legumes is a common phenomenon in Kenyan soils (Mungai and Karubiu, 2011). This could be due to the presence of effective indigenous rhizobia or highly competitive but inefficient indigenous strains that lock out the inoculant strains from occupying the nodules. An understanding of rhizobial ecology requires their enumeration in natural habitats, and the relationship between rhizobial numbers and legume nodulation response is of considerable practical importance for legume inoculation.

2.6 Methods of studying rhizobial diversity and phylogenetics

Generally, numerical taxonomy comprising biochemical, nutritional and serological characterization of the rhizobia together with the host ranges was used as the main method for classification and identification of root-nodulating bacteria until the molecular era. The rhizobial taxonomy has been improved considerably after the development of molecular techniques, such as sequencing of the 16S rRNA gene (Woese, 1987). Molecular genetic markers in general provide better information, are more sensitive and relatively more accurate to study the relationship of closely related bacterial strains and in detection of higher rhizobial diversity than the phenotypic techniques. Phenotypic features such as colony morphology, and physiological or biochemical responses of a bacterium may vary depending on the media and conditions used for growing it. Nevertheless, each of the molecular markers and techniques has also its own limitation in determining the diversity and phylogeny of bacteria (Pontes et al., 2007). Some of these methods are described in this section.

2.6.1 Phenotypic, cultural and metabolic methods

There are a wide range of morphological, cultural and metabolic properties that have been used in the characterization and identification of rhizobia. The phenotypic characteristics that are routinely used for this purpose are growth rate, colony characteristics on yeast extract mannitol agar (YEMA) media, utilization of carbon and nitrogen substrates as sole sources of nutrition (Maatallah et al., 2002).

Different laboratories also use additional methods to characterize rhizobia including cell protein banding pattern, multilocus enzyme electrophoresis and tolerance to stress, acidity, salinity, heavy metals and high temperatures (Bala and Giller, 2007). These set of tests by which the root nodule bacteria could be characterized was suggested as a way to resolving the taxonomic difficulties within the genus *Rhizobium* (Lange, 1961). However the methods were later considered to be impracticable (Graham and Parker, 1964). Despite this criticism, cultural and metabolic parameters are still used for phenotypic characterization and are frequently carried out in combination with other techniques. The use of phenotypic characteristics provides the primary basis for rhizobial species classification (Dlodlo, 2012).

2.6.2 Cross inoculation concept

Cross inoculation concept is based on the ability of *Rhizobium* strains to specifically nodulate a group of legume host species (Giller, 2001). In this concept, rhizobial strains have long been described as being specific when they are restricted in their host range or promiscuous when they have a very broad host range (Provorov et al., 2013). However, a wide range of legume species has shown that many legumes are nodulated by rhizobia outside their own groups (Bationo, 2007). Promiscuity of some rhizobial strains has also been found to be so broad that it goes beyond closely related legumes, to include legumes that are so distantly related as to be placed in different sub-families within the family Leguminosae (Gnanamanickam, 2007). Cross inoculation concept like other earlier methods for characterizing *Rhizobium* strains pays no attention to nitrogen fixation abilities.

It is thus common to find strains of rhizobia that can form nodules on say 10 different legume host species and yet in association with perhaps five of those host plants fix nitrogen only weakly or not at all (Perotto and Baluška, 2011). Due to these setbacks associated with the cross inoculation concept, its use in the classification of rhizobia has reduced. Continued use of this method by some scientists can only be justified on the basis of convenience and agronomic significance (Velázquez and Rodriguez-Barrueco, 2007). The concept actually has some practical use for selecting rhizobial strains which have the potential to be used as inoculants for particular legume crops (Amarger, 2001).

2.6.3 Serological method

The method is based on the principle of antigen- antibody reaction to study rhizobial diversity. Serological studies of indigenous rhizobia have revealed considerable diversity within and among geographic locations. In some instances it has been possible to correlate the presence of particular serogroups within a restricted region to soil properties such as pH or total nitrogen content (Dommergues, 2012; Pongslip, 2012). Apart from using serology to study rhizobial diversity, the practical importance of serology is to identify groups that have practical importance to the management of symbiosis. Although many studies have documented serological diversity within rhizobial populations, relatively few have assessed the value of the resulting groupings in predicting symbiotic performance. The main weakness of using serology to characterize rhizobia is the presence of strains that do not react with all antisera tested and the frequency of non-reactive strains is often significant (Zhang et al., 2014). Cases of cross-reaction of strains with antiserum derived from reference strains are also common.

2.6.4 Antibiotic resistance method

Environmental studies of micro-organisms in their natural habitat require the recovery of either the resident population or added cell on a selective medium to exclude contaminants. The lack of a suitable medium that allows the selective, recovery of rhizobia in soil has hindered studies on the behavior of rhizobia in soil (Dommergues, 2012). Rhizobia like other bacteria contain small numbers of naturally occurring mutants that are resistant to high concentrations of certain antibiotics.

The growth of antibiotic or toxin resistant mutants in media containing elevated levels of anti-microbial agents has been used for recognizing rhizobial strains in ecological studies and other bacteria (Spriggs and Dakora, 2009). Repeated culturing on YEMA plates containing these antibiotics marks target rhizobial strains with resistant strains retaining their N_2 fixing capabilities. Antibiotic-resistant marked strains of rhizobia may be identified by their ability to grow on media containing the antibiotics to which they are resistant, while non-marked ones are unable to grow. The antibiotic marker technique is applied in ecological studies where strain identification is not possible by serology due to cross reaction of strains or because of unavailability of antisera. The popularity of the technique is due to the ease with which the mutants, in particular those resistant to streptomycin have been obtained (Baldani et al., 2014).

2.6.5 16S rRNA gene sequence

The ribosomal ribonucleic acid (rRNA) gene is considered as a model genetic marker for studying the evolutionary history of life. Comparison of the rRNA sequences is used for construction of the "universal tree of life", the tree which divides all organisms on earth into three main domains; *Eukarya, Bacteria* and *Archaea* (Woese et al., 1990). Bacteria contain genes coding for 5S, 16S, and 23S rRNAs and 16S–23S rRNA internal transcribed spacer (ITS) regions, in which all are typically structured into a segment of genome, called rRNA operon. The rRNAs are vital components of ribosomes that are involved in translation of messenger RNA and protein synthesis (Acinas et al., 2004). Predominantly, the 16S rRNA gene sequence has been used as a standard genetic marker in identification and taxonomic classification of prokaryotes including rhizobia (Harris and Hartley, 2003).

This marker has several properties that make it suitable for phylogenetic inference. Firstly, 16S rRNA is universally found in all living organisms, this enables the comparison of the phylogenetic relationship of all organisms and allows the construction of a tree of life. Secondly, it is relatively highly conserved, thus the function of this gene over time has not changed, indicating that random sequence variations that may occur among organisms are more exact measure of evolution. Thirdly, the bacterial 16S rRNA gene sequence is a long stretch (about 1500 bp) that includes both conserved and variable regions, which can offer enough information for taxonomic purpose.

Nine “hyper-variable” regions (labeled as V1 to V9) that are flanked by conserved stretches exhibit significant sequence variability among different bacterial species and can be used for bacterial phylogenetic studies (Aserse et al., 2012).

Conserved regions are used in designing universal primers for PCR amplification of 16S rRNA gene and these also allow alignment of sequences of distantly related organisms (Chakravorty et al., 2007). Due to these benefits, 16S rRNA gene sequence has been used extensively as a main criterion for phylogenetic classification of prokaryotes. In general, 97% 16S rRNA gene sequence similarity has been considered as a threshold for species delineation (Stackebrandt and Goebel, 1994). Subsequently, large numbers of 16S rRNA gene sequences have been deposited in the nucleotide databases. This facilitates the comparisons between different species and makes the 16S rRNA sequences further a choice of marker for identification and in building bacterial phylogenies (Woese et al., 1990).

2.6.6 DNA-DNA hybridization (DDH)

The DNA-DNA hybridization technique is a common genetic method used to study bacterial heterogeneity, speciation and taxonomy (Degefu et al., 2013). The DNA-DNA hybridization (DDH) method has been used in determining specific differences between closely related bacterial species. This technique has been used as a regular criterion for description of new bacterial species (Krieg, 1988).

It is a technique by which the entire sequence similarity of different organisms calculated from the pairwise whole genome comparisons (Rosselló-Mora, 2006). In practice DDH has three main steps: sheering of the genomic DNAs of test organism and reference strain, mixing the DNA of both strains and heating to dissociate the DNA double strands, and cooling the temperature down until fragments re-anneal.

The melting temperature value varies according to the base pair matching of the two strands and thereby giving a clue for genetic relatedness of the two strains (Auch et al., 2010). It is recommended that test bacterial strains to be considered as different species should show 70% or less DDH relatedness value from the references (Moore et al., 1987). Methods used for measuring the DDH values can be varied in different laboratories (Rosselló-Mora, 2006). Practically all the methods are based on the same principle but the DNA quality, concentration or tagging and washings steps may vary from one laboratory to the other. These differences can lead to prominent errors or give conflicting result (Rosselló-Mora, 2006).

An additional main weakness of this technique is that it is not possible to build comparative database since the method gives non-cumulative relative DNA similarity values (Rosselló-Mora, 2006). The DDH technique needs also a large amount and high quality DNA, technically challenging, time consuming and labor intensive. This technique is therefore limited to a few specialized laboratories and mostly applied if the bacteria under study are known to have closely related 16S rRNA gene sequences (Tindall et al., 2010).

Though it has several limitations, the DDH technique is still considered as the gold standard in delineating bacteria at species level. However, the rapid progress of sequencing techniques and with the ever decreasing of its costs, most likely the DDH will be swapped by the more reproducible and accurate whole genome sequencing technique in the near future.

2.6.7 Whole genome sequencing

The complete genome sequencing is a molecular technique that involves sequencing of the entire chromosomal DNA of an organism as well as DNA found in the mitochondria (for higher organisms, eukaryotic), chloroplast (for plants) or plasmids (for single cell organisms, prokaryotic) at a single time. Recently, due to several whole genomes sequencing efforts, a large numbers of bacterial genome are sequenced and available in public nucleotide databases Whole genome sequencing provides the complete genetic variation of the organisms and the sequence data can be used in comparative genomic studies for identification and taxonomic purpose. Due to the sharp falling in the price of the technology, in the future many more bacteria including rhizobial complete genomes will be sequenced.

Nevertheless, at the moment sequencing the whole genome is far from applicable in many laboratories since its price is still expensive and it needs skilled personnel to analyze the sequence data. Consequently, the current phylogenetic and diversity studies are still based on nucleotide sequences of various genes and genetic fingerprints though these markers contain limited molecular information.

2.6.8 Housekeeping protein coding gene sequences

These genes are chromosomal in origin and are constitutively expressed in all cells under normal and patho-physiological conditions in order to maintain basic cellular functions of an organism. In order to overcome the limitations of rRNA genes and DNA–DNA hybridization techniques, the sequence analysis of multiple protein coding genes, known as multilocus sequence analysis (MLSA), has been recently considered as a preferred method to study closely related species and to discriminate strains of the same species (Aserse, 2013). The housekeeping protein coding genes seem to be the most appropriate markers for phylogenetic analysis of bacteria. The genes have higher level of sequence divergence compared to the 16S rRNA gene but are conserved enough to retain genetic information, and therefore their sequences show better discrimination than 16S rRNA gene sequences. The protein coding genes are widely distributed, unique within a given genome, long enough and phylogenetically informative but short enough to be sequenced economically, that has acceptable accuracy in predicting the whole-genome relationships and that located separately in the chromosome of the genome are recommended in studying bacterial taxonomy (Zeigler, 2003).

2.6.9 Amplified fragment length polymorphism (AFLP) fingerprinting

AFLP is among the most widely used DNA fingerprinting methods since it was developed (Vos et al., 1995). The technique has been used in several applications including genetic diversity, phylogeny, and ecological studies of plants, animals, fungi and bacteria (Bensch and Åkesson, 2005).

AFLP fingerprints are produced by restriction of intact genomic DNA with restriction endonuclease and ligation of them with double strand oligonucleotide adapters. The restricted fragments are then selectively amplified and separated either using polyacrylamide gel electrophoresis or more commonly with capillary electrophoresis using automatic sequencer. Usually one or two of the PCR primers are fluorescently labeled in order to produce labeled AFLP-fragments that can be detected and separated according to their size by automatic sequencer. In this case, once the fragment passes in the capillary electrophoresis of the sequencer, the primer that is labeled with fluorescent dye provides signal and the differently sized fragments are then converted to peaks on the computer software. The peaks are equivalent to the bands on silver stained polyacrylamide gel, which can also be employed to separate the AFLP-fragments based on their size in an electrical field. In comparison to the polyacrylamide gel electrophoresis, the capillary electrophoresis provides more resolute fingerprint pictures and the data are better manageable.

In addition, the silver staining in the polyacrylamide gel electrophoresis is time consuming and demanding. Therefore, the capillary electrophoresis has been used as the preferred method for separation of AFLP fragments (Terefework et al., 2001). With the AFLP technique, multilocus fingerprints representing the whole genome of an organism can be produced with a limited number of primer combinations. As a result, this method can be used to assess both core and accessory genes that are biologically and ecologically important for the organisms.

This indicates that the technique helps in studying the species from ecological species points of view and to identify bacterial strains that may be adapted to the same ecological niches or geographic origins (Cohan, 2001). The great number of polymorphic bands that are produced with the AFLP method shows different genetic fingerprints and provide excellent power in resolving differences between very closely related strains compared to other techniques (Boudon et al., 2005). In comparison to other molecular methods, AFLP is reported to have higher discriminatory power and to show better intraspecific genetic diversity between *Pseudomonas* species (Clerc et al., 1998). Other studies have shown that AFLP clustering present much more diversity between *Bradyrhizobium* species compared to 16S-23S rRNA intergenic sequence (ITS) and *recA* phylogenies, though all the three methods showed consistent grouping between the species (Gueye et al., 2009). Generally, the AFLP method has been comprehensively used in genetic diversity studies of root nodule bacteria and to confirm if rhizobial strains in the same species are genetically identical or different (Li et al., 2012).

The AFLP technique has also been applied in studying the taxonomic relationship of various groups of bacterial species (Costechareyre et al., 2010). However several restriction enzymes might be needed in order to produce composite datasets for comparison and for clear similarity or difference among the taxa (Terefework et al., 2001). The technique has been reported to have similar discriminating power as DNA-DNA hybridization and therefore can be used as an alternative to the DNA- DNA hybridization for identifying bacterial species (Costechareyre et al., 2010).

AFLP is used to effectively delineate the genomic species of the genus *Agrobacterium* that were previously classified by DNA-DNA hybridization relatedness (Costechareyre et al., 2010). Recently, the AFLP method has also been applied in studying the phylogenetic structure in identifying genospecies of the genus *Frankia* (Aserse, 2013).

2.6.10 Restriction fragment length polymorphism (RFLP)

In RFLP technique, DNA purified from rhizobial isolates is cut with a restriction endonuclease (often *Eco*R1 or *Hin*dIII). The variation (polymorphism) in the length of resulting DNA fragments is visualized by running the DNA fragments on an electrophoretic gel and staining the gel with ethidium bromide followed by illumination under UV light. These polymorphisms are then used to differentiate between rhizobial isolates. If chromosomal DNA is cut with restriction endonucleases then visualization of fragment polymorphism is difficult because of the large number of fragments generated. RFLP is rarely used on its own for diversity studies. It is most commonly used in conjunction with Southern blotting followed by nucleic acid hybridisation with an oligonucleotide or gene probe or in the restriction digestion of amplified ribosomal genes.

2.6.11 Metagenomics: Application of Genomics to Uncultured Microorganisms

Metagenomics is the genomic analysis of microorganisms by direct extraction and cloning of DNA from an assemblage of microorganisms (Handelsman, 2004). The development of metagenomics stemmed from the ineluctable evidence that as-yet-uncultured microorganisms represent the vast majority of organisms in most environments on earth. This evidence was derived from analyses of 16S rRNA gene sequences amplified directly from the environment, an approach that avoided the bias imposed by culturing and led to the discovery of vast new lineages of microbial life (Huson et al., 2007). Although the portrait of the microbial world was revolutionized by analysis of 16S rRNA genes, such studies yielded only a phylogenetic description of community membership, providing little insight into the genetics, physiology, and biochemistry of the members. Metagenomics provides a second tier of technical innovation that facilitates study of the physiology and ecology of environmental microorganisms. Novel genes and gene products discovered through metagenomics include the first bacteriorhodopsin of bacterial origin; novel small molecules with antimicrobial activity; and new members of families of known proteins, such as an $Na^+(Li^+)/H^+$ antiporter, RecA, DNA polymerase, and antibiotic resistance determinants (Schleper et al., 2005). Reassembly of multiple genomes has provided insight into energy and nutrient cycling within the community, genome structure, gene function, population genetics and microheterogeneity, and lateral gene transfer among members of an uncultured community (DeLong et al., 2006). The application of metagenomic sequence information will facilitate the design of better culturing strategies to link genomic analysis with pure culture studies.

2.6.12 Ribotyping

Ribotyping makes use of differences in the chromosomal positions or structure of rRNA genes to identify or group isolates of a particular genus or species (Laguerre et al., 2001). Ribotyping has been shown to be reproducible and hence has gained popularity for strain fingerprinting (Nicolas-Chanoine et al., 2008). The most frequently used ribotyping method is to identify RFLPs of rRNA genes by probing a Southern transfer of restricted genomic DNA (Bouchet et al., 2008). Bouchet et al. (2008) used the technique to characterise isolates of non nodulating, Gram-negative soil bacteria that gained the capacity to nodulate clover once they were crossed with *E. coli* strain PN200, which contains the cointegrative plasmid pPN1. Ribotyping combined with DNA-DNA hybridisation and partial 16S rRNA sequencing enabled them to classify these isolates of soil bacteria into 5 different species of *Rhizobium*. In this way, Bouchet et al. (2008) demonstrated that *nod* – rhizobia bacteria are an integral part of the soil microbial community regardless of either the presence of a compatible host legume or the capacity of the bacteria to nodulate it. Similarly, Laguerre et al. (2001) described non-symbiotic *R. etli* isolates that outnumber *R. etli* symbiotic bacteria in the field.

2.6.13 Single nucleotide polymorphism (SNPS)

Single-nucleotide polymorphisms (SNPs) have become of standard use in genetics and in model organisms and are particularly relevant genetic markers for evolutionary and conservation genetic analyses (Dereeper et al., 2011). Interest in SNP markers in evolutionary genetics comes primarily from their abundance and widespread distribution throughout the genome (Deschamps and Campbell, 2010). SNPs are usually biallelic so that more SNP markers are required compared to other molecular markers, especially microsatellites (Hauser et al., 2011). The binary nature makes genetic variation easy to score and comparisons of patterns of variation between markers and populations straightforward.

SNPs are easily amenable to automation, and during the last decade, many medium- to high-throughput SNP genotyping methods have been developed (Gupta et al., 2008). The choice between all these technologies depends strongly on the number of samples and markers to be analyzed. As SNPs occur both in coding and noncoding regions it is possible to build sets of SNPs containing 'anonymous' SNPs and 'candidate' SNPs (Brumfield et al., 2003). The availability of both categories is particularly relevant for candidate gene association studies and to look for footprints of selection. Anonymous SNPs can be used to capture a genome-wide picture of the demographic history of the studied sample. This in turn constitutes a control/reference to detect abnormal patterns of diversity on candidate SNPs reflecting a potential response to specific constraints and especially selection (Shen et al., 2014).

Whole-genome resequencing or SNPs arrays of very high density (>50 000 SNPs) are needed for genome-wide association studies (GWAS).

However, given the current cost of such kind of analysis, smaller sets of SNPs allowing to genotype large samples may be very valuable tools for the survey and/or the monitoring of natural populations and the study of patterns of local adaptation. One important complication when using SNPs to study the demographic and selective history of a population or species is the potential occurrence of a bias towards analyzing only the most variable genomic regions. This artefact, known as 'ascertainment bias', may arise when the discovery panel is too small and/or unrepresentative of the assayed sample and is of special concern for biallelic markers such as SNPs (Clark et al., 2005). Ascertainment bias affects the site frequency spectrum (SFS) of a population sample, that is, the distribution of allele frequencies of polymorphic sites) and may lead to false evolutionary inferences (Ewens, 1972).

2.7 Breeding for enhanced nitrogen fixation in crop legumes

The concept that legume N fixation could be enhanced through selection and breeding has been around for decades (Nutman, 1984). Previous studies showed that there was little understanding of how N fixation breeding programs might be undertaken and that the basic genetic information necessary for understanding the expression of desired traits was largely absent (Graham and Temple, 1984). Intensive research during the past 25 to 30 years has successfully addressed many of the challenges associated with legume breeding. Some, such as dissecting carbon and nitrogen allocation within the legume plant in relation to nodule function, were apparently useful, whilst others were clearly more critical (Herridge and Rose, 2000).

There was a need to develop alternative strategies for genetically improving legume N fixation and to clearly define protocols for selection and breeding. N fixation activity of individual plants and plant populations had to be quantified in a rapid, simple and cost effective manner.

2.8 Strategies for breeding legumes with enhanced N fixation

2.8.1 Legume biomass and seed yield

Maximizing legume (biomass) and seed yield within the constraints imposed by agronomic management and the environment. This is because larger biomass crops require more N and therefore N fixation will be increased as biomass yield is increased. The approach assumes a capacity for N fixation sufficient to satisfy increased N demand of larger plants. It has particular application to lower-yielding species such as common bean, lentil (*Lens culinaris*), mung bean (*Vigna radiata*) and chickpea (*Cicer arietinum*), but less relevance for the larger, vigourously growing species like fababean (*Vicia faba*), pea (*Pisum sativum*) and soybean (Attewell and Bliss, 1985).

2.8.2 Symbiotic nitrate tolerance

Enhancing symbiotic nitrate tolerance and the ability of the legume to nodulate and fix N in the presence of soil nitrate. This strategy may not result in greater yields (either biomass or grain). Rather, the principal impact of symbiotic nitrate tolerance is to increase the percentage of crop N derived from N fixation (Pfix) and therefore total N fixed by the legume.

This should lead in improved residual benefits of the legume on soil N fertility. Variation for symbiotic nitrate tolerance exists in natural plant populations and has also been created through plant mutagenesis (Park and Buttery, 1988). It may be impossible and even undesirable, to produce a legume solely dependent upon N for growth. There is scope, however, to improve the levels of symbiotic tolerance to nitrate for the most commonly grown crop legumes.

2.8.3 Legume nodulation through specific nodulation traits

Optimizing legume nodulation through specific nodulation traits (mass and duration), and depending on the circumstances, for promiscuous or selective nodulation (Cregan and Keyser, 1986). Continued improvements in the effectiveness of legume inoculants and the matching of strains with host genotypes should also be sought. There appears to be scope to select rhizobia for specific environmental niches such as acid tolerant strains for acidic soils (Hungria and Vargas, 2000).

2.9 Soil fertility and legume nitrogen requirements

The continued decline in soil fertility and high fertilizer costs are major limitations to crop production in smallholder farms in Kenya (Chemining'wa et al., 2004). The problem has been augmented by intensification of agriculture coupled with the reduction in farm sizes (Otieno et al., 2007; Saha and Muli, 2000).

Among the major nutrients, requirements for nitrogen (N) exceed any other and rarely do soils in the tropics have enough of this nutrient to produce high sustainable yields (Akter et al., 2013; Mourice and Tryphone, 2012). This lack of adequate amounts of nitrogen in most soils puts a limitation on the farmers' goals of increasing yield per unit area. Rebuilding soil fertility in traditional agricultural systems has been achieved through long-duration fallow periods (Kekeunou et al., 2006; Poubom et al., 2005). However, with increased human population and land pressure, long fallow periods are no longer feasible (Miriti et al., 2007; Poubom et al., 2005).

There are several options which are available to manage nitrogen in farmers' fields with chemical fertilizers often considered to be an immediate answer to current nutrient deficiencies in soils (Bationo, 2004; Chemining'wa et al., 2004). The quantity of nitrogen fertilizer needed for agriculture is projected to increase in the period to 2030 (Tillman, 1999) and this is likely to lead to greater environmental degradation (Lal and Stewart, 2013; Tilman, 1999). Reduced dependence on N fertilizer and adopting farming practices that favour the more economically viable and environmentally prudent BNF will therefore benefit both agriculture and the environment (Vance, 2001). Apart from symbiotic BNF that is already described, N requirement of legumes can also be met through mineral N assimilation and incorporation of organic materials (George and Singleton, 1992; Salvagiotti et al., 2008).

2.9.1 Organic nitrogen sources

Traditionally, organic inputs were used to replenish soil fertility but later emphasis shifted to the use of mineral fertilizers (Odhong' et al., 2014; Opala, 2011). However, due to increasing costs of chemical fertilizers and concerns for sustainability, there is renewed interest on the use of organic materials such as animal manures, green manures, composts and crop residues to improve soil fertility (Bationo, 2004). Organic materials are derived from plant and animal droppings such as weed residues, tree prunings, urine, green manure, farmyard manure and crop residues. However, their availability as nutrient sources is limited by their alternative uses as fuel, feed and fiber. In addition, the labour required to collect and process these materials affects their utilization (Jama et al., 1997).

Recent research efforts have therefore focused on increasing the generation of non-traditional organic resources using agroforestry interventions such as improved fallows and biomass transfers (Opala, 2011) and water hyacinth (Woomer, 1997; Woomer et al., 2000) to increase the amount of nutrients supplied by organic inputs. These sources have been recognized as alternative nutrient sources to resource poor smallholder farmers (Bala et al., 2011). The organic materials improve the soil fertility, promote good soil aggregation, improve moisture infiltration and increase the water holding capacity of the soil, increase the soil organic carbon, soil available nutrients (Bala et al., 2011; Mucheru-Muna et al., 2010).

Additionally, they have the ability to increase the P availability of the already present P by rendering it more accessible to crops through reducing the soil P absorption capacity, increasing the pH by decreasing the exchangeable acidity and aluminum in soil solution through chelation, and increasing the soil biological activity (Bationo et al., 2007; Mukuralinda et al., 2010).There are however some challenges in the use of OMs to manage acid soils and replenish soil fertility (Kisinyo et al., 2014; Nziguheba, 2007). The quantities and qualities of organic materials available to farmers are limiting factors to their use in Kenya (Kisinyo et al., 2014; Opala, 2011). Due to their low nutrient content, large amounts have to be applied thus increasing the labour cost (Jama and Pizarro, 2008). The high costs in some cases cannot be offset by the extra yields obtained by applying some of the organic materials such as tithonia (Opala et al., 2007), calliandra and maize stover (Nyambati and Opala, 2014). However, OMs such as FYM of high quality have in most cases been shown to be economically attractive under most smallholder situations (Forster et al., 2013; Opala et al., 2007). This shows the need for high quality OMs as sources of nutrients in acid soils.

2.9.2 Utilization of water hyacinth as a resource in agriculture

The water hyacinth is a free floating aquatic weed originated in the Amazon in South America (Bolenz et al., 1990) where it was kept under control by natural predators (Lee, 1979). The plant has, through introduction by man, spread throughout the whole tropical zone (Aweke, 1993). Due to its fast growth and the robustness of its seeds, the water hyacinth has since then caused major problems in the whole area.

For example, a reduction of fish has been reported among East African countries particularly in Kenya (Kateregga and Sterner, 2009). Other reported effects are physical interference with fishing, obstruction of shipping routes and losses of water in irrigation systems due to higher evaporation and interference with hydroelectric schemes and increased sedimentation by trapping silt particles (Villamagna and Murph, 2009). It also restricts the possibilities of fishing from the shore with baskets or lines and can cause hygienic problems (Aweke, 1993; Tham and Udén, 2013).

On health aspects, the increased growth rate of the water hyacinths has led to worsened health conditions for the people living in the affected areas. The floating water hyacinth mats can serve as a breeding ground for vector organisms carrying malaria, bilharzias and river blindness (Abdelhamid and Gabr, 1991). At some places precautions against water snakes, hippos and crocodiles need to be taken. The water hyacinths consume so much oxygen when decaying that it leads to less oxygen remaining in these waters. The decreased oxygen content in the water leads to less oxygen for the fish (Gunnarsson and Petersen, 2007). This combined with fewer algae and other food sources for the fish, cause the meat of the fish to go bad faster than before. Decreased possibility to store fish leads to lower income and food security (Gunnarsson and Petersen, 2007). This means that decreasing the amount of water hyacinths could hopefully improve the health situation.

Despite the problems associated with the weed, it has been recognized to have significant effects on sustainability of soil nutrient resources and yield of agricultural produce (Oyetunji et al., 2003). Composting of water hyacinth readily degrades organic matter in the substrate and gradually turns it into less increasingly degradable humic material (Epstein, 2011; Schaub and Leonard, 1996).

However, if the compost product is not mature enough, it may contain metabolites that are toxic to plants (Gunnarsson and Petersen, 2007). The major nutrients that are important for the fertilizing qualities of the compost are nitrogen (N), phosphorus (P) and potassium (K). The oxidation of ammonia form of nitrogen in the compost process ultimately yields nitrate, which is not normally lost from the compost pile (Edwards, 1980). Since phosphorus and potassium are physico-chemically less mobile than nitrogen, these compounds remain in the compost unless lost through leaching

The other option is to use the water hyacinths directly as green manure. Green manuring involves spreading plant material on the fields and incorporating into the soil (Bending et al., 2004). The chemical analyses indicate a high nutrient content of the water hyacinth, 20% crude protein (Lu et al., 2008), but values as low as 7.26% have been reported (Wu and Sun, 2010). However drying the water hyacinths before spreading them on the fields ought to be done primarily to minimize the risk of bilharzia and secondly to decrease the labour required for transportation (Gunnarsson and Petersen, 2007).

The N in water hyacinth leaves and stems is 3.7% and 2.7%, respectively (Gunnarsson and Petersen, 2007). The water hyacinth would, therefore, have a great potential for increasing crop yields if incorporated into agricultural practice either as organic compost or green manure.

2.9 .3 Inorganic nitrogen sources

Inorganic fertilizers have a high concentration of nutrients that are readily available for plant uptake and they can be formulated to supply the appropriate ratio of nutrients to meet plant growth requirements. Most farmers are aware that without inorganic fertilizers the productivity of their crops and pastures will drop and soil nutrient levels will decline rapidly (Waswa et al., 2007). Currently, there is a wide range of inorganic N fertilizers that are required to maintain soil fertility and sustainable agricultural systems including urea. Urea is a nitrogen fertilizer which belongs to amides chemical family. Its molecular formula is presented as $CO(NH_2)_2$. Its active ingredient is 46% N (Khan et al., 2007). Nitrogen in it becomes available for plants uptake when it is converted to ammonium (NH_4^+) and nitrate (NO_3^-). The fertilizer can be applied as top dressing, starter and broadcast in different field crops. However, it has high potential to caking if not properly managed in store and easily volatilized in the specified field conditions; if applied too close to the plants and seed it can cause crop and seedling damage (Verma et al., 2013). Proper application in annual crops is in the inter-row space as this allows better access of fertilizer by the roots and avoids contact with seeds and seedlings.

However its continuous use may lead to deterioration in soil chemical, physical, and biological properties, and soil health (Mahajan et al., 2008). In general contued use of inorganic fertilizers may contribute to eutrophication, which results in explosive growth of algae resulting in disruptive changes to biological equilibrium (Scheren et al., 2000). The negative impacts of chemical fertilizers, coupled with escalating prices, have led to growing interests in the use of organic fertilizers as a source of nutrients (Mahajan et al., 2008; Satyanarayana et al., 2002).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Site

The study was conducted at two sites; in Korando B (0^o 05´35″ S, 0^o 34^o 41.32´´E), Kisumu county and Masinde Muliro University of Science and Technology (MMUST) (0° 17′ 25.57″ N, 34° 45′ 50.02″), Kakamega county, selected based on agro-climatic conditions and prevalence of common bean cultivation. MMUST is located at an altitude of 1585 metres above sea level, within a high potential agro-ecological zone and has an annual rainfall of 1200-2100mm. Korando B is located at an altitude of 1300 meters above sea level and has an annual relief rainfall of 1200-1300 mm.

Soils at MMUST and Korando B are classified as Nitisols and Arenosols respectively (Jaetzold et al., 2009). The main crops grown in both areas include beans, maize, sorghum, finger millet, sweet potatoes, groundnuts and kales. Some of the initial soil properties at the sites are presented in Table 1.

Table 1: Initial surface (1-20 cm) soil properties in MMUST and Korando B

Soil property	MMUST	Kisumu
	Value	
pH	4.98	6.10
EC dS/m	0.7	0.2
% Nitrogen (N)	0.24	0.11
% Organic Carbon (OC)	2.66	1.32
Potassium (cmol/kg)	0.93	1.39
Sodium (cmol/ kg)	0.7	0.6
Magnesium (cmol/kg)	1.39	1.17
Calcium (cmol/kg)	3.08	3.02
Aluminum (cmol/kg)	2.56	0.62
Zinc (ppm)	7.6	9.8
Copper (ppm)	5.1	1.6
Iron (ppm)	10.1	22
Manganese (ppm)	75.2	38.3
Phosphorus (ppm)	28	35
Bulk density	1.07	1.42
Moisture	35.31	11.32
% Sand	15.68	71.84
% Silt	17.44	10.0
% Clay	66.88	18.16
Texture	Clay	Sandy Loam
Temperature	20.4 °C	22.9 °C

3.2 Soil sampling and analysis

Soil samples were taken from four farms at each site. This consisted of three farms with at least two seasons of growing common beans and one fallow farm that had never planted common bean. In Korando B, the sampled farms had beans, maize, maize-bean intercrop or fallow while in MMUST; soil was taken from maize, beans, napier and fallow land.

Using a 2.5 cm diameter soil probe, 20 soil cores were randomly collected from each farm to a depth of 15 cm and thoroughly mixed into a composite sample. Each sample was asceptically collected to avoid cross-contamination between soils from different sampling points. The soil samples were then divided in two; one part for the determination of rhizobia population while the other part was used for the chemical and physical analyses. Soil samples for chemical analysis were air-dried, passed through a 2 mm sieve and analyzed for pH, soil texture, density, organic carbon (% C), total N (% N), available P, exchangeable bases and Al using standard procedures at the College of Agriculture & Veterinary Sciences (CAVS), Upper Kabete Campus (Okalebo et al., 2002).

3.3 Determination of abundance of Indigenous Rhizobia in Soil

3.3.1 Assembly of the Leonard Jars and Rooting Media

The jars with plastic cups measuring 8 cm in diameter at the top tapered to the bottom diameter of 4 cm were assembled and used (Maingi et al., 2006). The cup was filled with vermiculite as the rooting media and inserted into a larger plastic vessel containing the nutrient solution. The larger vessel was filled with 800 ml of plant nutrient solution. A sponge connecting the upper and the lower units of the jar irrigated vermiculite with the nutrient solution.

The whole set up of the Leonard jar was insulated with a brown khaki bag. Vermiculite was used as the rooting medium in this experiment. The medium was washed thoroughly for three successive days by changing the water three times per day and stirring frequently and finally rinsed with distilled water and the pH of the medium adjusted to about 6.8. Water was drained off and the vermiculite packed into the small plastic cups of the Leonard jar assemblies and covered with aluminum foil to reduce contamination and entry of water. The whole set up was steamed twice for one hour in an autoclave to get rid of microorganisms.

3.3.2 Seed germination and greenhouse rhizobia trapping experiment

Greenhouse experiments were conducted at the department of Plant Science, Kenyatta University. The bean variety *Rose coco* was used as the trapping host. The seeds were purchased from the Kenya Seed Company Limited, Nairobi, Kenya and be pre-tested to determine the germination period and the information used to stagger pre-germination time of seeds to ensure synchronized germination among the seeds (Chemining'wa et al., 2012). Thereafter the seeds were surface sterilized in 3% sodium hypochlorite solution for 3 –5 minutes, then rinsed in 95% alcohol to remove waxy material on the surface and trapped air, followed by rinsing in at least 6 changes of sterile water. The seeds were then soaked in water in a refrigerator for four hours to imbibe water then placed in a germination chamber for pre-germination. After germination, two to three healthy seedlings were planted in every Leonard jar then inoculated with the diluted soil samples and later thinned to 1 plant per jar 7 days after emergence (Chemining'wa et al., 2012).

Soil inocula was prepared by suspending 10 g of soil sample in 90 ml of sterile water in a 160-ml dilution bottle and shaken for 20 min in a wrist-action shaker at room temperature (25ºC). One ml of each suspension was aseptically pipetted into 9 ml sterile water diluent in McCartney bottle and shaken for 2 min. The resulting suspension was serially-diluted tenfold from 10^{-1} to 10^{-6} with four replications at each dilution level.

An aliquot of 1 ml of diluent was then used to inoculate each seedling in the Leonard jars (Broughton and Dilworth, 1971). The seedlings were irrigated with sterile nitrogen free plant nutrient solution prepared (Beck et al., 1993). The nutrient consisted of 5 stock solutions containing in g/L: 0.1 $CaCl_2$, 0.12 $MgSO_4.7H_2O$, 0.1 KH_2PO_4, 0.15 $Na_2HPO_4.2H_2O$, 0.005 Ferric citrate and 1.0 ml of trace elements stock solution. The trace elements stock solution contained: 2.86 $H3BO_3$, 2.03 $MnSO_4.7H_2O$, 0.22 ZnSO4.$7H_2O$, 0.08 $CuSO_4.5H_2O$, and 0.14 $NaMoO_2$.2H2 O in g/L. The pH of the solution was adjusted to 6.8 using NaOH (1.0 M) or HCL (1.0 M). All solutions were sterilized by autoclaving at 121˚C for 15 minutes.

Checking of levels of nitrogen–free nutrient solution was done daily to ensure that the seedlings were adequately moistened. The plants in the Leonard jars were scored for the presence or absence of nodules 28 days after inoculation. The presence of a single nodule in a Leonard jar was considered a positive score. The most probable number technique was used to determine rhizobia cells per gram of dry soil (Somasegaran and Hoben, 1994).

3.4 Determination of symbiotic efficiency

3.4.1 Nodule sampling and isolation of rhizobia

Nodule sampling was done during the late flowering and early pod setting stages. A total of 40 representative flowering bean plants were carefully uprooted from farms in Korando B and MMUST and dug out 7 weeks after of germination. Fresh, red and large nodules were carefully removed from the roots. The nodules were surface sterilized in 1% NaOCl for 6 min, rinsed in several changes of sterile water, and then crushed with a flame-sterilized blunt-tipped pair of forceps. A loopful of the crushed nodule was streaked across the surface of Petri dish containing Yeast Extract Mannitol mineral salts agar (Vincent, 1985).

Single colonies were marked and checked for purity by repeated streaking on YEM agar medium and verifying a single type of colony morphology, absorption of Congo red (0.00125 mg kg-1) and a uniform Gram-stain reaction. Colony morphology characteristics such as color, mucosity, margin, transparency, elevation and acid/ alkaline reaction were evaluated on YEMA containing bromothymol blue (0.00125 mg kg^{-1}) as indicator. The pure rhizobial isolates were coded as KSM001, KSM002, KSM003, KSM004, KSM005, KSM006, KSM007, KSM008 and MMUST001, MMUST002, MMUST003, MMUST004, MMUST005, MMUST006 to represent Kisumu and Kakamega respectively. All the isolates were incubated at 28^0C and stored at -20 ^{0}C in 25% glycerol-YEM broth.

3.4.2 Authentication of the isolates

Each of the pure isolates (KSM001, KSM002, KSM003, KSM004, KSM005, KSM006, KSM007, KSM008, MMUST001, MMUST002, MMUST003, MMUST004, MMUST005 and MMUST006) was authenticated as root nodulating bacteria by re-inoculating 1 ml on the host plant grown in a controlled environment using growth pouches filled with sterilized vermiculite in Leonard jars (Somasegaran and Hoben 1994). The jars were arranged in a Randomized Complete Block Design (RCBD) with four replications. The plants were watered with Nitrogen-free nutrient solution. Treatments without inoculation and chemical nitrogen fertilizer served as negative control (NCTL) while treatments without inoculation but with nitrogen fertilizer applied at a rate of 70 μg N ml^{-1} as KNO_3 solution (PCTL) were used as positive control.

The isolates were also compared with commercial rhizobia strain 446 and CIAT 899 as references strains. After forty five days, plants shoot dry weight (SDW), number of nodules (NN) and nodules dry weight (NDW) were measured. SDW and NDW were determined from material dried at 65˚C for 24 to 48 hours (Argaw, 2012). Tissue N concentration per plant was analyzed using the Kjeldahl method (Niste et al., 2013) and the N content per plant calculated by multiplying the SDW with the tissue N concentration. Symbiotic efficiency (SE) was determined by comparing each of the native isolates with N applied control (plant N content in inoculated pots / plant N content in N application) × 100 (Beck et al., 1993).

3.5 Genetic characterization by 16S rRNA nucleotide sequencing of Rhizobia

3.5.1 DNA extraction

The rhizobia isolates were cultivated on YEM agar plates at 28˚C for 5-7 days. Single colonies of the isolates were picked and washed in 100 ul Tris-EDTA buffer (TE) at pH 7.5 to obtain pelleted cells (Sally et al., 2010). A volume of 250 ul of Cetyl Trimethylammonium Bromide (CTAB) buffer was added to the washed pelleted cells; vortexed for 30 seconds, and incubated at 65˚C for 15 minutes and then cooled to room temperature (Sally et al., 2010). Then 250 ul of 24:1 chloroform: isoamyl alcohol was added to the samples and vortexed until the solution was homogenous with the suspension appearing white in colour. The suspension was then centrifuge for 10 minutes at 12000 rpm using a fixed angle rotor. The aqueous phase was transferred to a new sterile 1.5 ml microcentrifuge tube and equal amounts of cold isopropanol added and mixed gently (Sally et al., 2010). The DNA was precipitated at -20˚C for 30 minutes and centrifuged for 10 minutes at 12,000 rpm. The DNA was re-suspended in 30 ul TE buffer at pH 7.4 and the concentration of the extracted DNA assessed at 260 nm using the Nanodrop Spectrophotometer (Sally et al., 2010).

3.5.2 Polymerase Chain Reaction (PCR), sequencing and sequence Analysis

PCR amplification was done using the primer pair fD1 (5-AGAGTTTGATCCTGGCTCAG-3) and rD1 (5-AAGGAGGTGATCCAGCC-3), an approximately 1500 bp product specific to nearly full length of 16S rRNA gene (Weisburg et al., 1991). The PCR reaction was performed in a 30 µl volume containing Taq polymerase (pre-mix), 14.4 µl PCR water, 0.3 µl each of the forward and reverse primers and 0.5 µg template of DNA. Amplifications were carried out as follows: an initial denaturation at 95°C for 3 min followed by 35 cycles of denaturation at 94°C for 1min, annealing at 55°C for 1 min, extension at 72°C for 2 min and a final extension at 72°C for 3 min (Gisèle et al., 1994; Hassen et al., 2014).

Amplicons were resolved on a 1.5% agarose (1 X TBE, 90mM Tris pH 8.0, 90mM boric acid, 2mM EDTA) gel, stained with SYBR green, and visualized with UV light. The amplicons were purified using a QIAquick PCR Purification kit (QIAGEN Inc, CA) following manufacturer's instructions, and the purified DNA samples were sequenced in both orientations using the ABI PRISM 377 DNA Sequencer (Applied Biosystems Inc, CA). Sequencing of the 16S PCR products was performed at Inqaba Biotech. (Pretoria, South Africa). The sequences were edited on Bioedit and Chomas Lite programs and aligned using MAFFT version 6 (http://mafft.cbrc.jp/alignment/server/) online program. After all gaps were treated by the pair wise deletion method, Neighbor joining (NJ) and Un-weighed Pair wise Group (UPGMA) phylogenetic trees were constructed using the Jukes Cantor and Kimura 2 models respectively.

The resulting sequences data were blast searched on the NCBI data library (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to establish the identity of the isolates. Phylogenetic analyses by the neighbor-joining method and a consensus tree were generated using Molecular Evolutionary Genetics Analysis (MEGA) software version 6.0 (Tamura et al., 2007).

3.6 Effect of organic and inorganic sources of nitrogen on the yield of common bean

The field experiments were carried out for two consecutive seasons: during the short rains in October to December, 2013 and long rains in April to August, 2014 at Masinde Muliro University of Science and Technology (MMUST), Kakamega and small holder farm in Kisumu. The two common bean cultivars used for this experiment were varieties *Rose coco* and *Yellow bean*. *Rose coco* variety is a commercial cultivar commonly grown along the Lake Victoria basin while the *Yellow bean* variety is farmer preferred local cultivar.

Soil samples from the fields were collected and analyzed for pH, organic carbon, total N, available P and the micronutrients using established procedures (Okalebo et al., 2002), before planting and at six weeks after planting. There were a total of 16 treatments arranged in a randomized complete block design (RCBD) with four replications as shown in Table 2. Triple Superphosphate (TSP) at a rate of 60 kg P ha^{-1} was applied to all treatments with no compost input to ensure that P was not limiting to bean yield while assessing the N effects.

Water hyacinth plants were manually harvested; sun dried and chopped into small pieces of about 5 cm and composted using above ground closed aerobic heap design for fifty days. Both urea and water hyacinth compost were applied to provide 100 kg N ha^{-1}. In addition, each of the bean cultivars was grown with no P or N application either with or without inoculation. The commercial Rhizobium strain 446 used was supplied by the MIRCEN project, University of Nairobi and applied at the rate of 100 g for 15 kg of seeds in the appropriate treatments. The moist seeds were mixed with the inoculant in the shade, sown immediately and covered with soil to minimize rhizobia exposure to the sun. All the nutrient inputs were applied at the time of planting. Two to three seeds were placed in the planting holes at the recommended inter-plot spacing of 40 cm × 15cm on a 2.4 m × 3 m plots and managed using recommended agronomic practices (Adama et al., 2008).

Table 2: Experimental Treatments

No.	Treatments
1	Rose Coco-Inoculated
2	Rose Coco-Non-inoculated
3	Rose Coco Triple Superphosphate - Inoculated
4	Rose Coco Triple Superphosphate - Noninoculated
5	Rose Coco Triple Superphosphate UREA- Inoculated
6	Rose Coco Triple Superphosphate UREA- Noninoculated
7	Rose Coco Water hyacinth compost - Inoculated
8	Rose Coco Water hyacinth compost – Noninoculated
9	Yellow bean- Inoculated
10	Yellow bean- Non-inoculated
11	Yellow bean Triple Superphosphate - Inoculated
12	Yellow bean Triple Superphosphate - Noninoculated
13	Yellow bean Triple Superphosphate UREA- Inoculated
14	Yellow bean Triple Superphosphate UREA- Noninoculated
15	Yellow bean Water hyacinth compost - Inoculated
16	Yellow bean Water hyacinth compost - Noninoculated

Four plants were randomly selected from each plot and dug out at 7 weeks after emergence and separated into shoots and roots. Soil was carefully washed from the roots. The nodules were picked from the roots and their numbers recorded for each plant for further use in diversity studies. The plant shoots were oven-dried at 70°C for 48 h for dry weight determination. At maturity, pods were harvested from each experimental plot, excluding the outer rows and the outer guard plants in each row, shelled and tagged for yield assessment. The grains were sun-dried until a constant weight was established. Yield parameters determined included the number of pods per plant and total grain yield. Seed yield per hectare was extrapolated from the seed yield per plot.

3.7 Data analysis

All data on root dry weight (RDW), SDW, tissue N concentration content per plant and SE from greenhouse experiments were subjected to analysis of variance (ANOVA) using General Linear Models Procedure of SAS software version 9.1. (SAS, 2003) and means separated using the Least Significance Differences of means (LSD) at $p < 0.05$. Pearson correlation coefficients were calculated to establish the associative relations among the agronomic traits of the test crop.

Molecular data was blast searched on the NCBI data library to establish the identity of the isolates. Phylogenetic analyses was done by the neighbor-joining method and a consensus tree of the isolates was generated using Molecular Evolutionary Genetics Analysis (MEGA) software version 6.0 (Tamura et al., 2007).

CHAPTER FOUR

RESULTS

4.1 Characteristics of the soil at the study sites

Initial soil chemical and physical characteristics at the experimental plots in MMUST and Korando B are presented in Table 1. The soil at MMUST was acidic (4.98) with a clay texture compared to sandy loamy soil in Korando B. The levels of Al and Cu were lower in Korando B than MMUST. The sandy loamy soil in Korando B was characterized by low clay (%), silt (%) and moisture. The clay soil in MMUST had lower sand (%) and bulk density. Results of the chemical analysis of the water hyacinth compost are shown in Table 3.

Table 3: Mean chemical composition of water hyacinth compost used in this study

Chemical property	Value
pH	8.37
% Nitrogen (N)	1.33
% Organic Carbon (OC)	12.23
Potassium (cmol/kg)	25
Sodium (cmol/kg)	2.1
Phosphorus (ppm)	280
Calcium (cmol/kg)	20.65
Magnesium (cmol/kg)	9.33
Zinc (ppm)	2.96
Iron (ppm)	1.29

4.2 Abundance of rhizobia in different land use systems

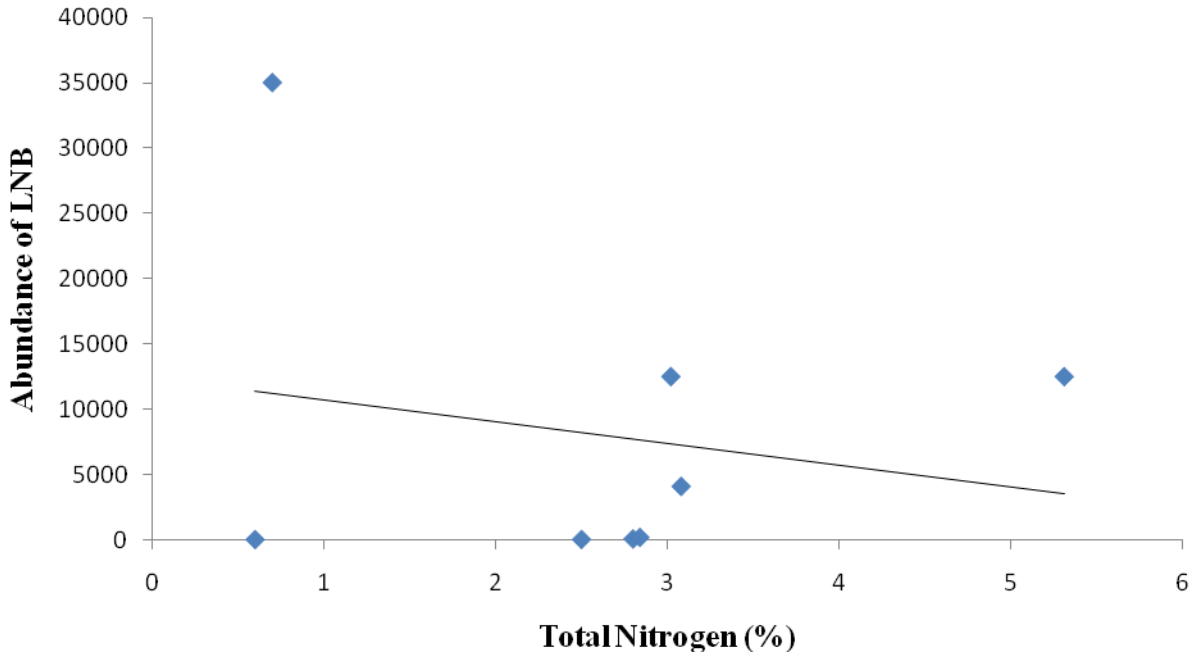
The soil fertility parameters varied in land use systems across the two study sites (Table 4). Farms with beans in MMUST and bean-maize intercrop in Kisumu had lowest soil pH. Total nitrogen in maize and napier in MMUST was higher than the other farms. Most of the farms in Kisumu were characterized by low N compared to MMUST. The levels of Aluminum (Al), Copper (Cu) and organic carbon (OC) in Kisumu were lower than MMUST. Maize farm in MMUST had higher levels Al compared to bean and fallow farms in Kisumu. Fallow soil had the least amount of organic carbon in MMUST but the highest in Kisumu. Potassium levels were generally higher in all the farms at the two study sites. The levels of available P were higher across all the farms except fallow soil in MMUST.

Maize-bean intercrop in Kisumu recorded the highest available P in all the farms. The general classification of soil texture in MMUST was clay and sandy loam in Kisumu. All the soils from Kisumu and MMUST farms had contained bacteria capable of nodulating common bean. Generally sandy loam soils of farms in Kisumu had higher population of native bacteria than clay soil in farms at MMUST. Maize farm in Kisumu harboured higher abundance of bacteria count than similar land use systems in MMUST. Higher N and Al in clay soils of MMUST farms recorded lower population of bacteria compared to the other farms. Farms with low OC, Al and Cu in Kisumu contained more bacteria. The effect of selected soil properties on the population of legume nodulating bacteria (LNB) are presented in Figure 2 and 3. The population of the bacteria was positively correlated ($r=0.78$) with soil P. Soil nitrogen, organic carbon and aluminium negatively correlated ($r=-0.54$, $r=-0.65$ and $r=-0.57$ respectively) with the bacteria population.

Table 4: Rhizobia population and soil characteristics in Kisumu and MMUST

Soil Properties /Landuse settings	MMUST farms				Korando B farms			
	Maize	Fallow	Bean	Napier	Bean	Maize	Fallow	Maize-Bean
MPN (No. rhizobia in gm^{-1} of soil)	7.8×10^{1}	1.98×10^{2}	4.102×10^{3}	3.9×10^{1}	1.25×10^{4}	3.5×10^{4}	3.2×10^{1}	1.25×10^{4}
pH (1:2.5 soil water ratio)	5.12	5.01	4.98	5.4	6.1	6.06	5.98	5.24
EC (dS/m)	0.3	0.7	0.7	0.4	0.2	0.2	0.4	0.4
Total N (%)	0.35	0.18	0.24	0.31	0.11	0.13	0.15	0.2
Organic Carbon (%)	2.6	1.58	2.66	2.8	1.32	1.15	1.78	1.39
K(cmol/ kg)	1.75	0.75	0.93	1.2	1.39	1.42	1.39	1.42
Na (cmol/ kg)	0.6	0.5	0.7	0.8	0.6	0.7	0.6	0.8
Mg (cmol/ kg)	1.19	1.01	1.39	1.53	1.17	1.4	1.08	1.77
Ca (cmol/ kg)	2.74	2.84	3.08	3.7	3.02	2.66	2.56	5.31
Al (cmol/ kg)	2.8	1.9	2.6	2.5	0.6	0.7	0.6	0.9
Zn (ppm)	7.2	4.5	7.6	20	9.8	6	6.2	1.7
Cu (ppm)	5.6	5.4	5.1	5.6	1.6	1.8	1.4	1.6
Fe (ppm)	16.1	13.3	10.1	24.6	22	12.5	20.4	43.2
Mn (ppm)	72.4	43.3	75.2	91.1	38.3	48.6	32.3	94.1
P (ppm)	24	6	28	35	35	62	11.8	15
Soil Texture	Clay	Clay	Clay	Clay	SL	SL	SL	SL

Note: SL-sandy loam
Land use systems: Maize farms, fallow land, bean farm, napier farm, maize bean intercrop farm



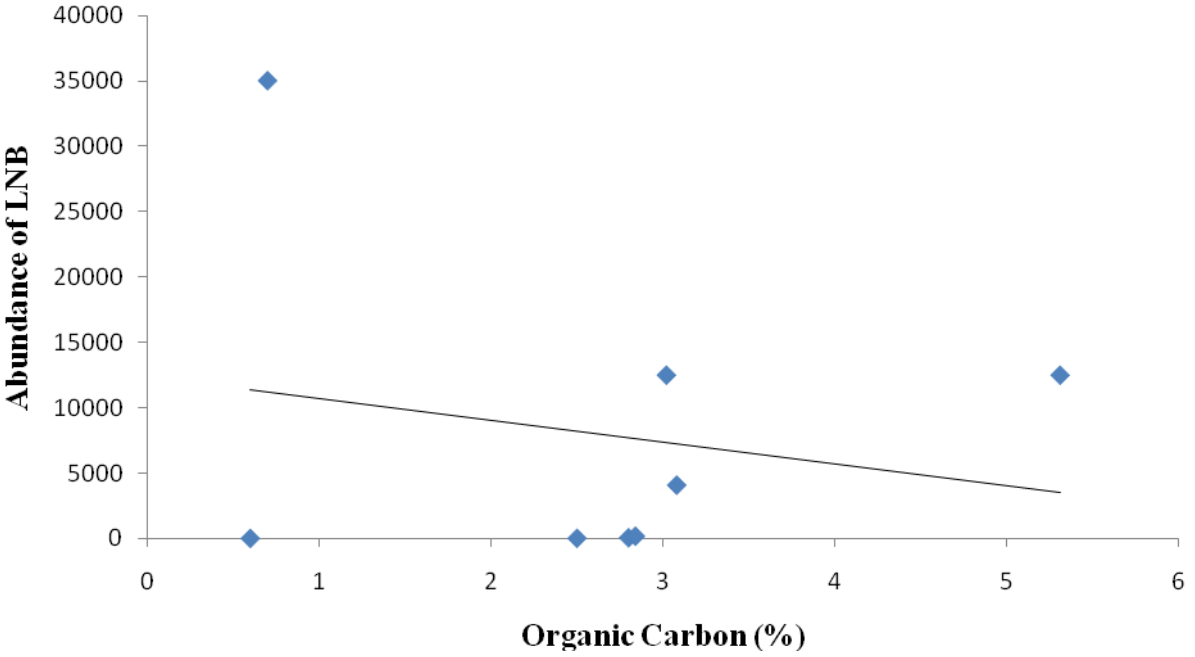


Figure 2: Relationship between Nitrogen and Organic Carbon on abundance of LNB

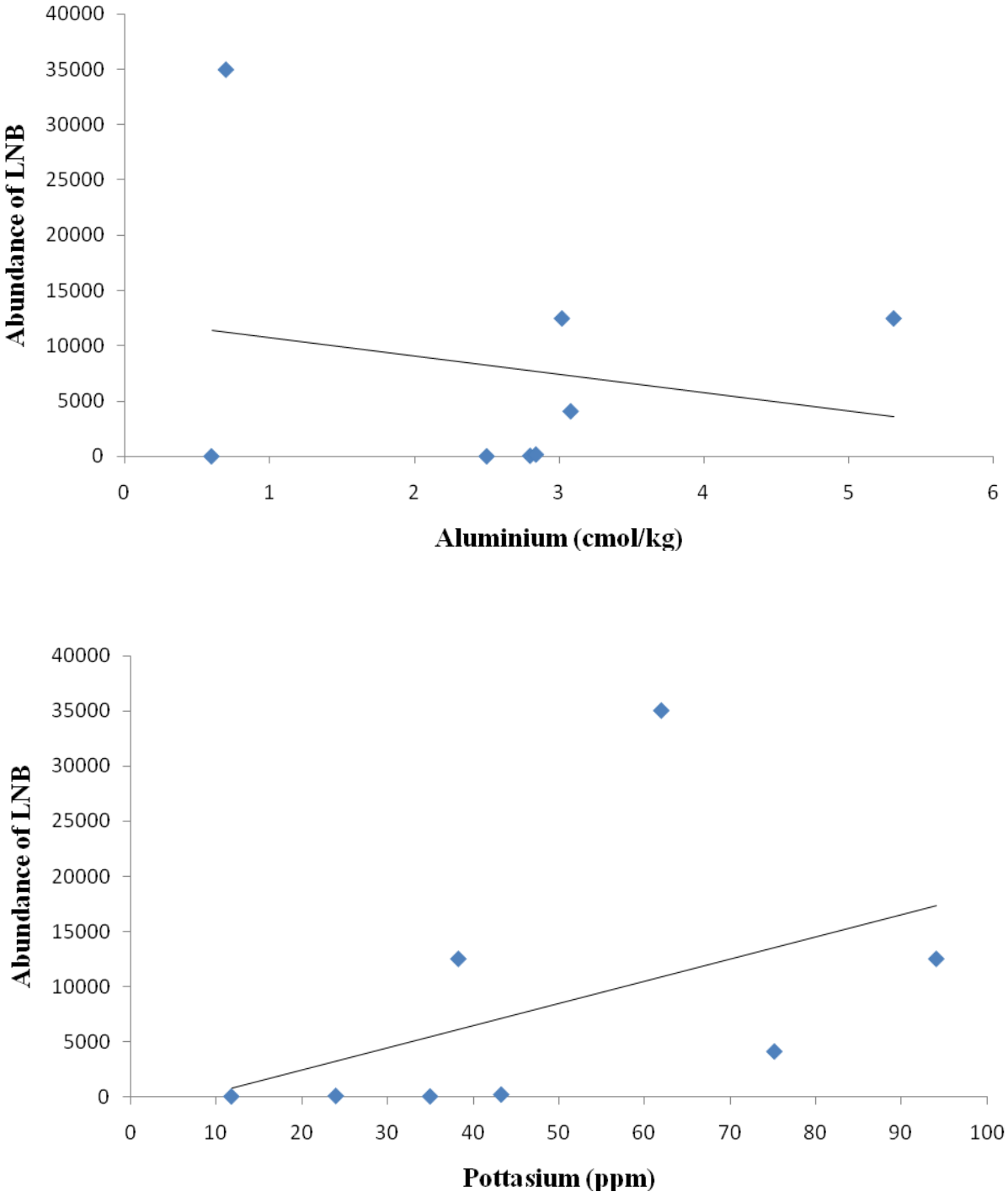


Figure 3: Relationship between of Aluminium and Pottasium on abundance of LNB

4.3 Morphology and cultural characteristics

A total of 328 bacterial isolates were obtained from the roots of common bean grown in soils from different farms in Kisumu and MMUST. Morphological and cultural characterization clustered the pure isolates into 12 groups (Table 5). The isolates were gram negative with entire colony margin and convex elevation. Screening the isolates on YEMA media containing Congo red dye indicated that the groups either did not absorb the red dye or absorbed it lightly under incubation in the dark. The isolates further turned YEMA media substituted with bromothymol blue (BTB) into moderately yellow to deep yellow color and thus considered to be acid producers and fast growers.

The colonies were categorized as creamy yellow, creamy white and milky white that were either opaque or translucent. The texture of the 3 categories was either smooth viscous or firm and dry. As shown in Table 5, all the groups produced extracellular polysaccharide (EPS) except the milky white colonies and constituted 67.7 % of all the isolates. The colony shapes were either circular or oval with diameters lying between 1 mm to 5.7 mm.

Table 5: Morpho-cultural characteristics of bacterial isolates from Kisumu and MMUST

Characteristics	Isolates											
	KSM 1	*KSM 2*	*KSM3*	*KSM 4*	*KSM 5*	*KSM 006*	*KSM 7*	*KSM 8*	*MMUST 3*	*MMUST 4*	*MMUST 5*	*MMUST 6*
Congo Red Absorption	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
BTB Reaction	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Colony Colour	cream yellow	cream white	cream white	milky white	milky white	cream yellow	cream white	cream white	milky white	cream yellow	milky white	cream yellow
Colony Transparency	opaque	translucent	opaque	opaque	translucent	translucent	opaque	translucent	opaque	translucent	opaque	opaque
Colony appearance	shiny	shiny	shiny	dull	dull	shiny	Shiny	shiny	shiny	shiny	dull	dull
EPS Production	✓	✓	✓	x	x	✓	✓	✓	x	✓	x	✓
Colony Texture	firm dry	smooth viscous	smooth viscous	firm dry	firm dry	smooth viscous	smooth viscous	smooth viscous	smooth viscous	smooth viscous	firm dry	firm dry
Colony Shape	circular	oval	oval	circular	circular	circular	Oval	oval	circular	circular	circular	circular
Colony Elevation	convex	convex	convex	convex	convex	convex	convex	convex	convex	convex	convex	convex
Colony Diameter (mm)	3.7	4.7	5.7	3.7	4.0	3.7	5.0	3.3	4.7	3.3	3.0	1.0
Gram Stain	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Colony Margin	entire	entire	entire	entire	entire	entire	Entire	entire	entire	entire	entire	entire

BTB-Bromothymol Blue, EPS- Exopolysaccharides, ✓-positive reaction, ×-negative

Key: *KSM 001-Enterobacter hormaechei, KSM 002- Rhizobium tropici, KSM 003- Klebsiella variicola, KSM 004- Rhizobium leguminosarum, KSM 005- Klebsiella variicola, KSM 006- Rhizobium sp., KSM 007- Bacillus aryabhattai, KSM 008- Klebsiella sp.; MMUST 001- Pantoea dispersa, MMUST 002- Klebsiella sp., MMUST 003- Rhizobium leguminosarum, MMUST 004- Klebsiella variicola, MMUST 005-Klebsiella variicola, MMUST 006- Rhizobium tropici*

4.4 Authentication and symbiotic efficiency

Of the 14 clustered groups of isolates evaluated in the authentication and symbiotic efficiency (SE) experiments, 85.7 % initiated nodulation in common bean and were hence confirmed as root nodule bacteria. Controls with and without nitrogen supplementation did not however form nodules. The nodules were pink and the leaves of the nodulated plants were dark-green, while uninoculated and unfertilized control plants turned yellow after 21 days (Figure 4).

In Kisumu all the plants inoculated with native bacterial isolates had higher root dry weight (RDW) compared to the reference strain CIAT 899. *Klebsiell variicola* had the highest SDW among all the isolates (Table 6). The isolates significantly increased the nitrogen (N) concentration and content compared to the uninoculated control. The symbiotic efficiency (SE) of the native isolates ranged from 74% to 170 %. The SE values of 50% of the isolates were equal to or greater than those of N-fertilized plants. The native isolates had higher SE compared to CIAT 899 while 37.5 % of the isolates recorded SE greater than strain 446. There were significant differences on the RDW, N concentration, N content and SE of plants inoculated with native bacterial isolates, reference strains, N fertilized and uninoculated control. Similarly, in MMUST significant differences were observed on the RDW, N concentration, N content and SE of the inoculated plants ($p < 0.05$). CIAT 899 had the lowest RDW (0.56 g plant^{-1}), SDW (1.5 g plant^{-1}) and SE (67 %) compared to the native isolate *Klebsiella variicola* (MMUST 005), N-fertilized, uninoculated control and strain 446 (Table 7).

There was positive correlation between RDW and SDW, SDW and N concentration ($p<0.05$, $r=0.3$) and SDW and N content, SDW and SE, nodule number and nodule dry weight, N concentration and N content, N concentration and SE ($p<0.01$, $r=0.6$) (Table 8).

Figure 4: Sample nodules formed by isolates from Kisumu (A) and MMUST (B)

Table 6: Effect of inoculation on growth and SE of common bean in Kisumu

Isolate Code	Isolate Identity	RDW	SDW	N Concentration	N Content	SE (%)
KSM001	*Enterobacter hormaechei*	0.90ab	1.59bc	1.82ab	2.89abc	125.0abc
KSM002	*Rhizobium tropici*	0.94ab	1.52c	1.50abc	2.31bc	100.0bc
KSM003	*Klebsiella variicola*	0.96ab	1.84bc	2.06a	3.94a	170.0a
KSM004	*Rhizobium leguminosarum*	0.82b	1.64bc	1.22c	2.07bcd	89.0bcd
KSM005	*Klebsiella variicola*	1.03ab	2.01a	1.45bc	3.01ab	130.0ab
KSM006	*Rhizobium sp.*	0.86ab	1.65ab	1.04cd	1.72bcd	74.0bcd
KSM007	*Bacillus aryabhattai*	0.86ab	1.65bc	1.31bc	2.17bcd	94.0bcd
KSM008	*Klebsiella sp.*	0.90ab	1.88ab	1.01cd	1.89bcd	81.0bcd
Strain 446	-	1.07a	1.69abc	1.51abc	2.56abc	110.0abc
CIAT 899	-	0.56c	1.50c	1.08c	1.55cd	67.0cd
PCNTL	-	1.07a	1.67abc	1.37bc	2.32bc	100.0bc
NCTL	-	1.00ab	1.63bc	0.46d	0.75d	-
LSD (5%)		0.22	0.36	0.59	1.43	62.0

NCTL-Negative control, PCNTL-Positive control, LSD is the least significant difference of means; Strain 446 and CIAT 899 are reference commercial inoculants. Means within a column followed by the same letter (s) are not significantly different at $p<0.05$.
SE- Symbiotic efficiency

Table 7: Effect of inoculation on growth and SE of common bean in MMUST

Isolate Code	Isolate Identity	RDW	SDW	N Concentration	N Content	SE (%)
MUST003	*Rhizobium leguminosarum*	0.89ab	1.76a	1.32b	2.33b	100.0b
MUST004		0.80b	1.78a	1.41b	2.48b	107.0b
MUST005	*Klebsiella variicola*	1.08a	1.85a	2.02a	3.80a	164.0a
MUST006		1.07a	1.73a	1.06b	1.80bc	78.0bc
Strain 446	*Klebsiella variicola*	1.07a	1.69a	1.51ab	2.56b	110.0b
CIAT 899		0.56c	1.50a	1.08b	1.55bc	67.0bc
PCNTL	*Rhizobium tropici*	1.07a	1.68a	1.37b	2.32b	100.0b
NCTL		1.00ab	1.63a	0.46c	0.75c	-
LSD (5%)		0.24	0.39	0.56	1.13	49.0
	-					

NCTL-Negative control, PCNTL-Positive control, LSD is the least significant difference of means Strain 446 and CIAT 899 are reference commercial inoculants. Means within a column followed by the same letter (s) are not significantly different at $p<0.05$.
SE- Symbiotic efficiency

Table 8: Correlation coefficients of common bean growth parameters with SE

Variables	**SDW**	**NN**	**NDW**	**N Concentration**	**N Content**	**SE**
Root Dry Weight	0.245*	0.166	-0.018	0.095	0.175	0.175
Shoot Dry Weight		0.056	0.096	0.275*	0.545**	0.546**
Nodule Number			0.703**	-0.032	-0.042	-0.043
Nodule Dry Weight				-0.106	-0.087	-0.087
Nitrogen Concentration					0.948**	0.948**
Nitrogen Content						1.000**

*, ** Correlation is significant at $p<0.05$, $r=0.25$; $p<0.01$, $r=0.55$ respectively

4.5 Molecular characterization of bean nodulating bacteria

4.5.1 Polymerase chain reaction and analysis of 16S rRNA genes

The PCR amplification of 16S rRNA genes of the 14 pure bacterial isolates from Kisumu and MMUST produced a single band of approximately 1500 bp (Appendix 1). The comparison of the partial 16S rDNA sequences of the isolates with the NCBI database showed varying sequence lengths ranging from 1254 bp to 1307 bp and 1283 bp to 1324 bp for Kisumu and MMUST respectively (Table 9 and 10). The sequences were deposited in the Genbank and assigned accessions numbers KP027678-KP027691 and their identity available on online on NCBI website (Appendix 6).

In Kisumu, 37.5 % of the isolates were closely related to *Rhizobium* genus: *Rhizobium tropici*, *Rhizobium sp.* and *Rhizobium leguminosarum*. *Klebsiella* genus: *Klebsiella sp.* and *Klebsiella variicola* constituted 25 % of the total isolates. The remaining isolates were identified as *Enterobacter hormaechei* and *Bacillus aryabhattai*. In MMUST BLAST results confirmed that the isolates were closely related to *Pantoea dispersa, Klebsiella sp., Rhizobium leguminosarum, Rhizobium tropici* and *Klebsiella variicola*. The two isolates, *Enterobacter hormaechei* and *Bacillus aryabhattai* in Kisumu were not present in MMUST. *Pantoea dispersa* was only found in MMUST. The two non nodulating isolates (MMUST 001 and MMUST 002) in this study were confirmed to be *Pantoea dispersa, Klebsiella sp.* However in Kisumu *Klebsiella sp* initiated nodulation.

Table 9: Genetic relationship between 16S rRNA of Kisumu and Genbank sequences

Isolate	Accession No.	Sequence length (bp)	Species identity	Similarity (%)
KSM001	KP027682	1295	*Enterobacter hormaechei*	99
KSM002	KP027680	1254	*Rhizobium tropici*	99
KSM003	KP027683	1295	*Klebsiella variicola*	100
KSM004	KP027679	1254	*Rhizobium leguminosarum*	100
SM005	KP027685	1295	*Klebsiella variicola*	99
KSM006	KP027681	1258	*Rhizobium sp.*	98
KSM007	KP027678	1307	*Bacillus aryabhattai*	100
KSM008	KP027684	1295	*Klebsiella sp.*	99

Table 10: Genetic relationship between 16S rRNA of MMUST and Genbank sequences

Isolate	Accession No.	Sequence length (bp)	Species identity	Similarity (%)
MMUST 001	KP027686	1324	*Pantoea dispersa*	100
MMUST 002	KP027689	1324	*Klebsiella sp.*	99
MMUST 003	KP027691	1283	*Rhizobium leguminosarum*	100
MMUST 004	KP027687	1324	*Klebsiella variicola*	100
MMUST 005	KP027688	1324	*Klebsiella variicola*	99
MMUST 006	KP027690	1283	*Rhizobium tropici*	99

4.5.2 Diversity of the 16S rRNA genes within the bacterial genome

The number of base substitutions per site between bacterial sequences analyzed by Jukes-Cantor model is shown in Table 11. The analysis involved 14 bacteria nucleotide sequences and the codon positions included were 1^{st} + 2^{nd} + 3^{rd} + non-coding. All the gaps and missing data were eliminated to give a final dataset of 1244 positions. The evolutionary relationship estimated by calculating the matrix pairwise genetic distances for the 16S rRNA genes showed that most of the bacterial isolates were closely related. The results showed that *Bacillus aryabhattai* had a longer genetic distance of 0.26 compared to *Pantoea dispersa* and *Enterobacter hormaechei*. Further analysis based on the genetic distance indicated that *B. aryabhattai* has the least evolutionary relationship with *P. dispersa* and *E. hormaechei.*

Table 11: Evolutionary genetic distance between each of the eleven of LNB populations

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
1. *P. dispersa* MMUST 001														
2. *E. hormaechei* KSM 001	0.02													
3. *K. variicola* MMUST 004	0.03	0.002												
4. *K. variicola* MMUST 005	0.03	0.02	0.00											
5. *K. variicola* KSM 003	0.03	0.02	0.00	0.00										
6. *K. variicola* KSM 005	0.03	0.02	0.00	0.00	0.00									
7. *Rhizobium* sp. KSM 008	0.03	0.02	0.00	0.00	0.00	0.00								
8. *Klebsiella* sp. MMUST 002	0.03	0.02	0.00	0.00	0.00	0.00	0.00							
9. *R. tropici* MMUST 006	0.22	0.22	0.23	0.23	0.23	0.23	0.23	0.23						
10. *R. leguminosarum* MMUST 003	0.22	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.02					
11. *R. leguminosarum* KSM 004	0.22	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.02	0.00				
12. *R. tropici* KSM 002	0.22	0.22	0.23	0.23	0.23	0.23	0.23	0.23	0.00	0.02	0.02			
13. *Rhizobium* sp. KSM 006	0.23	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.06	0.06	0.06	0.06		
14. *B. aryabhattai* KSM 007	0.26	0.26	0.25	0.25	0.25	0.25	0.25	0.25	0.12	0.12	0.12	0.12	0.22	

4.5.3 Evolutionary relationships of native bacterial isolates in MMUST

An optimal tree with the sum of branch length (0.2398) was inferred using the Neighbor-Joining method to show the evolutionary relationship of the taxa in MMUST. The replicate (%) trees associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Figure 4). The analysis used 6 nucleotide sequences and codon positions including 1^{st} + 2^{nd} + 3^{rd} + non-coding. Gaps and missing data were eliminated to give a total of 1279 positions in the final dataset.

Phylogenetic tree derived from the bacterial partial sequences of 16S rRNA gene by neighbor-joining analysis confirmed higher relationship between *Rhizobium leguminosarum* and *Rhizobium tropici*. The *Klebsiella* strains including the non nodulating *Klebsiella* sp. (KP027691) clustered together indicating their genetic relatedness. However the non nodulating *Pantoea dispersa* showed the least relationship with the other bacterial isolates. The scale (0.02) represents the number of nucleotide changes per site.

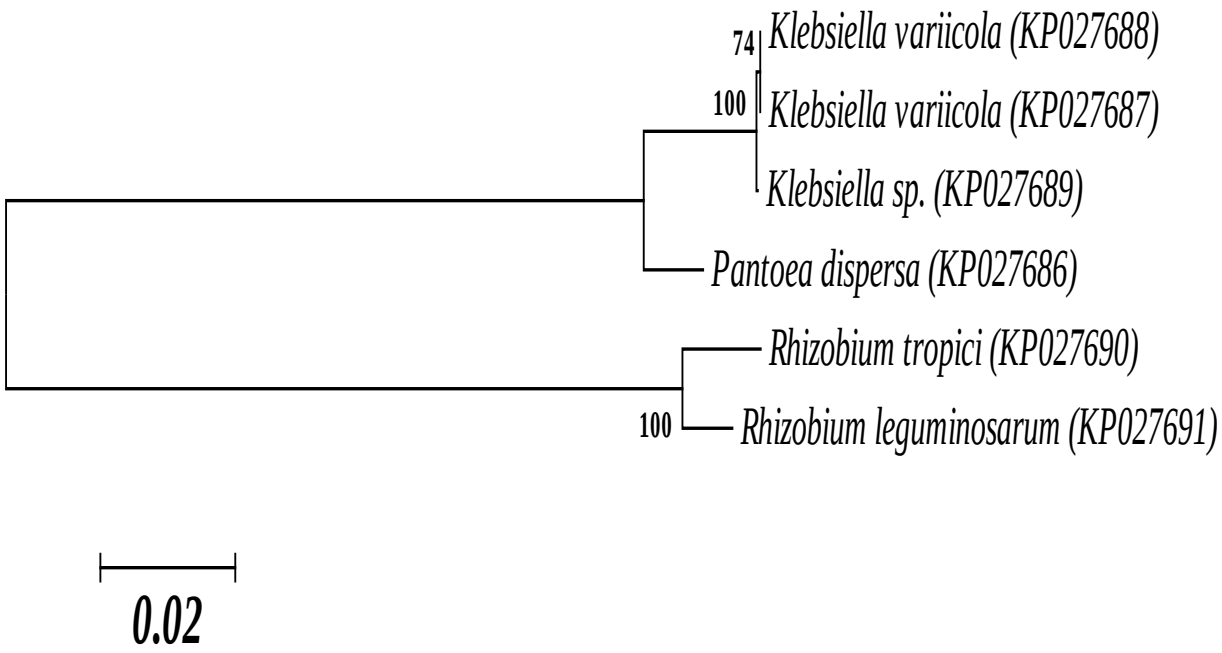


Figure 5: Neighbor Joining (NJ) tree of 16S rRNA sequences of isolates from MMUST

4.5.4 Evolutionary relationships of native bactetial isolates in Kisumu

Inference using the neighbor-joining method produced an optimal tree with the sum of branch length (0.4074) revealing an evolutionary relationship of the taxa (Figure 5). The replicate (%) trees associated taxa clustered together in the bootstrap test (1000 replicates) are indicated next to the branches. The analysis used codon positions including 1^{st} + 2^{nd} + 3^{rd} + non-coding and 8 nucleotide sequences. Gaps and missing data were eliminated to give a total of 1253 positions.

Phylogenetic tree derived from the partial sequences of 16S rRNA gene by neighbor-joining analysis clustered the isolates into *Rhizobium strains, Bacillus aryabhattai, Klebsiella* strains and *Enterobacter hormaechei*. *Rhizobium* sp. clustered with *Rhizobium leguminosarum* and *Rhizobium tropici* however *R. leguminosarum* and *R. tropici* had a closer genetic closeness compared to *Rhizobium* sp. *Klebsiella* strains were grouped with *Enterobacter hormaechei* but with a closer association among the *Klebsiella variicola* (KP027685), *Klebsiella variicola* (KP027683) and *Klebsiella* sp. (KP027684).

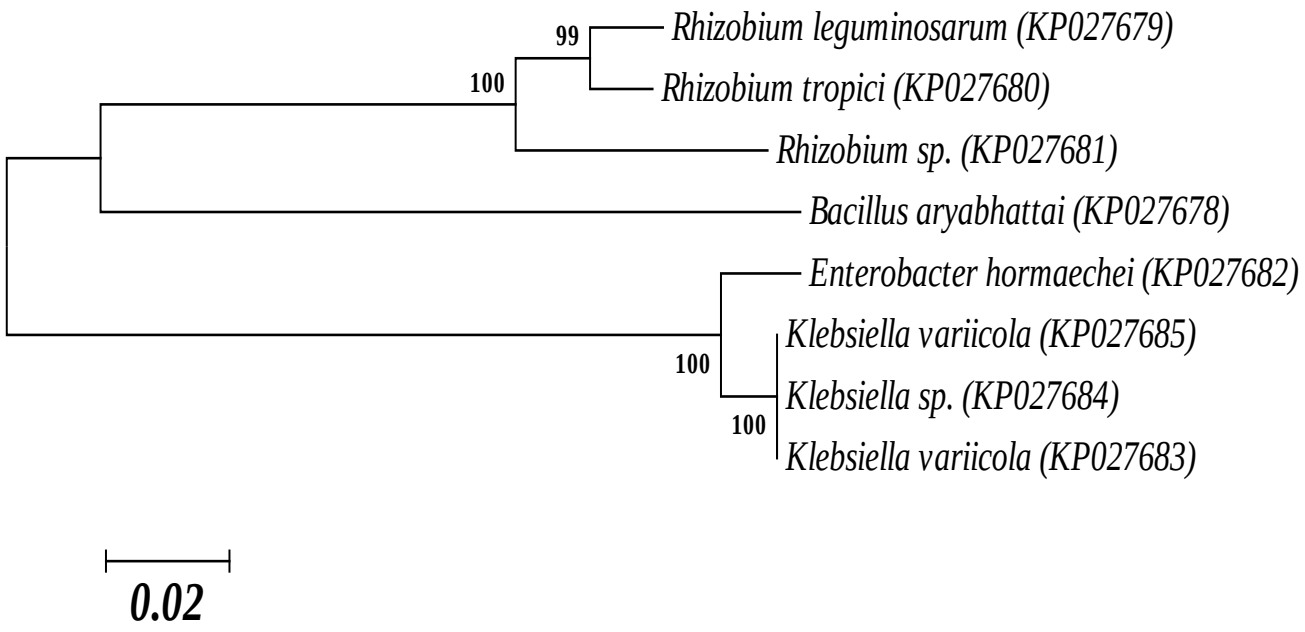


Figure 6: Neighbor Joining (NJ) tree of 16S rRNA sequences of isolates from Kisumu

The genetic relationship of the sequences and analysis of the isolates from the two sites produced an overall optimal tree with the sum of branch length (0.4143). The percentage replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Figure 6). The bootstrap values tested reliability based on evolutionary distances and base substitutions per site to infer phylogenetic tree. The genetic analysis involved 14 nucleotide sequences including codon positions of 1^{st} + 2^{nd} + 3^{rd} + non-coding regions. In total there were 1244 positions in the final dataset representing both Kisumu and MMUST. Partial sequences of 16S rRNA gene produced a phylogenetic tree that clustered the isolates into three major groups. Location of isolation did not affect clustering of different strains in this study. The first group consisted of *Rhizobium tropici*, *Rhizobium leguminosarum, Rhizobium sp.* and *Rhizobium leguminosarum*.

The second cluster had *Bacillus aryabhattai* while the last group clustered *Pantoea dispersa, Klebsiella* sp., *Enterobacter hormaechei* and *Klebsiella variicola* together. *Rhizobium* sp. had the least genetic relationship in the group compared to the closely associated *R. tropici* and *R. leguminosarum*. In the last cluster *Pantoea dispersa* had the least evolutionary relationship with *Enterobacter hormaechei* and *Klebsiella* sp. *E. hormaechei* and *Klebsiella* sp. clustered together revealing their close genetic association.

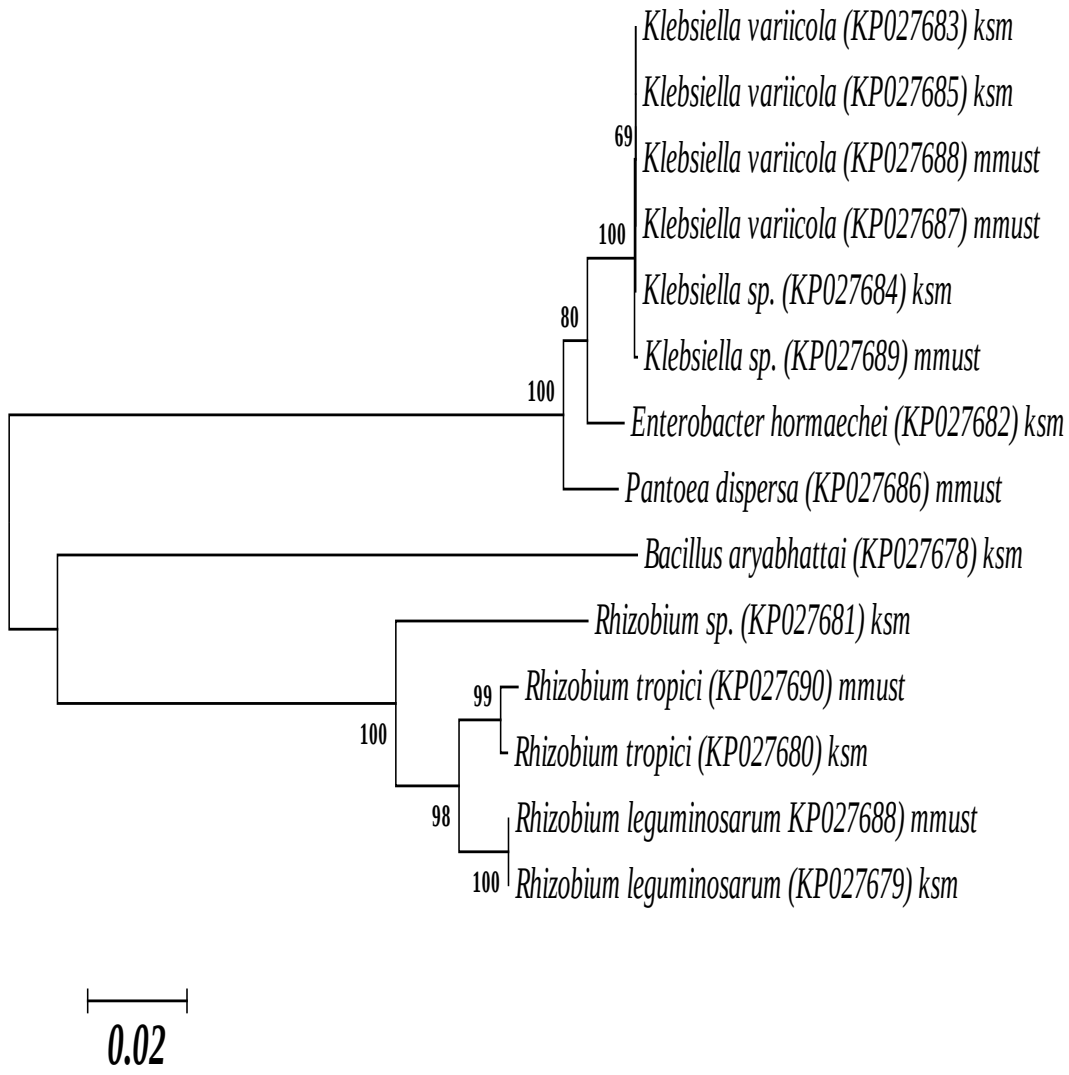


Figure 7: Neighbor Joining (NJ) tree of 16S rRNA sequences of all isolates

4.6 Effect of water hyacinth compost on soil properties

The results for the effect of water hyacinth compost and mineral fertilizer inputs on soil properties in the SR and LR at Kisumu are presented in Table 12 and 13. There was pH increase in water hyacinth compost plots after six weeks in both the SR and LR seasons at the two sites. The soil N was influenced by the application of water hyacinth compost, although the increase was not significant in the SR. The amount of Ca and P increased in the LR compared to the SR in all treatments. Soil Mg, OC and Al were influenced by the application of the fertilizers in the LR season. There was a general reduction in soil K in all the treatments in both the SR and LR seasons.

Table 12: Mean soil characteristics in the SR season at Kisumu

Treatment	pH	N	OC	Ca	Mg	K	Al	Mn	P
1 Rose Coco-I	5.63b	0.09abc	0.92ab	8.87bc	1.26bc	1.23ab	2.05bcde	39.73bcd	14.17abcd
2 Rose Coco NI	5.58ab	0.10a	1.22ab	6.31bc	1.20bc	0.81ab	1.58cde	46.59bcd	14.60abcd
3 Rose Coco TSP-I	5.59b	0.12c	1.31ab	5.38a	1.30bc	1.00ab	1.13a	53.65cd	19.47cd
4 Rose Coco TSP-NI	5.75b	0.14bc	1.32b	4.58ab	1.24	1.07ab	1.00ab	47.78bcd	22.85bcd
5 Rose Coco UREA-I	6.03a	0.15ab	1.45a	2.66bc	1.23bc	1.40a	0.64e	42.57bcd	36.36a
6 Rose Coco UREA-NI	5.58a	0.11c	1.29ab	6.24ab	1.45a	0.96ab	0.74a	60.33a	15.37d
7 Rose Coco WH-I	6.10ab	0.13a	1.37ab	5.14c	1.25bc	1.60ab	0.74e	47.90bcd	44.61abc
8 Rose Coco WH-NI	6.13b	0.09bc	1.03ab	7.30ab	1.56ab	0.93ab	1.97e	86.80bc	7.78bcd
9 Yellow bean-I	5.61ab	0.13ab	1.42ab	6.58bc	1.12bc	0.54ab	1.20bcde	37.56bc	30.60abcd
10 Yellow bean-NI	5.55ab	0.11ab	1.34ab	5.18ab	1.21c	0.71ab	1.31cde	50.62b	15.98abc
11 Yellow bean TSP-I	5.68b	0.12ab	1.33a	5.39ab	1.21c	0.86b	1.12bcd	60.60d	32.98abcd
12 Yellow bean TSP-NI	5.72b	0.12bc	1.31ab	6.51bc	1.14bc	0.80ab	1.04bc	64.25bcd	39.59bcd
13 Yellow bean UREA-I	5.70ab	0.13ab	1.34ab	6.61ab	1.23c	0.94ab	1.12bcd	63.39b	40.53abc
14 Yellow bean UREA-NI	5.64ab	0.12ab	1.32ab	6.12ab	1.23bc	0.84ab	1.20bcde	58.99bcd	30.95abcd
15 Yellow bean WH-I	6.12ab	0.13ab	1.32ab	6.13ab	1.18	0.90ab	1.17bcde	62.25b	38.35ab
16 Yellow bean WH-NI	6.17ab	0.12ab	1.32ab	6.00ab	1.23bc	0.90ab	1.14bcd	57.86bcd	31.43abcd
LSD (5%)	0.50	0.03	0.42	3.09	0.24	0.91	0.50	21.85	26.08

Means within a column followed by the same letter(s) are not significantly different at $p < 0.05$; LSD is the Least Significant Difference of means, I-Rhizobia Inoculation; NI-Non Rhizobia Inoculation, TSP- Triple Superphosphate; WH-Water Hyacinth Compost

Table 13: Mean soil characteristics in the LR at Kisumu

Treatments		pH	N	OC	Ca	Mg	K	Al	Mn	P
1	Rose Coco-I	5.70a	0.24d	1.77b	4.47a	1.26abc	1.24abc	2.00c	78.90abcde	44.00abcd
2	Rose Coco NI	5.63ab	0.16d	1.44b	5.65a	1.08a	0.70ab	1.76c	54.15abcde	31.00abcd
3	Rose Coco TSP-I	6.26ab	0.12ab	1.30ab	7.31a	1.29abcd	1.10a	0.99a	64.10abc	65.00bcd
4	Rose Coco TSP-NI	5.93ab	0.13cd	1.34b	7.60a	1.41bcd	1.19cd	0.96ab	60.30bcde	64.50d
5	Rose Coco UREA-I	6.04ab	0.14a	1.49a	5.88a	1.07abcd	1.02abc	0.99ab	53.65a	98.50bcd
6	Rose Coco UREA-NI	5.65ab	0.19cd	1.31b	6.81a	1.29bcd	0.99bcd	0.93bc	87.00cde	42.50cd
7	Rose Coco WH-I	6.18ab	0.25cd	2.15b	5.55a	1.24bcd	0.98abc	1.76c	86.05e	52.00a
8	Rose Coco WH-NI	6.12ab	0.16bc	1.48b	4.60a	1.06abc	0.79abc	1.29c	55.55a	34.31bcd
9	Yellow bean-I	5.68ab	0.13cd	1.44b	4.44a	1.03d	0.55cd	1.09bc	66.55abc	38.60bcd
10	Yellow bean-NI	5.63ab	0.13cd	1.37b	4.30a	1.11d	0.89abcd	1.20c	69.00abc	41.85ab
11	Yellow bean TSP-I	5.98ab	0.16d	1.44b	6.58a	1.02cd	0.77d	1.25c	78.08abcde	61.22bcd
12	Yellow bean TSP-NI	5.88b	0.13d	1.33b	7.56a	1.02bcd	0.86abcd	1.06bc	78.44abcde	72.34bcd
13	Yellow bean UREA-I	6.00ab	0.13cd	1.36b	7.75a	1.29bcd	1.05bcd	0.98bc	69.20abcd	67.17bcd
14	Yellow bean UREA-NI	5.84ab	0.13d	1.34b	6.12a	1.03bcd	0.69bcd	0.99c	77.19ab	55.60ab
15	Yellow bean WH-I	6.14ab	0.16cd	1.43b	5.61a	1.08ab	0.81abc	1.22c	77.65abcde	58.20abc
16	Yellow bean WH-NI	6.12ab	0.13d	1.27b	7.05a	1.07cd	0.78cd	0.95c	83.42abcde	72.07bcd
LSD (5%)		0.63	0.06	0.56	4.12	0.26	0.41	0.58	23.72	35.22

Means within a column followed by the same letter(s) are not significantly different at $p < 0.05$; LSD is the Least Significant Difference of means, I-Rhizobia Inoculation; NI-Non Rhizobia Inoculation, TSP- Triple Superphosphate; WH-Water Hyacinth Compost

In MMUST soil properties in the SR and LR after application of compost are shown in Table 14 and 15 repectively. Application of water hyacinth compost generally reduced soil Al but increased Ca and K. In the LR, soil Ca and K increased significantly in response to water hyacinth compost while Al reduced significantly. However the application of the fertilizers did not significantly influence soil Mg, OC and N in the LR.

Table 14: Mean soil characteristics in the SR at MMUST

Treatment		pH	N	OC	Ca	Mg	K	Al	Mn	P
1	Rose Coco-I	5.39bcde	0.26abcd	2.21a	2.87de	1.27ef	1.12a	1.68d	86.90abc	20.00ab
2	Rose Coco NI	5.15bcde	0.21abcd	1.94b	3.16abc	1.19f	1.21a	2.13abcd	55.10bcd	15.96b
3	Rose Coco TSP-I	5.22abcd	0.21a	2.43ab	2.63cde	1.14def	1.26a	1.34bcd	75.30a	23.54ab
4	Rose Coco TSP-NI	5.29bcde	0.20abcd	1.64ab	4.10bcde	1.09ef	1.19a	2.30abcd	56.70bcd	14.67b
5	Rose Coco UREA-I	5.88bcde	0.23abcde	1.97ab	4.36cde	1.36def	1.33a	2.07cd	80.90abc	18.67ab
6	Rose Coco UREAN-I	5.54bcde	0.17abcde	1.91ab	2.86bcde	1.28ef	1.25a	2.16abcd	49.10cd	29.92ab
7	Rose Coco WH-I	5.19a	0.18ab	1.91ab	2.89ab	1.23bcdef	1.24a	1.62abcd	69.30ab	22.58ab
8	Rose Coco WH-NI	5.21ab	0.17abcde	2.27ab	3.16cde	1.18cdef	1.28a	2.29abcd	50.70cd	26.67ab
9	Yellow bean-I	4.88abc	0.21e	2.11ab	4.63bcde	1.80ab	1.20a	2.15abcd	62.11abcd	19.73ab
10	Yellow bean-NI	4.81bcde	0.15cde	1.70ab	3.89e	1.71abcd	1.25a	1.84bcd	60.02d	19.11ab
11	Yellow bean TSP-I	5.42de	0.08abcd	1.89ab	3.07a	1.67a	1.25a	2.05abcd	66.40abcd	22.11ab
12	Yellow bean TSP-NI	5.18e	0.12bcde	2.14ab	2.53abcde	1.61ab	1.34a	1.75abcd	39.33abcd	32.00ab
13	Yellow bean UREA-I	5.25bcde	0.11abc	1.74ab	4.01abcd	1.70ef	1.39a	2.48a	66.30bcd	27.96ab
14	Yellow bean UREA-NI	4.98de	0.21abcde	2.09b	3.73abcde	1.65abcde	1.32a	2.68abcd	51.77bdc	35.54ab
15	Yellow bean WH-I	5.16bcde	0.22de	2.31ab	4.01abcd	1.15ab	1.34a	2.78ab	54.54abcd	26.67ab
16	Yellow bean WH-NI	4.98cde	0.18abcd	1.57ab	3.92abcde	1.47abc	1.30a	2.10abc	58.15cd	28.63a
LSD (5%)		0.52	0.10	0.76	1.42	0.38	0.52	1.01	28.52	19.11

Means within a column followed by the same letter(s) are not significantly different at $p < 0.05$; LSD is the Least Significant Difference of means, I-Rhizobia Inoculation; NI-Non Rhizobia Inoculation, TSP- Triple Superphosphate; WH-Water Hyacinth Compost

Table 15: Mean soil characteristics in the LR at MMUST

Treatment		pH	N	OC	Ca	Mg	K	Al	Mn	P
1	Rose Coco-I	4.94fg	0.18abc	2.49h	6.88e	1.52b	1.33abc	1.78abcd	75.98def	21.98a
2	Rose Coco NI	5.31h	0.29abc	2.15fg	7.05g	1.85gh	1.42ab	1.80ab	89.00cd	30.00d
3	Rose Coco TSP-I	4.93fg	0.25bc	1.59abc	7.97g	1.62bcd	1.47a	1.49bcde	75.97def	58.97k
4	Rose Coco TSP-NI	4.56ab	0.20abc	2.11ef	7.05g	1.16a	0.93cd	1.45bcde	81.00b	44.00h
5	Rose Coco UREA-I	4.75abc	0.23abc	2.27abc	7.83b	1.29cde	1.21abc	1.04efg	78.00a	41.67b
6	Rose Coco UREA-NI	5.20fg	0.27c	2.53g	8.80g	1.58gh	1.02bcd	1.42bcde	79.66cde	38.00h
7	Rose Coco WH-I	5.29gh	0.22abc	2.47def	9.84ef	1.44efg	1.25abc	0.63gh	98.24cde	48.04e
8	Rose Coco WH-NI	4.90bcde	0.15abc	1.95ab	6.72cd	1.12bc	1.22abc	0.39h	76.87cde	29.97f
9	Yellow bean-I	5.08bcd	0.35abc	2.26h	5.61bc	1.26bcd	1.00cd	1.87ab	79.71g	31.11g
10	Yellow bean-NI	5.25cdef	0.21abc	2.40bcde	10.70de	1.20def	1.09abcd	2.15a	79.00fg	46.00i
11	Yellow bean TSP-I	5.23def	0.23a	1.72def	9.15h	1.52fg	0.76d	1.08efg	69.07cde	33.07h
12	Yellow bean TSP-NI	5.10bcd	0.24abc	2.34abcd	8.30a	1.36fgh	1.12abcd	1.26cdef	70.80cde	27.00c
13	Yellow bean UREA-I	5.01a	0.31abc	2.51cdef	7.21g	1.21h	1.04bcd	1.55bcde	82.02efg	39.91g
14	Yellow bean UREA-NI	4.56def	0.23ab	2.55ef	7.95h	1.13gh	0.96cd	1.80abc	77.95g	23.95bc
15	Yellow bean WH-I	5.47ef	0.23ab	2.30ab	7.01fg	1.06fgh	1.29abc	1.24def	74.36c	34.01e
16	Yellow bean WH-NI	5.06h	0.30abc	2.18a	5.60e	1.06gh	0.92cd	0.73fgh	70.00cde	47.00j
LSD (5%)		0.21	0.15	0.19	0.73	0.18	0.41	0.54	5.93	1.83

Means within a column followed by the same letter(s) are not significantly different at $p < 0.05$; LSD is the Least Significant Difference of means, I-Rhizobia Inoculation; NI-Non Rhizobia Inoculation, TSP- Triple Superphosphate; WH-Water Hyacinth Compost

4.7 Field experiments

4.7.1 Yield of of common bean

The results of the effect of water hyacinth compost and inorganic fertilizers on the growth and yield of bean in the SR and LR in Kisumu and MMUST are presented in Table 16 and 17 respectively. Application of compost and inorganic fertilizer significantly ($p < 0.05$) influenced the growth parameters of beans in the SR and LR seasons. In Kisumu, high DW was recorded in Rose Coco TSP-I (17 g plant^{-1}) compared to Yellow bean Urea-I (14 g plant^{-1}) in MMUST in the SR. In Kisumu, inoculated beans grown with urea and compost recorded lower DW than non-inoculated in SR and LR seasons. In the SR, beans in Kisumu had high DW compared to the LR. However in MMUST, the DW was higher in LR than in the SR. In the LR, Yellow Bean-NI (Kisumu) and Yellow Bean-I (MMUST) gave the highest DW (14.2 g plant^{-1} and 18.4 g plant^{-1} respectively).

The highest number of nodules was recorded in Yello Bean WH-I (Kisumu) and Rose Coco WH-NI (MMUST) in the SR. Inoculation increased the number of nodules in Yellow bean treated with compost at the two sites. High number of nodules was recorded in MMUST than in Kisumu between similar treatments. In the LR, high number of nodules was recorded in Yellow Bean WH-I in Kisumu while Rose coco TSP-I and Yellow Bean WH-I gave higher nodules compared to other treatments in MMUST. Urea treatments gave the least number of nodules in both the SR and LR seasons.

The number of pods was high in Rose Coco WH-I and Rose Coco TSP-I in Kisumu and MMUST respectively in the SR. In the LR, Yellow Bean-NI in Kisumu had high number of pods compared to other treatments. Inoculation significantly increased the number of pods in all the treatments across the two sites except in Rose Coco-I in Kisumu. In MMUST, no significant effect was observed on the number of pods under different treatments.

Bean yields ranged from 120.7 kg ha^{-1} (Rose Coco TSP-I) to 382 kg ha^{-1} (Yellow Bean Urea-I) in Kisumu and 152.8 kg ha^{-1} (Yellow Bean TSP-I) to 341.0 kg ha^{-1} (Rose Coco Urea-I) in MMUST in the SR. Bean yields were generally high in MMUST compared to Kisumu between similar treatments. In Kisumu, yields were higher in none inoculated than inoculated plots treated with water hyacinth compost. However in MMUST inoculation increased bean yields in plots treated with compost and urea. In the LR, bean yields ranged from 92.2 kg ha^{-1} (Yellow Bean-NI) to 530.9kg ha^{-1} (Rose Coco WH-I) in Kisumu. In MMUST, the yields ranged from 656.8 kg ha^{-1} (Yellow bean-I) to 1583.4 kg ha^{-1} (Rose Coco WH-NI). Plots with no fertilizer input recorded the lowest yield in the LR season.

Table 16: Yield of common bean in the short rains (SR)

		KISUMU				MMUST			
Treatments		DW (g plant^{-1})	Nodules per plant	Pods per plant	Yield (kg ha^{-1})	DW (g plant^{-1})	Nodules per plant	Pods per plant	Yield (kg ha^{-1})
1	Rose Coco-I	12.4bc	60ed	13efgh	129.1fg	5.5c	87a	14abc	323.5abc
2	Rose Coco NI	7.9efg	14f	15cdefg	266.2b	4.7c	73ab	12bcde	182.5fg
3	Rose Coco TSP-I	17.0a	78ab	13fgh	120.7g	6.0bc	61b	16ab	250.4def
4	Rose Coco TSP-NI	11.4bcd	77abc	11h	181.1defg	4.9c	59b	13abcd	269.3bcd
5	Rose Coco UREA-I	6.9fg	9f	18bc	256.7bc	5.8bc	3c	14abc	341.0a
6	Rose Coco UREA-NI	14.0ab	19f	14defgh	265.0b	4.5c	7c	11cde	326.8ab
7	Rose Coco WH-I	5.7g	63cde	23a	193.0de	5.5c	60b	17a	244.0def
8	Rose Coco WH-NI	10.1cdef	72bcd	16cdef	202.3cd	5.6c	92a	9def	238.9def
9	Yellow bean-I	8.3defg	91a	21ab	133.8efg	5.0c	74ab	11bcde	221.4defg
10	Yellow bean-NI	7.5efg	16f	14defgh	195.9cd	7.0bc	11c	9def	235.4def
11	Yellow bean TSP-I	7.1efg	59e	18bc	143.3defg	8.7b	86a	13abcd	152.8g
12	Yellow bean TSP-NI	12.0bc	84ab	15cdefg	198.3cd	4.9c	81a	10cdef	254.2cde
13	Yellow bean UREA-I	5.6g	6f	16cde	382.0a	14.0a	7c	11cde	290.0abcd
14	Yellow bean UREA-NI	10.3cde	10f	15cdefg	312.1b	4.9c	4c	8ef	287.1abcd
15	Yellow bean WH-I	6.8fg	90a	17cd	184.2def	5.7bc	74ab	14abc	245.8def
16	Yellow bean WH-NI	8.0efg	55e	12gh	202.3cd	4.8c	60b	6f	191.3efg
LSD (5%)		3.4	14.4	3.6	61.7	3.1	19.4	4.7	71.4
CV (%)		22	17	14	18	30	22	25	17

Means within a column followed by the same letter (s) are not significantly different at $p < 0.05$; LSD is the Least Significant Difference of means, CV-Coefficient of Variation; I-Rhizobia Inoculation; NI-Non Rhizobia Inoculation, TSP- Triple Superphosphate; WH-Water Hyacinth Compost

Table 17: Yield of common bean in the long rains (LR)

		KISUMU				MMUST			
Treatment		DW (g plant^{-1})	Nodules per plant	Pods per plant	Yield (kg ha^{-1})	DW (g plant^{-1})	Nodules per plant	Pods per plant	Yield (kg ha^{-1})
1	Rose Coco-I	6.3defg	13cd	7g	95.7h	13.3bcd	19c	11a	1069.6bc
2	Rose Coco NI	6.0fg	4e	9efg	223.4fg	14.8bc	18cd	13a	691.7g
3	Rose Coco TSP-I	7.8cdef	13cd	13cdefg	251.9efg	16.5ab	37a	13a	1419.1cd
4	Rose Coco TSP-NI	6.2efg	10d	13cdefg	423.4bc	13.9bcd	12de	16a	1006.0b
5	Rose Coco UREA-I	9.3bcdef	3e	16cdef	258.4ef	12.0cd	4f	14a	1196.9bc
6	Rose Coco UREA-NI	9.5bcdef	13cd	11defg	486.1ab	13.3bcd	4f	12a	1063.9bc
7	Rose Coco WH-I	9.8bcd	17ab	6g	530.9a	15.0abc	16cde	12a	957.6cde
8	Rose Coco WH-NI	10.9abc	14bc	8fg	383.6cd	15.9ab	28b	13a	1583.4a
9	Yellow bean-I	3.8g	3e	18cd	177.7g	18.4a	16cde	15a	656.8g
10	Yellow bean-NI	14.2a	13cd	29a	92.2h	10.9d	10ef	14a	807.2efg
11	Yellow bean TSP-I	12.0ab	12cd	19bcd	188.6fg	14.2bcd	10ef	15a	698.2fg
12	Yellow bean TSP-NI	9.1bcdef	12cd	16cdef	319.1de	14.0bcd	18cd	13a	893.4cde
13	Yellow bean UREA-I	12.4ab	3e	17cde	423.4bc	13.2bcd	5f	15a	695.0g
14	Yellow bean UREA-NI	9.9bc	3e	27ab	426.2bc	14.9abc	5f	13a	796.2efg
15	Yellow bean WH-I	8.9bcdef	18a	21abc	470.2ab	16.6ab	30b	14a	879.4def
16	Yellow bean WH-NI	9.6bcde	4e	19bcd	453.2abc	13.3bcd	21c	15a	1191.0b
LSD (5%)		3.6	3.8	9	80.4	3.7	6.4	4.2	182.1
CV (%)		24	24	35	15	15	24	18	11

Means within a column followed by the same letter(s) are not significantly different at $p < 0.05$; LSD is the Least Significant Difference of means, CV-Coefficient of Variation; I-Rhizobia Inoculation; NI-Non Rhizobia Inoculation, TSP- Triple Superphosphate; WH-Water Hyacinth Compost

4.7.2 Correlation of selected soil properties with yield components of common bean

Relationship of the effect of selected soil properties with the growth and yield of beans after six weeks of compost application in SR and LR are shown in Table 18 and 19. The number of pods were positively correlated with soil pH (r=0.29) and N (r=0.35) but negatively correlated with Ca (r=-0.37) and Al (r-0.46).

In MMUST, the number of pods were positively correlated with soil pH (r=0.34) but negatively correlated with Al (r=-0.29). Bean yields were found to be negatively associated with Mg (r=-0.42). In Kisumu during the LR only soil pH (r=0.29) showed a positive correlation with yield. However negative associations were observed in the number of pods with Mg (r=-0.33), yield with OC (r=-0.31), K (r=-0.44). The number of pods was positively associated with soil Ca, Al and P while bean yields had a positive correlation with pH. However yields at the two study sites were negatively correlated with Al (r=-0.28 and r=-0.26).

Table 18: Correlation of soil properties with yield of common bean in SR

Soil properties	**KISUMU**			**MMUST**		
	Nodules	Pods	Yield	Nodules	Pods	Yield
pH (1 : 2.5 soil water ratio)	-0.08	0.29*	0.07	0.10	0.34*	-0.01
N (%)	-0.18	0.35*	0.17	0.08	0.22	0.02
Organic carbon (%)	0.01	0.27	0.04	-0.07	0.21	0.18
Ca (cmol/kg)	0.02	-0.37**	-0.03	0.06	0.08	-0.27
Mg (cmol/kg)	-0.04	-0.02	0.01	0.23	-0.23	-0.42**
K (cmol/kg)	-0.09	0.12	0.01	-0.04	-0.09	0.09
Al (cmol/kg)	0.08	-0.46**	-0.06	-0.02	-0.29*	-0.18
P (ppm)	-0.19	0.26	0.17	-0.01	-0.17	0.16

*, **Correlation is significant at $p < 0.05$, 0.01, respectively

Table 19: Correlation of soil properties with yield of common bean in LR

Soil properties	**KISUMU**			**MMUST**		
	Nodules	Pods	Yield	Nodules	Pods	Yield
pH (1 : 2.5 soil water ratio)	0.03	-0.09	0.29*	0.02	0.11	0.33*
N (%)	0.03	0.19	0.18	0.01	-0.04	0.20
Organic carbon (%)	-0.17	0.12	-0.31*	-0.04	-0.24	0.05
Ca (cmol/kg)	-0.08	-0.26	-0.04	-0.05	0.31*	-0.02
Mg (cmol/kg)	-0.01	-0.33*	0.23	0.12	0.28	-0.22
K (cmol/kg)	-0.07	-0.08	-0.44**	-0.04	0.04	-0.23
Al (cmol/kg)	0.11	0.08	-0.28*	0.24	0.30*	-0.26*
P (ppm)	0.11	0.08	0.28	0.24	0.30*	0.23

*, **Correlation is significant at $p < 0.05$, 0.01, respectively

CHAPTER FIVE

DISCUSSION

5.1 Soil properties and abundance of indigenous rhizobia

There was variation in soil fertility properties at the farms and this could be due to different fertility management practices. Most of the farms in MMUST had low soil pH compared to Kisumu. Farmers' fields in Kisumu generally had low soil nitrogen than MMUST indicating need for external N input to for improved crop production (Okalebo et al., 2002). The soils in Kisumu also had low organic carbon that could be attributed to warmer temperatures compared to MMUST and the sandy loam soil texture. This is in agreement with Alvarez et al. (2008), who reported that organic matter decays more rapidly at higher temperatures and warmer climates than in cooler regions that contain more organic matter.

Similarly, different authors have reported that warmer temperatures and high moisture levels result in higher rates of decomposition, faster litter turnover, and less organic matter accumulation (Alvarez et al., 2008; Moore et al., 2007; Yule and Gomez, 2009). In addition, common farm management practices such as repetitive tillage or burning of crops remains and vegetation that are common in Kisumu have been shown to lower soil organic carbon (Carter et al., 2002; Curry and Good, 1992). On the other hand, fine-textured clay soils similar to those in MMUST tend to have more organic matter than coarse sandy soils; they hold nutrients and water better, thus providing good conditions for plant growth.

Farms at Kisumu and MMUST except those of fallow land had levels of available P above the critical value of 15 ppm as described by Okalebo et al. (2002). The high levels of available P in these soils may have resulted from continuous on farm organic amendment or external P fertilizer input. The abundance of native bacteria population nodulating common bean in the soils at MMUST and Kisumu ranged from 3.2×10^1 to 3.5×10^4 cells per gram of soil. These results show presence of native bacteria in soils of western Kenya. High population levels of native bacteria in some of the farms could be attributed to the widespread integration of legumes in the cropping system in the region (Katungi et al., 2009). Similarly, Mathu et al. (2012) and Chemining'wa et al. (2011) reported an increase in soil native bacteria population when legumes were cultivated in the field.

The lower population of native bacteria observed in farms at MMUST compared to Kisumu could be due to the low pH that has been shown to adversely affect nodulation in common bean (Soares et al., 2014). The clay texture of soil in MMUST could have also affected nodule bacteria population. Several others authors have demonstrated that hand fine clay soil reduces nodule bacterial population and diversity in different cropping systems (Loureiro et al., 2007; Mothapo et al., 2013). The light sandy soil in Kisumu could have also promoted the proliferation and survival of root-nodule bacteria (Martyniuk and Oron, 2008).

Lower population of LNB in soils at MMUST could further be attributed to the high N content. This is in agreement with Gage (2004), who reported that elevated levels of N in the soil delayed symbiotic process through decreased multiplication of free-living rhizobia. Similarly, many authors have shown that N fertilizers inhibit root hair infection, nodule initiation, growth and development, nitrogenase activity and promote premature nodule senescence (Kiers et al., 2006; Liu et al., 2010; Watanabe et al., 2014). Nahed-Toral et al. (2013) further pointed out that legume nodulation is reduced or eliminated when soils have high supplies of ammonium and nitrate.

The presence of native bacteria nodulating legume in all soils at the two sites may be due to soil pH that ranged from 5.2 to 6.1. This pH range is considered optimal for rhizobial growth (Niste et al., 2013; Soares et al., 2014). Similar studies have shown that fewer root nodule bacteria grow well at pH less than 5.0 (Soares et al., 2014). However certain strains of root nodule bacteria are known to tolerate varying range of pH than others and tolerance may vary within species (Kannaiyan, 2002). Generally low pH increases the solubility of Al, Mn, and Fe in soil causing toxicity to plants in excess by slowing or stopping the growth of roots (Morón et al., 2005). The population variation of root nodule bacteria observed among the different farms has been reported by several authors. Abaidoo et al. (2007) and Woomer et al. (1997) reported similar population size variations in different soils from Africa including Kenya.

The farms in Kisumu were characterized by low Cu, Al and N levels and higher population of legume nodulating bacteria (LNB) than MMUST. Similarly, Ahmad (2012), Arora (2013) and Cage (2004), and reported that high levels of soil N, Al and Cu have detrimental effects on legume nodulation.

High Al levels are extremely toxic and reduce the various enzymatic activities like nitrite reduction, nitrogenase and hydrogenase uptake during biological nitrogen fixation. Arora (2013) and Zahran (1999) further pointed out that high Al levels have deleterious effect on the LNB DNA both *in vitro* and *in vivo* conditions. Copper plays a critical role in physiological processes such as respiration, photosynthesis, nitrogen and cell wall metabolism, carbohydrate distribution and seed production. However, higher levels inhibit growth, morphology and activities of various groups of soil microorganisms including LNB (Ahmad et al., 2012)

In the current study, LNB were isolated from fallow and Napier soils that had no history of growing legumes. This is in agreement with Anyango et al. (1995) who demonstrated the occurrence of LNB in soils with no history of growing legumes or deliberate inoculation. A similar study by Pongslip (2012) showed that the occurrence of LNB in different soils is independent of the host crop. Most of the farms at both MMUST and Kisumu had P levels above the critical values described by Okalebo et al. (2002), and this could have favoured the occurrence of LNB.

World Bank (2006), reported that soil P is an important nutrient in the process of nodulation and nitrogen fixation. The population of LNB reported in this study is high compared to those reported by other authors in different regions of Kenya (Chemining'wa et al., 2011; Mwenda et al., 2011; Waswa, 2013).

5.2 Morphology and cultural characteristics

Legume nodulating bacteria were isolated from all the sampled farms at MMUST and Kisumu. These LNB had varying morphological and cultural characteristics that could be an indication of diversity. Similarly, many authors pointed out that nodule sampling directly from the fields is the best method for assessing the diversity of rhizobia compared to soil dilutions under greenhouse conditions (Giongo et al., 2008; Loureiro et al., 2007). Alberton et al.(2006) reported high diversity of LNB when nodules were sampled from plants inoculated with soil dilutions.

The general growth appearance of the isolates on yeast extract mannitol agar (YEMA) media were typical phenotypic characteristics of LNB (Somasegaran and Hoben, 1994). The LNB were gram negative and further failed to absorb Congo red dye, which are typical characteristics of nodule based bacteria (Abere et al., 2009). The absorption of Congo red dye has been used to distinguish Rhizobia from other root nodule bacteria in leguminous plants that take up Congo red dye.

The LNB turned YEMA-BTB media into moderately yellow to deep yellow color that is considered typical colour change for fast growing and acid producers. The colour change reaction has been used by several authors as a classical phenotypic characteristic of *Rhizobia* sp. that is often used when classifying unknown strains (Jida and Assefa, 2011; Wei et al., 2008; Wolde-meskel et al., 2004).

However the absorption depends on concentration of the dye and other studies have shown that rhizobia will only absorb the red dye if plates are exposed to light during the incubation or exposed to light for an hour or more after growth has occurred (Elkoca et al., 2010; Prévost and Antoun, 2007). The isolation of fast growing LNB in this study is contrary to the findings of other authors in Kenya who reported the occurrence of both slow and fast growing bacteria (Mwenda et al., 2011; Odee et al., 1997). The presence of fast growing LNB in soils at MMUST and Kisumu shows their suitability in developing inocula adapted to local conditions. Studies have demonstrated that fast growing LNB have a shorter time of inoculant production and easier establishment in soil (Werner and Newton, 2006). The diameter of the colonies for all the LNB isolated from the two sites ranged from 1 mm to 5.7 mm with circular margins. This is consistent with Somasegaran and Hoben, (1994), who described the colony size of LNB to lie between 1 mm and 5 mm for both slow and fast growing strains except in crowded plates.

The colonies of the LNB were cream yellow, milky white and cream white colonies that were either opaque or translucent. Similar typical phenotypic characteristics have been used to describe LNB (Odee et al., 1997). The production of surface polysaccharides such as exopolysaccharides and lipopolysaccharides by some of the LNB isolates are features associated with rhizobia (Nandal et al., 2005).

These features show the ability of the bacteria to withstand environmental stresses and could be suitable for the development of commercial inoculants. The surface polysaccharides are adaptive features that provide protection to bacteria against environmental factors like temperature, salinity and pH fluctuations in the soil (de Carvalho and Fernandes, 2010; Miranda-Ríos et al., 2015). The cultural and phenotypic characteristics have been used to separate impurities and contaminants from pure LNB (Vincent, 1970). However it is important to use phenotypic characterization in combination with more informative techniques for species identification.

5.3 Authentication and symbiotic efficiency

The bacterial isolates from soils in MMUST and Kisumu that initiated nodulation when inoculated on the host crop grown in sterile vermiculite were considered as LNB. According to Vincent (1970), nodule formation in a host crop is a confirmatory test for LNB. Similarly, many authors have demonstrated that no bacterial isolate can be regarded as LNB until its identity has been confirmed through plant infection test on an appropriate host crop (Brockwell, 1980; Howieson and Brockwell, 2005; Weir, 2006).

However, most of the results from authentication experiments have described LNB as the only soil inhabiting bacteria and excludes non nodulating bacteria.

In the present study, authentication experiment together with morphological and cultural characteristics was used as a test for LNB. However the results showed that *Pantoea dispera* and *Klebsiella* sp. with typical cultural characteristics of LNB failed to initiate nodulation on the parent host. This finding is similar to that of Sullivan et al. (1996) who associated nodulation failure in a host crop to loss of plasmids or lack of genes responsible for nodulation in the bean variety used. Plasmids play an important role in the development of nodules and N fixation in legumes (Laranjo et al., 2002; Sullivan et al., 1996). In other studies, different authors have reported that LNB that lack genes for infecting legumes are common in rhizosphere of some suitable host legumes (Gage, 2004; Van der Putten et al., 2007). In China, bacterial isolates from soils in different geographical regions failed nodulation test but were later confirmed to be LNB (Acton, 2012). This shows that nodulation test alone should not be used as a confirmatory test for LNB.

In our experiment, control plants grown with and without N supplementation in sterilized modified Leonard jar assemblies did not form nodules. Lack of nodules in the control plants is an indication that no external contaminations occurred during experimental set up in the greenhouse. Hassen et al.(2014) previously described lack of contamination as a requirement in authentication experiment.

The method is widely accepted as a standard for testing nodulation and nitrogen fixation under greenhouse conditions (Beck et al., 1993; Öğütcü and Algur, 2014).

Inoculated plants produced pink nodules and had dark-green leaves compared to the yellow leaves of uninoculated and unfertilized control plants. This is consistent with the findings of Chouhan et al. (2008) who reported the presence of pink nodules and dark green leaves in inoculated bean plants. The typical colour change is an indication of effective symbiotic legheamoglobin content in the nodules that is crucial for N fixation in legume root nodules (Chouhan et al., 2008). Plants inoculated with LNB isolates from soils in Kisumu had high RDW than CIAT 899 but lower compared to strain 446 while in MMUST a single LNB, *Klebsiella variicola* had a higher RDW than strain 446. The increase in RDW could be an indication that native LNB promoted bean root development than CIAT 899. The LNB isolates produced exopolysaccharides which could have further contributed to the development of roots of inoculated plants. According to Hirsch, (1999) and Kelly et al. (2013), LNB surface exopolysaccharides are required for nodule formation and root development of legumes but the mechanisms by which they act still remains unknown. Other LNB are also known to produce plant root growth promoting hormone (Erum and Bano, 2008; Hirsch, 1999; Kelly et al., 2013). However, these results are in contrast to those reported by other authors elsewhere. Mungai and Karubiu, (2011) in Kenya and Argaw, (2012) in Ethiopia demonstrated that inoculation with native LNB did not increase RDW.

Inoculated plants had SDW that were higher compared to the uninoculated negative controls and this could be attributed to the effectiveness of the native LNB in biomass accumulation in the tissue. Peoples et al. (1995) and Somasegaran (1994) and pointed out that SDW is a good indicator of relative LNB effectiveness.

In a similar study, Argaw (2012) explained that the increase in SDW could also be due to production of plant growth promoting hormone by the LNB in addition to N fixation. The RDW, N concentration, N content and SE of the inoculated plants were significantly different. This is in accordance with previous results reported by Graham (2007) in pulse legumes. Further, Abere et al. (2002) observed significant differences in agronomic growth of beans inoculated with native LNB under growth room, greenhouse and field conditions. The results of the SDW of 1.70 g plant^{-1} (MMUST) and 1.68 g plant^{-1} (Kisumu) reported in this study were higher compared to those reported by other authors (Abere et al., 2009; Kelly et al., 2013; Laranjo et al., 2002). Öğütcü and Algur (2014) also observed lower SDW in high altitudes in Erzurum, Turkey.

In the current study, *Enterobacter hormaechei* and *Klebsiella variicola* from soils in Kisumu and MMUST had a higher SE than CIAT 899 and strain 446. This shows that these bacteria are well adapted to the local environmental conditions than the commercial inoculant. Similarly, Anyango et al. (1995) isolated superior native LNB compared to CIAT 899 from soils with contrasting pH in Kenya.

These results suggest the presence of native LNB in western Kenya with superior characteristics in BNF fixation than commercial inoculant strains, CIAT 899 and strain 446 (Anyango et al., 1995). The less competitive native LNB with low SE could be attributed to the soil dilution during the authentication experiment. Alberton et al. (2006) reported that dilution of the soil used as a source of LNB inoculum decreases the proportion of the most competitive strains, enabling the less competitive strains to nodulate.

High proportion of the native LNB were found to be effective N fixers and performed as good as the N supplemented controls based on differences in SDW. The finding corroborates results reported by Prasad et al. (2010), who used differences in SDW of inoculated and nitrogen-fertilized plants as a measure of symbiotic efficiency.

The RDW was positively correlated with SDW and N concentration while SDW was highly correlated with N content and SE ($r=0.25$, $p< 0.05$ and $r=0.55$, $p< 0.01$ respectively). The positive correlation between the legume growth parameters with N content is considered as one of the most reliable screening parameters for the selection of superior LNB isolates (Abere et al., 2009; Meghvansi et al., 2010). Abere et al.(2009) demonstrated that N fixation is a function of photosynthesis and translocation interactions between fixed and soil N. Further, other authors have shown that the strong association between N content with SDW justifies the use of N content to measure symbiotic N fixation under field and greenhouse conditions (Abere et al., 2009; Atici et al., 2005).

The occurrence of native and superior LNB strains at MMUST and Kisumu is indicative of the benefits which could be achieved by rigorous screening of large numbers of isolates from natural environment for inoculum development.

5.4 Polymerase chain reaction and analysis of 16S rRNA genes

There were a total of 14 pure LNB isolates obtained from MMUST and Kisumu. The amplification of the 16S rRNA gene of the isolates using specific primers generated a single band of approximately 1500 bp. This is the expected band size for most endophytic bacteria including LNB as previously described by several other authors (Dilworth, 2008; Hassen et al., 2014; Rajendhran and Gunasekaran, 2011). The single band size generated in this study confirms that 16S rRNA is a conserved region within the LNB genome. Yang et al. (2014) pointed out that the degree of conservation observed in the 16S rRNA is due to its importance as a critical component of cell function. Several authors have demonstrated that few other genes are as highly conserved as the 16S rRNA gene (Chakravorty et al., 2007; Clarridge, 2004; Yang et al., 2014). Although the absolute rate of change in the 16S rRNA gene sequence still remains unknown, it marks evolutionary distance and relatedness of organisms (Guindon and Gascuel, 2003; Kimura, 1980; Nayak et al., 2011). This is in contrast to other genes such as those needed to make enzymes that are frequently affected by mutations (Clarridge, 2004). Mutations in these genes can usually be tolerated more frequently since they may affect structures not as unique and essential as rRNA (Chakravorty et al., 2007).

The variation in size of the amplified PCR products observed in this study further shows that 16S rRNA gene is considerably different among species (Rajendhran and Gunasekaran, 2011). The size and sequence polymorphisms of 16S rRNA can therefore be used to distinguish different species. The amplification products of the 16S rRNA region were sequenced and the resulting nucleotide sequences edited and BLAST searched to reveal the identity of the LNB.

The BLAST results confirmed the identity of the LNB as similar to *Rhizobium tropici, Rhizobium leguminosarum* bv. *viciae, Rhizobium* sp., *Rhizobium leguminosarum, Klebsiella sp., Klebsiella variicola, Enterobacter hormaechei, Bacillus aryabhattai* and *Pantoea dispersa.* These results are similar to those previously reported by Anyango et al. (1995) and Mwenda et al. (2011), who isolated different strains of LNB in other regions of Kenya. The two LNB, *Pantoea dispersa* and *Klebsiella* sp. in MMUST failed to initiate nodulation on the test crop but in Kisumu *Klebsiella* sp. initiated nodulation. This failure to initiate nodulation by *Klebsiella* sp. at one site could be attributed to loss of viability during laboratory storage and sub-culturing (Elbanna et al., 2009).

For the first time in western Kenya, the current study has demonstrated the occurrence of N fixing nonrhizobial bacteria in the root nodules of common bean. These nonrhizobial bacteria had typical morphological and cultural characteristics similar to those of rhizobia. Recently, Ghosh et al. (2015) reported the production of ascorbic acid by *Enterobacter* sp isolated from root nodules of legumes (*Abrus precatorius L.).*

These authors pointed out that ascorbic acid is an antioxidant and plays a significant role to protect against activated form of O_2 for N fixation in root nodules of legume plants. This bacterium plays an important role particularly in facilitating root infection for nodulation, N fixation and delay nodule senescence (Ghosh et al., 2015).

The other two LNB, *Klebsiella* sp and *Pantoea* sp have been reported in the root nodules of different legumes including soybean (de Meyer et al., 2013; Silva et al., 2012). Silva et al. (2012) explained that these bacteria promote plant cell growth by fixing N and producing thiamin, riboflavin, nicotin, indole-3- acetic acid (IAA) and gibberellins. *K. variicola* is a novel bacterial species discovered in 2004 with both clinical and plant-associated characteristics (Rosenblueth et al., 2004). This bacterium has previously been reported to occur in the roots of monocots such as wheat, banana, rice and corn where it supplies biologically fixed nitrogen to the host plant (Vacheron et al., 2014).

5.5 Evolutionary relationship between the LNB

The genetic distance of the 16S rDNA sequences ranged from 0.00 to 0.26, revealing least and highest differentiation respectively. In MMUST analysis of the sequences clustered the LNB into three distinct groups consisting of *Klebsiella* sp., *Klebsiella variicola* and *Klebsiella variicola* with a genetic distance of 0.00. This genetic distance indicates a common ancestor with minimum recombination rates (Rosenblueth et al., 2004).

The second group had *Pantoea dispersa* with a genetic distance of 0.03 compared to the first cluster showing that two are related but not similar. The third group consisted of *R. leguminosarum* and *R. tropici* with a genetic distance of 0.22, indicating the highest divergence in relation to *Pantoea dispersa*.

In Kisumu, the LNB clusters included *Rhizobium* sp., *Bacillus aryabhattai*, *Enterobacter hormaechei* and *Klebsiella* sp. This clustering shows a remarkable level of genetic diversity in the studied population. The high genetic variation observed between *Bacillus aryabhattai* and the other LNB in this study could have contributed to its adaptability. High genetic variation is necessary to allow organisms to adapt to ever changing environments with some of this variation stemming from introduction of new alleles by the random and natural process of mutation (Sharma et al., 2015). This is consistent with the findings of several authors who reported similar genetic relatedness of LNB (Baraúna et al., 2014; Rosenblueth et al., 2004). The bacteria had bootstrap values greater than 69% indicated at the nodes further confirmed the reliability of the phylogenetic tree by clustering similar strains together irrespective of the region.

The presence of *Bacillus aryabhattai* and *Enterobacter hormaechei* in Kisumu could be attributed to the low levels of Al, Cu and sandy loam texture of soil. Similarly, clay soil in MMUST could have favoured *P. dispersa*. This study further confirms that partial 16S rRNA sequencing can demonstrate phylogenetic diversity within genera and sometimes give an indication of species (McInroy et al., 1999).

However clustering of LNB strains together under one group shows that it cannot clearly discriminate between species (Sprent, 2007; Zahran, 2001). In contrast, other authors have showed that even full length 16S rRNA sequencing lacks the sensitivity to distinguish between closely related species (Lindström et al., 1998; University, 2009).

Many other genomic regions have also been used to examine the phylogenetic relationships among bacteria (Clarridge, 2004). Different authors have used whole-genome analysis but found it quite difficult because gene duplication, gene transfer, gene deletion, gene fusion, and gene splitting are common (Bansal and Meyer, 2002; Coenye et al., 2005; Yang et al., 2005). The use of 16S rRNA gene sequence can discriminate far more finely among strains of bacteria than is possible with phenotypic methods, it can allow a more precise identification of poorly described, rarely isolated, or phenotypically aberrant strains (Chakravorty et al., 2007; Clarridge, 2004). However, it has been observed that the phylogenetic trees based on whole-genomic analysis and the 16S rRNA gene trees are similar (Didelot et al., 2012; Mothershed and Whitney, 2006).

5.6 Effect of water hyacinth compost application on soil properties

Application of water hyacinth compost significantly increased soil pH in the SR and LR at MMUST and Kisumu. Similarly, Mucheru-Muna et al.(2010) and Opala et al.(2012), reported an increase in soil pH on plots treated with compost. This increase in soil pH resulting from the added compost could be attributed to increase in the levels of exchangeable bases like K and Ca in compost treatments. The level of Al significantly reduced in plots treated with water hyacinth compost in MMUST. This is consistent with the findings of other authors who observed an increase in soil pH with concomitant decrease in exchangeable Al during decomposition of organic residues in soils (Naramabuye and Haynes, 2006; Opala et al., 2012; Wong et al., 1998).

Similarly, Mokolobate and Haynes (2002), pointed out that increase in soil pH results in precipitation of exchangeable and soluble Al as insoluble Al hydroxides thus reducing concentration of Al in soil solution. Other intricate mechanisms involved in the reactions of Al with organic composts have also been described by other authors (Ritchie and Dolling, 1985; Xu et al., 2006). These mechanisms involve complex formation with low-molecular weight organic acids, such as citric, oxalic, and malic acids and humic material produced during the decomposition of organic materials and adsorption of Al on to the decomposing organic residues (Opala et al., 2012; Ritchie and Dolling, 1985; Xu et al., 2006).

Most soil chemical properties were not influenced after application of compost and this could be attributed to the long term effect of compost application.Studies have shown that N mineralization from compost is very limited in the short term and the residual effect becomes visible after 4 to 5 years of repeated application (Leroy et al., 2007). In this study, soil properties were analyzed after 6 weeks of compost application for two consecutive seasons and this may be considered too soon to determine the influence of compost on soil properties. Similar studies have also pointed out that only a part of the N and P in compost is readily available for plant uptake and a large part needs to be mineralized (Diacono and Montemurro, 2011; Miao et al., 2010). Therefore organic compost can only improve soil nutrients if applied over long periods of time because many nutrients become more available in less acid soils (Chianu et al., 2012).

5.7 Effect of compost on growth and yield of common bean

Urea and TSP treated plants had high DW compared to the other treatments. This observation could be attributed to the immediate release of nutrients by inorganic fertilizers that supported the vegetative growth. A similar study showed pointed out that P significantly increased dry matter yield in Common Bean (Turuko and Mohammed, 2014). Turuko and Mohammed (2014) demonstrated that P is required by plants for cell division leading to the increase of the number of branches per plant and consequently increased the plant dry weight.

Several studies have also reported an increase in dry weight of most crops in response to N application (Henson and Bliss, 1991; Kimura et al., 2004; Otieno et al., 2007). Lack of significant increase in DW in response to rhizobial inoculation observed in Kisumu is consistent with those reported by Mungai & Karubiu (2011). These authors showed that inoculation did not result in significant changes in above ground biomass in cowpeas and groundnuts.

Water hyacinth compost and TSP treated plants produced more nodules compared to other treatments. This could be due to the slow mineralization of water hyacinth compost hence slow release of N. In addition, high number of nodules in TSP treated plants could be attributed to the positive effects of P on nodulation (Ganeshamurthy and Sammi Reddy, 2000). This is in agreement with a previous study by Ganeshamurthy and Sammi Reddy (2000), who reported that P and compost improve both the total and active nodules and nodule dry weight.

Since nodulation being is an energy driven process, availability of soil P provides nutrition required for the N fixation (Amba et al., 2013). This study showed that inoculation significantly increased the number of nodules in the LR. This observation is in agreement with the findings of Otieno et al. (2007), who reported higher number of nodules in response to rhizobial inoculation.

Similarly, an increase in the number of nodules per plant after inoculating soy bean plants with *Bradyrhizobium japonicum* has been reported (Mweetwa et al., 2014). The least number of nodules recorded in plants grown under urea treated plots in the SR and LR seasons could be attributed to the inhibitory effects of N. Similar findings were reported by Gentili et al.(2006) in different legumes. These authors demonstrated that added N fertilizer inhibits nodulation and BNF in legume crops such as common beans. Nodule degeneration has also been observed in different bean varieties after application of N fertilizer (Taylor et al., 2005). Gentili et al. (2006) further reported that high N levels inhibited early cell divisions in the cortex of *Alnus incana* thus inhibiting nodulation.

The application of water hyacinth compost significantly increased the number of pods per plant. Other authors have also reported that addition of organic compost increases the number of pods in different crop legumes (Azimzadeh et al., 2014; Benjawan et al., 2007; Davari et al., 2012). All the treatments did not have any significant effect on the number of pods per plant in MMUST. This could be due to the fact that the soil had adequate amount of N above the critical value as described by Okalebo et al. (2002).

In field trials conducted in western Kenya, Amos et al. (2001) found that N fertilizer only increased seed count per plant but not the number of pods in common bean. The significant increase in the number of pods observed in plants grown in TSP treated plots shows that P is an important nutrient in the formation of pods in common bean. The result is similar to those of Turuku and Mohammed (2014), who reported significant increase in number of pods per plant due to increased P fertilization.

Bean plants treated with urea and TSP produced high yields compared to the other treatments in the SR. This high yield in the SR could be attributed to nutrients from inorganic fertilizers being readily available to plants. Rhizobia inoculation increased bean yields in water hyacinth compost and urea treatments in Kisumu. This is consistent with the findings of Lanier et al. (2005), who reported increase in yield after inoculation of peanut and soybean. In addition, similar studies in Kenya have documented significant yield improvement in legumes by rhizobia inoculation (Chemining'wa et al., 2004; Otieno et al., 2007).

However in Kisumu, lack of yield increment in response to rhizobia inoculation could be attributed to several factors. For example, the presence of native ineffective strains of rhizobia in the soil (Amos et al., 2001; Ham et al., 1971), soil pH (Shamseldin, 2007; Vinuesa et al., 2003), bean cultivar and strain interaction (Payakapong et al., 2004; Prévost et al., 2012) limit the effect of introduced rhizobia on yield. According to Amos et al. (2001), competition between strains of rhizobia in the soil is common as the introduced inoculum strains compete with indigenous rhizobia for nodule sites.

Further evidence also suggests that common bean cultivars are preferentially nodulated by different strains of bacteria (Aguilar et al., 2004; Pueppke et al., 1998). In this study, the Yellow and Rose coco bean cultivars used in the field trials at Kisumu could have been incompatible with the commercial inoculant used leading to lack of yield response.

These results are consistent with those of other authors who reported that certain Rhizobia strains only improve N fixation and yields in specific common bean cultivars (Amarger, 2001; Moawad et al., 1998). The findings in this study therefore indicate that bean cultivars that select strains, rather than nodulate with competitive and ineffective native rhizobia could offer a solution to the common lack of response to rhizobia inoculation problem. It is important to consider host variety and strain of rhizobia compatibility in order to minimize the frequently observed unsatisfactory responses to inoculation (Biederman and Harpole, 2013).

Furthermore, lack of rhizobia inoculation response on yield observed in urea treated plants could be due to the added N. Kiers et al. (2006) and Liu et al. (2010), showed that N fertilizers inhibit rhizobia root hair infection, nodule initiation, growth, multiplication, nitrogenase activity and promote premature nodule senescence.

In similar studies, different authors reported that legume root nodulation is reduced or eliminated when soils have high supplies of ammonium and nitrate (Nahed-Toral et al., 2013; Watanabe et al., 2014). The soil pH at the field trial sites was generally acidic and this could have further affected the activity of the introduced rhizobia through inoculation. Soil acidity interferes with early steps in the infection of process including exchange of molecular signals between symbiotic partners and attachment to the roots (Hungria and Vargas, 2000).

Bean yields were higher in water hyacinth compost treated plants compared to urea in the LR than in the SR. The LR season is usually characterized by heavy rainfall and the low yield in urea treated plants could be attributed to the rapid solubility leading to N loss through leaching and runoff. Similar studies have been reported that long-term use of mineral fertilisers does not increase crop yields but just sustain them (Bationo et al., 2004; Fatokun et al., 2002; Powell and Africa, 1994). High yields in compost treated plants could be due to the provision of additional benefits besides N by the organic compost to the soil chemical and physical properties that in turn influenced plant growth (Mutegi et al., 2012). Mucheru-Muna et al. (2007) stated that organic compost supplies essential plant nutrients by alleviating Al toxicity or by producing organic acids which complex with Al, thereby increasing nutrient availability and crop yield.

Organic composts contain high amount of organic matter which increases the moisture retention of soil and improves dissolution of nutrients such as N and P (Olupot et al., 2004; Otieno et al., 2007). In contrast, insufficient soil moisture has been reported to limit the response of crops to nutrients (Jama et al., 1997). Organic compost improves soil structure and in turn soil porosity allowing better root growth and hence better nutrient uptake (Tisserat et al., 2012).

In addition, application of readily decomposed organic matter such as water hyacinth compost used in this study has been shown to improve crop tolerance to root rots and hence crop yield (Hillocks et al., 2006; Mutitu et al., 1989; Otsyula et al., 1988). The positive response of legumes to organic composts has also been attributed to the quantity of soil N already available for the plants, amount of N that becomes available after mineralization during the season, release and availability of P,K and microelements (Bationo et al., 2006; Bocchi and Tano, 1994). High yield in the LR season can further be attributed to the fact that the nutrients in the water hyacinth compost were released gradually over prolonged periods of time (Bationo et al., 2004).

Other studies have also indicated that organic composts may act as chelates that help in the absorption of iron and other micro-nutrients (Altomare et al., 1999; Hülsebusch, 2007). In addition to the water hyacinth compost, sufficient and even distribution of rainfall in the LR compared to the low and unevenly distributed precipitation in SR season could have contributed to high yields. Sufficient and evenly distributed rainfall, more than soil fertility has been shown to be the most important yield-limiting factor (Clay et al., 2014; Clay, 2008; Munodawafa, 2012). Low yields in plots with no fertilizer input in the LR season could be attributed to plants continuously using the available nutrients without external nutrient replenishment.

In the SR, no significant correlations were observed after compost application between selected soil properties with the yield. However soil pH was positively correlated with yield while Al negatively correlated with yield. This shows that acidity and high Al in soils pose a challenge for legumes symbiotic as bacteria are sensitive to acidity. Aluminum (Al) toxicity is widespread in acidic soils where the common bean is produced and it is a limiting factor for crop production and symbiotic nitrogen fixation (Mendoza-Soto et al., 2015). Several studies have demonstrated reduced growth of different symbiotic bacteria in acidic and aluminium soils, both in the laboratory and field conditions (Ferreira et al., 2012; Mendoza-Soto et al., 2015).

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

- The study has demonstrated the occurrence of varying population levels of fast growing symbiotic bacteria in soils of western Kenya. The population levels were also influenced by some soil factors.
- High genetic diversity of indigenous bacteria nodulating common bean was observed in this study. The result indicates the high promiscuity of common bean in its symbiotic relationship with different strains of soil bacteria.
- Phenotypic identification of symbiotic bacteria should be carried out in combination with other more informative techniques. In this study, some of the LNB with typical morphological and cultural characteristics similar to those of rhizobia were later confirmed to be non rhizobial bacteria. This illustrates that phenotypic characteristics only provide primary basis for classification.
- In the current study, all the native symbiotic bacteria were highly effective in nitrogen fixation compared to the commercial inoculant, CIAT 899. The results demonstrate that *Enterobacter hormaechei* and *Klebsiella variicola* are potentially superior to the two commercial inoculants, CIAT 899 and strain 446.
- Bean yields in the SR were higher in Urea treated plants compared to water hyacinth compost. However in the LR water hyacinth compost recorded the highest yield increment than Urea. In addition water hyacinth compost promoted nodulation in both the SR and LR seasons.

6.2 Recommendations

- The knowledge on the abundance and characteristics of native symbiotic bacteria in soils of western Kenya should be exploited to develop strategies that will improve the BNF for increasing the yield of common bean.
- The results in this study emphasize the need to use a number of trap host species with varying symbiotic bacteria affinities in order to capture the full composition and diversity of indigenous populations.
- The current study has demonstrated the need to employ a combination of both phenotypic and molecular techniques for a proper identification of symbiotic bacteria.
- The native symbiotic bacteria isolated in this study with superior SE compared to the commercial inoculants should be subjected to further greenhouse and field trials to ascertain their potential and stability in N fixation. More emphasis is required to popularize this cheap and eco-friendly technology to be adopted by majority of smallholder farmers in western Kenya.
- The study has also shown the potential of water hyacinth compost in improving yield of beans and therefore local smallholder farmers should be encouraged to adopt its use as an alternative to the expensive inorganic N sources. Further research should focus on the evaluating the effect of combining the compost with mineral fertilizers on the yield of common bean.

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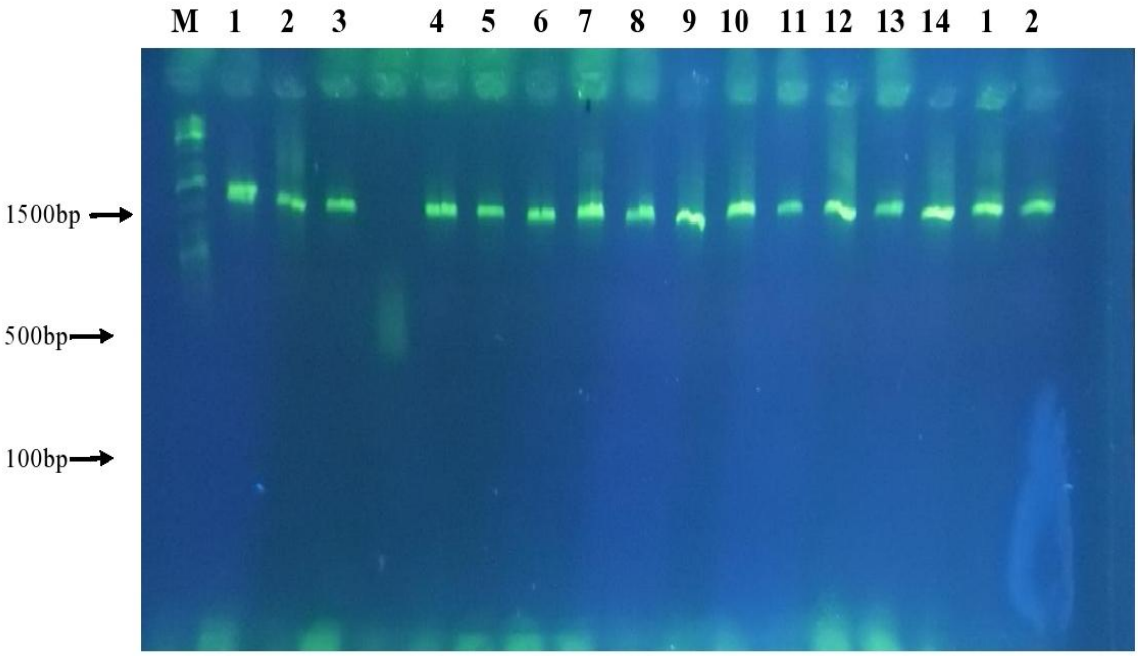
APPENDICES

Appendix 1: Gel photo of amplified 16S PCR products pure rhizobia isolates



Key: M-100bp Marker

Appendix 2: Consensus sequences for the Kisumu isolates

>*Bacillus aryabhattai* (KP027678)
CTATGACGTTAGCGGCGGACGGGTGAGTAACACGTGGGCAACCTGCCTGTAAG
ACTGGGATAACTTCGGGAAACCGAAGCTAATACCGGATAGGATCTTCTCCTTCA
TGGGAGATGATTGAAAGATGGTTTCGGCTATCACTTACAGATGGGCCCGCGGTG
CATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCATAGCCGACC
TGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGG
AGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCC
GCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAA
GTACGAGAGTAACTGCTCGTACCTTGACGGTACCTAACCAGAAAGCCACGGCTA
ACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTA
TTGGGCGTAAAGCGCGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCACGG
CTCAACCGTGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAGAGAAAA
GCGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGT
GGCGAAGGCGGCTTTTTGGTCTGTAACTGACGCTGAGGCGCGAAAGCGTGGGG
AGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAA
GTGTTAGAGGGTTTCCGCCCTTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCC
TGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCAC
AAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTC
TTGACATCCTCTGACAACTCTAGAGATAGAGCGTTCCCCTTCGGGGGACAGAGT
GACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTC
CCGCAACGAGCGCAACCCTTGATCTTAGTTGCCAGCATTCAGTTGGGCACTCTA
AGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCA
TGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGATGGTACAAAGGGCTG
CAAGACCGCGAGGTCAAGCCAATCCCATAAAACCATTCTCAGTTCGGATTGTAG
GCTGCAACTCGCCTACATGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGC
CGCGGTGAATACGTTCCCG

>*Rhizobium leguminosarum* (KP027679)
CCGCAAGGGGAGCGGCAGACGGGTGAGTAACGCGTGGGAATCTACCCTTGACT
ACGGAATAACGCAGGGAAACTTGTGCTAATACCGTATGTGTCCTTCGGGAGAAA
GATTTATCGGTCAAGGATGAGCCCGCGTTGGATTAGCTAGTTGGTGGGGTAAAG
GCCTACCAAGGCGACGATCCATAGCTGGTCTGAGAGGATGATCAGCCACATTGG
GACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTGGGGAATATTGGAC
AATGGGCGCAAGCCTGATCCAGCCATGCCGCGTGAGTGATGAAGGCCCTAGGG
TTGTAAAGCTCTTTCACCGGAGAAGATAATGACGGTATCCGGAGAAGAAGCCCC
GGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGGGCTAGCGTTGTTCGG
AATTACTGGGCGTAAAGCGCACGTAGGCGGATCGATCAGTCAGGGGTGAAATC
CCAGGGCTCAACCCTGGAACTGCCTTTGATACTGTCGATCTGGAGTATGGAAGA
GGTGAGTGGAATTCCGAGTGTAGAGGTGAAATTCGTAGATATTCGGAGGAACA
CCAGTGGCGAAGGCGGCTCACTGGTCCATTACTGACGCTGAGGTGCGAAAGCGT
GGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAATG
TTAGCCGTCGGGCAGTATACTGTTCGGTGGCGCAGCTAACGCATTAAACATTCC
GCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCCG

CACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCAG
CCCTTGACATGCCCGGCTACTTGCAGAGATGCAAGGTTCCCTTCGGGGACCGGG
ACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGT
CCCGCAACGAGCGCAACCCTCGCCCTTAGTTGCCAGCATTCAGTTGGGCACTCT
AAGGGGACTGCCGGTGATAAGCCGAGAGGAAGGTGGGGATGACGTCAAGTCCT
CATGGCCCTTACGGGCTGGGCTACACACGTGCTACAATGGTGGTGACAGTGGGC
AGCGAGCACGCGAGTGTGAGCTAATCTCCAAAAGCCATCTCAGTTCGGATTGCA
CTCTGCAACTCGAGTGCATGAAGTTGGAATCGCTAGTAATCGCGGATCAGCATG
CCGCGGTGAATACGTTCCCG

>*Rhizobium tropici* (KP027680)
CCGCAAGGGGAGCGGCAGACGGGTGAGTAACGCGTGGGAATCTACCTTTTGCT
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GATTTATCGGCAAGAGATGAGCCCGCGTTGGATTAGCTAGTTGGTGGGGTAAAG
GCCTACCAAGGCGACGATCCATAGCTGGTCTGAGAGGATGATCAGCCACATTGG
GACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTGGGGAATATTGGAC
AATGGGCGCAAGCCTGATCCAGCCATGCCGCGTGAGTGATGAAGGCCCTAGGG
TTGTAAAGCTCTTTCACCGGAGAAGATAATGACGGTATCCGGAGAAGAAGCCCC
GGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGGGCTAGCGTTGTTCGG
AATTACTGGGCGTAAAGCGCACGTAGGCGGATCGATCAGTCAGGGGTGAAATC
CCAGGGCTCAACCCTGGAACTGCCTTTGATACTGTCGATCTGGAGTATGGAAGA
GGTGAGTGGAATTCCGAGTGTAGAGGTGAAATTCGTAGATATTCGGAGGAACA
CCAGTGGCGAAGGCGGCTCACTGGTCCATTACTGACGCTGAGGTGCGAAAGCGT
GGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAATG
TTAGCCGTCGGGCAGTATACTGTTCGGTGGCGCAGCTAACGCATTAAACATTCC
GCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCCG
CACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCAG
CCCTTGACATCCTGTGTTACCTCTAGAGATAGGGGGTCCACTTCGGTGGCGCAG
AGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGT
CCCGCAACGAGCGCAACCCTCGCCCTTAGTTGCCAGCATTCAGTTGGGCACTCT
AAGGGGACTGCCGGTGATAAGCCGAGAGGAAGGTGGGGATGACGTCAAGTCCT
CATGGCCCTTACGGGCTGGGCTACACACGTGCTACAATGGTGGTGACAGTGGGC
AGCGAGCACGCGAGTGTGAGCTAATCTCCAAAAGCCATCTCAGTTCGGATTGCA
CTCTGCAACTCGAGTGCATGAAGTTGGAATCGCTAGTAATCGCGGATCAGCATG
CCGCGGTGAATACGTTCCCG

>*Rhizobium* sp. (KP027681)
CCGCAAGGGGAGTGGCAGACGGGTGAGTAACGCGTGGGAACATACCCTTTCCT
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GGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTGGGGAATATTGGA
CAATGGGCGCAAGCCTGATCCAGCCATGCCGCGTGAGTGATGAAGGCCTTAGG
GTTGTAAAGCTCTTTCACCGGAGAAGATAATGACGGTATCCGGAGAAGAAGCCC
CGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGGGCTAGCGTTGTTCG
GAATTACTGGGCGTAAAGCGCACGTAGGCGGATATTTAAGTCAGGGGTGAAAT
CCCAGAGCTCAACTCTGGAACTGCCTTTGATACTGGGTATCTTGAGTATGGAAG
AGGTAAGTGGAATTCCGAGTGTAGAGGTGAAATTCGTAGATATTCGGAGGAAC
ACCAGTGGCGAAGGCGGCTTACTGGTCCATTACTGACGCTGAGGTGCGAAAGCG
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CGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCC
GCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCA
GCTCTTGACATTCGGGGTATGGGCATTGGAGACGATGTCCTTCAGTTAGGCTGG
CCCCAGAACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGT
TAAGTCCCGCAACGAGCGCAACCCTCGCCCTTAGTTGCCAGCATTTAGTTGGGC
ACTCTAAGGGGACTGCCGGTGATAAGCCGAGAGGAAGGTGGGGATGACGTCAA
GTCCTCATGGCCCTTACGGGCTGGGCTACACACGTGCTACAATGGTGGTGACAG
TGGGCAGCGAGACAGCGATGTCGAGCTAATCTCCAAAAGCCATCTCAGTTCGGA
TTGCACTCTGCAACTCGAGTGCATGAAGTTGGAATCGCTAGTAATCGCAGATCA
GCATGCTGCGGTGAATACGTTCCCG

>*Enterobacter hormaechei* (KP027682)
TTTGCTGACGAGTGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAG
GGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAA
GAGGGGGACCTTCGGGCCTCTTGCCATCGGATGTGCCCAGATGGGATTAGCTAG
TAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATG
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AGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGCGATAAGGTT
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CGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGG
AGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTACTCTTGACATCC
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CAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTAC
GAGTAGGGCTACACACGTGCTACAATGGCGCATACAAAGAGAAGCGACCTCGC
GAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATTGGAGTCTGCAACT
CGACTCCATGAAGTCGGAATCGCTAGTAATCGTGGATCAGAATGCCACGGTGAA
TACGTTCCCG

>*Klebsiella variicola* (KP027685)
TCGGGTGACGAGCGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGA
GGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAA
AGTGGGGGACCTTCGGGCCTCATGCCATCAGATGTGCCCAGATGGGATTAGCTG
GTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGAT
GACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAG
TGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTGTGA
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CAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGT
AAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGGGCTCAACCT
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ACAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTGTGAGACAGGTGCTG
CATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGC
GCAACCCTTATCCTTTGTTGCCAGCGGTTAGGCCGGGAACTCAAAGGAGACTGC
CAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTAC
GACCAGGGCTACACACGTGCTACAATGGCATATACAAAGAGAAGCGACCTCGC
GAGAGCAAGCGGACCTCATAAAGTATGTCGTAGTCCGGATTGGAGTCTGCAACT
CGACTCCATGAAGTCGGAATCGCTAGTAATCGTAGATCAGAATGCTACGGTGAA
TACGTTCCCG

>*Klebsiella* sp. (KP027684)
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>*Klebsiella variicola* (KP027683)
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Appendix 3: Consensus sequences for the MMUST isolates

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>*Klebsiella variicola* (KP027688)
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CCATGAAGTCGGAATCGCTAGTAATCGTAGATCAGAATGCTACGGTGAATACGT
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>*Klebsiella variicola* (KP027687)
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>*Klebsiella* sp. (KP027689)
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>*Rhizobium tropici* (KP027690)
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>*Rhizobium leguminosarum* (KP027691)
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Appendix 4: Authentication and SE experiments

Appendix 5: Field trials

Appendix 6: NCBI released flat files for Kisumu and MMUST symbiotic bacteria

1. LOCUS KP027678 1307 bp DNA linear BCT 26-FEB-2015

DEFINITION Bacillus aryabhattai strain KSM-007 16S ribosomal RNA gene, partial sequence.
ACCESSION KP027678
VERSION KP027678
KEYWORDS .
SOURCE Bacillus aryabhattai
ORGANISM Bacillus aryabhattai
Bacteria; Firmicutes; Bacilli; Bacillales; Bacillaceae; Bacillus.
REFERENCE 1 (bases 1 to 1307)
AUTHORS Kawaka,J.F., Dida,M.M., Opala,P.A., Ombori,O., Maingi,J. and Muoma,J.O.
TITLE Diversity of Native Rhizobia Nodulating Common Bean (Phaseolus vulgaris L.) in Soils of Western Kenya
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 1307)
AUTHORS Kawaka,J.F., Dida,M.M., Opala,P.A., Ombori,O., Maingi,J. and Muoma,J.O.
TITLE Direct Submission
JOURNAL Submitted (23-OCT-2014) Pure and Applied, Technical University of Mombasa, Tom Mboya, Mombasa +254, Kenya
FEATURES Location/Qualifiers
source 1..1307
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/db_xref="taxon:412384"
/country="Kenya"
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/product="16S ribosomal RNA"
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2. LOCUS KP027679 1254 bp DNA linear BCT 26-FEB-2015
DEFINITION Rhizobium leguminosarum strain KSM-004 16S ribosomal RNA gene, partial sequence.
ACCESSION KP027679
VERSION KP027679
KEYWORDS .
SOURCE Rhizobium leguminosarum
ORGANISM Rhizobium leguminosarum
Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales; Rhizobiaceae; Rhizobium/Agrobacterium group; Rhizobium.
REFERENCE 1 (bases 1 to 1254)
AUTHORS Kawaka,J.F., Dida,M.M., Opala,P.A., Ombori,O., Maingi,J. and Muoma,J.O.
TITLE Diversity of Native Rhizobia Nodulating Common Bean (Phaseolus vulgaris L.) in Soils of Western Kenya
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 1254)
AUTHORS Kawaka,J.F., Dida,M.M., Opala,P.A., Ombori,O., Maingi,J. and Muoma,J.O.
TITLE Direct Submission
JOURNAL Submitted (23-OCT-2014) Pure and Applied, Technical University of Mombasa, Tom Mboya, Mombasa +254, Kenya
FEATURES Location/Qualifiers
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3. LOCUS KP027680 1254 bp DNA linear BCT 26-FEB-2015

DEFINITION Rhizobium tropici strain KSM-002 16S ribosomal RNA gene, partial sequence.

ACCESSION KP027680

VERSION KP027680

KEYWORDS .

SOURCE Rhizobium tropici

ORGANISM Rhizobium tropici
Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales; Rhizobiaceae; Rhizobium/Agrobacterium group; Rhizobium.

REFERENCE 1 (bases 1 to 1254)

AUTHORS Kawaka,J.F., Dida,M.M., Opala,P.A., Ombori,O., Maingi,J. and Muoma,J.O.

TITLE Diversity of Native Rhizobia Nodulating Common Bean (Phaseolus vulgaris L.) in Soils of Western Kenya

JOURNAL Unpublished

REFERENCE 2 (bases 1 to 1254)

AUTHORS Kawaka,J.F., Dida,M.M., Opala,P.A., Ombori,O., Maingi,J. and Muoma,J.O.

TITLE Direct Submission
JOURNAL Submitted (23-OCT-2014) Pure and Applied, Technical University of Mombasa, Tom Mboya, Mombasa +254, Kenya
FEATURES Location/Qualifiers
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241 cgggaggcag cagtggggaa tattggacaa tgggcgcaag cctgatccag ccatgccgcg
301 tgagtgatga aggccctagg gttgtaaagc tctttcaccg gagaagataa tgacggtatc
361 cggagaagaa gccccggcta acttcgtgcc agcagccgcg gtaatacgaa gggggctagc
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601 ggcggctcac tggtccatta ctgacgctga ggtgcgaaag cgtggggagc aaacaggatt
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841 gcgcagaacc ttaccagccc ttgacatcct gtgttacctc tagagatagg gggtccactt
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1021 aggggactgc cggtgataag ccgagaggaa ggtggggatg acgtcaagtc ctcatggccc
1081 ttacgggctg ggctacacac gtgctacaat ggtggtgaca gtgggcagcg agcacgcgag
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4.LOCUS KP027681 1258 bp DNA linear BCT 26-FEB-2015
DEFINITION Rhizobium sp. KSM-006 16S ribosomal RNA gene, partial sequence.
ACCESSION KP027681
VERSION KP027681
KEYWORDS .
SOURCE Rhizobium sp. KSM-006
ORGANISM Rhizobium sp. KSM-006
Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales;

Rhizobiaceae; Rhizobium/Agrobacterium group; Rhizobium.

REFERENCE 1 (bases 1 to 1258)
AUTHORS Kawaka,J.F., Dida,M.M., Opala,P.A., Ombori,O., Maingi,J. and Muoma,J.O.
TITLE Diversity of Native Rhizobia Nodulating Common Bean (Phaseolus vulgaris L.) in Soils of Western Kenya
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 1258)
AUTHORS Kawaka,J.F., Dida,M.M., Opala,P.A., Ombori,O., Maingi,J. and Muoma,J.O.
TITLE Direct Submission
JOURNAL Submitted (23-OCT-2014) Pure and Applied, Technical University of Mombasa, Tom Mboya, Mombasa +254, Kenya
FEATURES Location/Qualifiers
source 1..1258
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/mol_type="genomic DNA"
/strain="KSM-006"
/db_xref="taxon:1616816"
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rRNA <1..>1258
/product="16S ribosomal RNA"
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61 agctccggga aactggaatt aataccgcat acgccctacg ggggaaagat ttatcgggga
121 aggattggcc cgcgttggat tagctagttg gtggggtaaa ggcctaccaa ggcgacgatc
181 catagctggt ctgagaggat gatcagccac attgggactg agacacggcc caaactccta
241 cgggaggcag cagtggggaa tattggacaa tgggcgcaag cctgatccag ccatgccgcg
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421 gttgttcgga attactgggc gtaaagcgca cgtaggcgga tatttaagtc aggggtgaaa
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601 ggcggcttac tggtccatta ctgacgctga ggtgcgaaag cgtggggagc aaacaggatt
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901 gttaggctgg ccccagaaca ggtgctgcat ggctgtcgtc agctcgtgtc gtgagatgtt
961 gggttaagtc ccgcaacgag cgcaaccctc gcccttagtt gccagcattt agttgggcac
1021 tctaagggga ctgccggtga taagccgaga ggaaggtggg gatgacgtca agtcctcatg
1081 gcccttacgg gctgggctac acacgtgcta caatggtggt gacagtgggc agcgagacag
1141 cgatgtcgag ctaatctcca aaagccatct cagttcggat tgcactctgc aactcgagtg

1201 catgaagttg gaatcgctag taatcgcaga tcagcatgct gcggtgaata cgttcccg

5. LOCUS KP027682 1295 bp DNA linear BCT 26-FEB-2015
DEFINITION Enterobacter hormaechei strain KSM-001 16S ribosomal RNA gene, partial sequence.
ACCESSION KP027682
VERSION KP027682
KEYWORDS .
SOURCE Enterobacter hormaechei
ORGANISM Enterobacter hormaechei
Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales; Enterobacteriaceae; Enterobacter; Enterobacter cloacae complex.
REFERENCE 1 (bases 1 to 1295)
AUTHORS Kawaka,J.F., Dida,M.M., Opala,P.A., Ombori,O., Maingi,J. and Muoma,J.O.
TITLE Diversity of Native Rhizobia Nodulating Common Bean (Phaseolus vulgaris L.) in Soils of Western Kenya
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 1295)
AUTHORS Kawaka,J.F., Dida,M.M., Opala,P.A., Ombori,O., Maingi,J. and Muoma,J.O.
TITLE Direct Submission
JOURNAL Submitted (23-OCT-2014) Pure and Applied, Technical University of Mombasa, Tom Mboya, Mombasa +254, Kenya
FEATURES Location/Qualifiers
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/mol_type="genomic DNA"
/strain="KSM-001"
/db_xref="taxon:158836"
/country="Kenya"
rRNA <1..>1295
/product="16S ribosomal RNA"
ORIGIN
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61 aactactgga aacggtagct aataccgcat aacgtcgcaa gaccaaagag ggggaccttc
121 gggcctcttg ccatcggatg tgcccagatg ggattagcta gtaggtgggg taacggctca
181 cctaggcgac gatccctagc tggtctgaga ggatgaccag ccacactgga actgagacac
241 ggtccagact cctacgggag gcagcagtgg ggaatattgc acaatgggcg caagcctgat
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421 ctccgtgcca gcagccgcgg taatacggag ggtgcaagcg ttaatcggaa ttactgggcg
481 taaagcgcac gcaggcggtc tgtcaagtcg gatgtgaaat ccccgggctc aacctgggaa
541 ctgcattcga aactggcagg ctagagtctt gtagaggggg gtagaattcc aggtgtagcg
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841 ccgcacaagc ggtggagcat gtggtttaat tcgatgcaac gcgaagaacc ttacctactc
901 ttgacatcca gagaacttac cagagatggt ttggtgcctt cgggaactct gagacaggtg
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1021 acccttatcc tttgttgcca gcggttaggc cgggaactca aaggagactg ccagtgataa
1081 actggaggaa ggtggggatg acgtcaagtc atcatggccc ttacgagtag ggctacacac
1141 gtgctacaat ggcgcataca aagagaagcg acctcgcgag agcaagcgga cctcataaag
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1261 tcgtggatca gaatgccacg gtgaatacgt tcccg
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6. LOCUS KP027683 1295 bp DNA linear BCT 26-FEB-2015
DEFINITION Klebsiella variicola strain KSM-005 16S ribosomal RNA gene, partial sequence.
ACCESSION KP027683
VERSION KP027683
KEYWORDS .
SOURCE Klebsiella variicola
ORGANISM Klebsiella variicola
Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales; Enterobacteriaceae; Klebsiella.
REFERENCE 1 (bases 1 to 1295)
AUTHORS Kawaka,J.F., Dida,M.M., Opala,P.A., Ombori,O., Maingi,J. and Muoma,J.O.
TITLE Diversity of Native Rhizobia Nodulating Common Bean (Phaseolus vulgaris L.) in Soils of Western Kenya
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 1295)
AUTHORS Kawaka,J.F., Dida,M.M., Opala,P.A., Ombori,O., Maingi,J. and Muoma,J.O.
TITLE Direct Submission
JOURNAL Submitted (23-OCT-2014) Pure and Applied, Technical University of Mombasa, Tom Mboya, Mombasa +254, Kenya
FEATURES Location/Qualifiers
source 1..1295
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/mol_type="genomic DNA"
/strain="KSM-005"
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/country="Kenya"
rRNA <1..>1295
/product="16S ribosomal RNA"
ORIGIN

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 121 gggcctcatg ccatcagatg tgcccagatg ggattagctg gtaggtgggg taacggctca
 181 cctaggcgac gatccctagc tggtctgaga ggatgaccag ccacactgga actgagacac
 241 ggtccagact cctacgggag gcagcagtgg ggaatattgc acaatgggcg caagcctgat
 301 gcagccatgc cgcgtgtgtg aagaaggcct tcgggttgta aagcactttc agcggggagg
 361 aaggcggtga ggttaataac ctcatcgatt gacgttaccc gcagaagaag caccggctaa
 421 ctccgtgcca gcagccgcgg taatacggag ggtgcaagcg ttaatcggaa ttactgggcg
 481 taaagcgcac gcaggcggtc tgtcaagtcg gatgtgaaat ccccgggctc aacctgggaa
 541 ctgcattcga aactggcagg ctagagtctt gtagaggggg gtagaattcc aggtgtagcg
 601 gtgaaatgcg tagagatctg gaggaatacc ggtggcgaag gcggcccccc ggacaaagac
 661 tgacgctcag gtgcgaaagc gtggggagca aacaggatta gataccctgg tagtccacgc
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 781 taaatcgacc gcctggggag tacggccgca aggttaaaac tcaaatgaat tgacgggggc
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 961 ctgcatggct gtcgtcagct cgtgttgtga aatgttgggt taagtcccgc aacgagcgca
1021 acccttatcc tttgttgcca gcggttaggc cgggaactca aaggagactg ccagtgataa
1081 actggaggaa ggtggggatg acgtcaagtc atcatggccc ttacgaccag ggctacacac
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7. LOCUS KP027684 1295 bp DNA linear BCT 26-FEB-2015

DEFINITION Klebsiella sp. KSM-008 16S ribosomal RNA gene, partial sequence.
ACCESSION KP027684
VERSION KP027684
KEYWORDS .
SOURCE Klebsiella sp. KSM-008
ORGANISM Klebsiella sp. KSM-008
Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales;
Enterobacteriaceae; Klebsiella.
REFERENCE 1 (bases 1 to 1295)
AUTHORS Kawaka,J.F., Dida,M.M., Opala,P.A., Ombori,O., Maingi,J. and
Muoma,J.O.
TITLE Diversity of Native Rhizobia Nodulating Common Bean (Phaseolus
vulgaris L.) in Soils of Western Kenya
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 1295)
AUTHORS Kawaka,J.F., Dida,M.M., Opala,P.A., Ombori,O., Maingi,J. and
Muoma,J.O.
TITLE Direct Submission
JOURNAL Submitted (23-OCT-2014) Pure and Applied, Technical University of
Mombasa, Tom Mboya, Mombasa +254, Kenya

FEATURES Location/Qualifiers
source 1..1295
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/db_xref="taxon:1616814"
/country="Kenya"
rRNA <1..>1295
/product="16S ribosomal RNA"
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121 gggcctcatg ccatcagatg tgcccagatg ggattagctg gtaggtgggg taacggctca
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421 ctccgtgcca gcagccgcgg taatacggag ggtgcaagcg ttaatcggaa ttactgggcg
481 taaagcgcac gcaggcggtc tgtcaagtcg gatgtgaaat ccccgggctc aacctgggaa
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721 tgtaaacgat gtcgatttgg aggttgtgcc cttgaggcgt ggcttccgga gctaacgcgt
781 taaatcgacc gcctggggag tacggccgca aggttaaaac tcaaatgaat tgacgggggc
841 ccgcacaagc ggtggagcat gtggtttaat tcgatgcaac gcgaagaacc ttacctggtc
901 ttgacatcca cagaactttc cagagatgga ttggtgcctt cgggaactgt gagacaggtg
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1021 acccttatcc tttgttgcca gcggttaggc cgggaactca aaggagactg ccagtgataa
1081 actggaggaa ggtggggatg acgtcaagtc atcatggccc ttacgaccag ggctacacac
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8. LOCUS KP027685 1295 bp DNA linear BCT 26-FEB-2015

DEFINITION Klebsiella variicola strain KSM-003 16S ribosomal RNA gene, partial sequence.
ACCESSION KP027685
VERSION KP027685
KEYWORDS .
SOURCE Klebsiella variicola
ORGANISM Klebsiella variicola
Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales; Enterobacteriaceae; Klebsiella.
REFERENCE 1 (bases 1 to 1295)
AUTHORS Kawaka,J.F., Dida,M.M., Opala,P.A., Ombori,O., Maingi,J. and

Muoma,J.O.
TITLE Diversity of Native Rhizobia Nodulating Common Bean (Phaseolus vulgaris L.) in Soils of Western Kenya
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 1295)
AUTHORS Kawaka,J.F., Dida,M.M., Opala,P.A., Ombori,O., Maingi,J. and Muoma,J.O.
TITLE Direct Submission
JOURNAL Submitted (23-OCT-2014) Pure and Applied, Technical University of Mombasa, Tom Mboya, Mombasa +254, Kenya
FEATURES Location/Qualifiers
source 1..1295
/organism="Klebsiella variicola"
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241 ggtccagact cctacgggag gcagcagtgg ggaatattgc acaatgggcg caagcctgat
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9. LOCUS KP027686 1324 bp DNA linear BCT 26-FEB-2015
DEFINITION Pantoea dispersa strain MMUST-001 16S ribosomal RNA gene, partial

sequence.
ACCESSION KP027686
VERSION KP027686
KEYWORDS .
SOURCE Pantoea dispersa
ORGANISM Pantoea dispersa
Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales;
Enterobacteriaceae; Pantoea.
REFERENCE 1 (bases 1 to 1324)
AUTHORS Kawaka,J.F., Dida,M.M., Opala,P.A., Ombori,O., Maingi,J. and
Muoma,J.O.
TITLE Diversity of Native Rhizobia Nodulating Common Bean (Phaseolus
vulgaris L.) in Soils of Western Kenya
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 1324)
AUTHORS Kawaka,J.F., Dida,M.M., Opala,P.A., Ombori,O., Maingi,J. and
Muoma,J.O.
TITLE Direct Submission
JOURNAL Submitted (24-OCT-2014) Pure and Applied, Technical University of
Mombasa, Tom Mboya, Mombasa +254, Kenya
FEATURES Location/Qualifiers
source 1..1324
/organism="Pantoea dispersa"
/mol_type="genomic DNA"
/strain="MMUST-001"
/db_xref="taxon:59814"
/country="Kenya"
rRNA <1..>1324
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10. LOCUS KP027687 1324 bp DNA linear BCT 26-FEB-2015

DEFINITION Klebsiella variicola strain MMUST-005 16S ribosomal RNA gene, partial sequence.
ACCESSION KP027687
VERSION KP027687
KEYWORDS .
SOURCE Klebsiella variicola
ORGANISM Klebsiella variicola
Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales; Enterobacteriaceae; Klebsiella.
REFERENCE 1 (bases 1 to 1324)
AUTHORS Kawaka,J.F., Dida,M.M., Opala,P.A., Ombori,O., Maingi,J. and Muoma,J.O.
TITLE Diversity of Native Rhizobia Nodulating Common Bean (Phaseolus vulgaris L.) in Soils of Western Kenya
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 1324)
AUTHORS Kawaka,J.F., Dida,M.M., Opala,P.A., Ombori,O., Maingi,J. and Muoma,J.O.
TITLE Direct Submission
JOURNAL Submitted (24-OCT-2014) Pure and Applied, Technical University of Mombasa, Tom Mboya, Mombasa +254, Kenya
FEATURES Location/Qualifiers
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/db_xref="taxon:244366"
/country="Kenya"
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121 tcatgccatc agatgtgccc agatgggatt agctggtagg tggggtaacg gctcacctag
181 gcgacgatcc ctagctggtc tgagaggatg accagccaca ctggaactga gacacggtcc

241 agactcctac gggaggcagc agtggggaat attgcacaat gggcgcaagc ctgatgcagc
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361 ggtgaggtta ataacctcat cgattgacgt tacccgcaga agaagcaccg gctaactccg
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1321 ggag
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11. LOCUS KP027688 1324 bp DNA linear BCT 26-FEB-2015

DEFINITION Klebsiella variicola strain MMUST-004 16S ribosomal RNA gene, partial sequence.
ACCESSION KP027688
VERSION KP027688
KEYWORDS .
SOURCE Klebsiella variicola
ORGANISM Klebsiella variicola
Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales; Enterobacteriaceae; Klebsiella.
REFERENCE 1 (bases 1 to 1324)
AUTHORS Kawaka,J.F., Dida,M.M., Opala,P.A., Ombori,O., Maingi,J. and Muoma,J.O.
TITLE Diversity of Native Rhizobia Nodulating Common Bean (Phaseolus vulgaris L.) in Soils of Western Kenya
JOURNAL Unpublished
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TITLE Direct Submission
JOURNAL Submitted (24-OCT-2014) Pure and Applied, Technical University of Mombasa, Tom Mboya, Mombasa +254, Kenya
FEATURES Location/Qualifiers
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1321 ggag
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12. LOCUS KP027689 1324 bp DNA linear BCT 26-FEB-2015

DEFINITION Klebsiella sp. MMUST-002 16S ribosomal RNA gene, partial sequence.
ACCESSION KP027689
VERSION KP027689
KEYWORDS .
SOURCE Klebsiella sp. MMUST-002
ORGANISM Klebsiella sp. MMUST-002
Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales;
Enterobacteriaceae; Klebsiella.
REFERENCE 1 (bases 1 to 1324)
AUTHORS Kawaka,J.F., Dida,M.M., Opala,P.A., Ombori,O., Maingi,J. and
Muoma,J.O.
TITLE Diversity of Native Rhizobia Nodulating Common Bean (Phaseolus
vulgaris L.) in Soils of Western Kenya

JOURNAL Unpublished
REFERENCE 2 (bases 1 to 1324)
AUTHORS Kawaka,J.F., Dida,M.M., Opala,P.A., Ombori,O., Maingi,J. and Muoma,J.O.
TITLE Direct Submission
JOURNAL Submitted (24-OCT-2014) Pure and Applied, Technical University of Mombasa, Tom Mboya, Mombasa +254, Kenya
FEATURES Location/Qualifiers
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/strain="MMUST-002"
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13. LOCUS KP027690 1283 bp DNA linear BCT 26-FEB-2015
DEFINITION Rhizobium tropici strain MMUST-006 16S ribosomal RNA gene, partial sequence.
ACCESSION KP027690

VERSION KP027690
KEYWORDS .
SOURCE Rhizobium tropici
ORGANISM Rhizobium tropici
Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales;
Rhizobiaceae; Rhizobium/Agrobacterium group; Rhizobium.
REFERENCE 1 (bases 1 to 1283)
AUTHORS Kawaka,J.F., Dida,M.M., Opala,P.A., Ombori,O., Maingi,J. and
Muoma,J.O.
TITLE Diversity of Native Rhizobia Nodulating Common Bean (Phaseolus
vulgaris L.) in Soils of Western Kenya
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 1283)
AUTHORS Kawaka,J.F., Dida,M.M., Opala,P.A., Ombori,O., Maingi,J. and
Muoma,J.O.
TITLE Direct Submission
JOURNAL Submitted (24-OCT-2014) Pure and Applied, Technical University of
Mombasa, Tom Mboya, Mombasa +254, Kenya
FEATURES Location/Qualifiers
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14. LOCUS KP027691 1283 bp DNA linear BCT 26-FEB-2015

DEFINITION Rhizobium leguminosarum bv. viciae strain MMUST-003 16S ribosomal RNA gene, partial sequence.
ACCESSION KP027691
VERSION KP027691
KEYWORDS .
SOURCE Rhizobium leguminosarum bv. viciae
ORGANISM Rhizobium leguminosarum bv. viciae
Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales;
Rhizobiaceae; Rhizobium/Agrobacterium group; Rhizobium.
REFERENCE 1 (bases 1 to 1283)
AUTHORS Kawaka,J.F., Dida,M.M., Opala,P.A., Ombori,O., Maingi,J. and Muoma,J.O.
TITLE Diversity of Native Rhizobia Nodulating Common Bean (Phaseolus vulgaris L.) in Soils of Western Kenya
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 1283)
AUTHORS Kawaka,J.F., Dida,M.M., Opala,P.A., Ombori,O., Maingi,J. and Muoma,J.O.
TITLE Direct Submission
JOURNAL Submitted (24-OCT-2014) Pure and Applied, Technical University of Mombasa, Tom Mboya, Mombasa +254, Kenya
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