POTENTIAL USE OF ENDOPHYTIC BACTERIA AS BIOFERTILIZER FOR SUSTAINABLE BANANA (MUSA SPP.) PRODUCTION

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Abstract

Bananas and plantains are of special significance to human society and are ranked as fourth most important food in the world after rice, wheat, and maize. Increased trade in local, regional and international markets has also made them an important cash crop, and in some cases the only source of income for rural populations. In Kenya, production of bananas is constrained by among others declining soil fertility coupled with high cost of fertilizers. A sustainable complementary response to declining soil fertility would be to increase the biological inputs of nutrients by exploitation of microorganisms, which are largely untapped natural resources for plant growth promotion. Endophytic bacteria are known to enhance plant growth in non-leguminous crops and improve their nutrition through nitrogen fixation, phosphate solubilisation or siderophore production (iron chelation). Besides biofertilization, endophytic bacteria are also reported to promote plant growth and yield through direct production of phytohormones, or enzymes, or indirectly through biological control of plant pests and diseases or induced resistance response (biotization). In return, the plant protects endophytes and provides them with nutrients in form of photosynthates. Endophytes are increasingly gaining scientific and commercial interest because of this potential to improve plant quality and growth and their close association with internal tissues of host plant. This paper reviews the potential use of endophytic bacteria as biofertilizers for sustainable banana production.

Key words**:** microbial inoculants, diazotrophic bacteria, phosphate-solubilizing microorganisms, siderophores

Introduction

Bananas and plantains are of special significance to human society being the fourth most important food in the world after rice, wheat, and maize (Scot *et al*., 2006). Increased trade in local, regional and international markets has also made them an important cash crop, and in some cases the only source of income for rural populations (Frison and Sharrock, 2001). In Kenya, however, banana production is constrained by among others, declining soil fertility (Vanlauwe and Giller, 2006; Okumu, 2008). This is brought about by insufficient application of manure due to cost implications especially for the farmers without livestock, and limited use of inorganic fertilizers, which are expensive and therefore unaffordable for most banana farmers.

A sustainable complementary approach would be to increase the biological inputs of nutrients by exploitation of microorganisms, which are largely untapped natural resources for plant growth promotion (Thomas and Soly, 2009). Notably, there is a renewed scientific and commercial interest in the use of microbes especially the endophytes because of their potential to improve plant quality and growth and their close association with internal tissues of host plant (Schulz *et al*., 1998 & 1999). According to Azevedo *et al*., (2000), most researches on endophytes have been carried out using hosts from temperate countries, while data available from tropical regions remain scarce. Tropical plants host a great diversity of endophytic microorganisms, many of them not yet classified and possibly belonging to new genera and species. This review will document and highlight the potential use of endophytic bacteria as biofertilizers in banana production in line with the principles guiding sustainable agricultural development.

Banana production in Kenya

In Kenya, area under banana production is about 63,290 ha with an estimated average yield of 19 tonnes per hectare as opposed to an average potential yield of 35-45 tonnes per hectare (FAOSTAT, 2011; HortiNews, 2013). Banana production in Kenya is constrained by several factors that include; insect pests such as the banana weevil (*Cosmopolites sordidus*), nematodes (*Radopholus similis*, *Pratylenchus goodeyi* and *Helicotylenchus multicintus*), fungal diseases such as Fusarium wilt, poor crop husbandry, soil moisture deficits and declining soil fertility (Vanlauwe and Giller, 2006; Okumu, 2008; Kahangi, 2010). Okumu (2008) identified soil fertility as the major problem in tissue cultured banana production in Central Kenya, where yields depended more on soil fertility (67%) than either farm management (23%) or pests and diseases (10%). In Kenya, farmyard manure and mulching are used to maintain or increase soil organic matter reserves in banana production. However, according to Vanlauwe and Giller (2006), organic inputs alone cannot sustain crop production in resource-poor farming systems due to limitations in their quality and availability. Use of inorganic fertilizers is limited because they are expensive and therefore unaffordable for most banana farmers. A sustainable alternative approach would be to improve nutrient uptake by plants through utilization of microbial inoculants such as endophytic bacteria, which are in the plant system and once the seedlings have them, they will keep the banana plants healthy through auto fertilization.

Endophytic bacteria

Plants are naturally associated with mutualistic microbes that include endophytes. Endophytes have been defined as diverse microbes, most commonly fungi and bacteria (Wilson, 1995; Strobel and Daisy, 2003), which spend the entire or part of their life cycle living in internal plant tissues causing no apparent or immediate disease symptoms (Hallmann *et al*., 1997, Bacon and White, 2000; Long *et al*., 2008). These non-pathogenic associations could be beneficial, neutral or detrimental (Sturz *et al*., 2000).

Endophytic bacteria enter plants mainly through wounds, naturally occurring as a result of plant growth or through root hairs and at epidermal conjunctions. Besides providing entry avenues, wounds also create favourable conditions for the bacteria by allowing leakage of plant exudates that serve as a nutrient source for the bacteria (Hallmann *et al*., 1997). Other entry sites for endophytic bacteria include flowers, stomata and lenticels (Kluepfel, 1993). Once in the plant, the endophytic bacteria have an ecological advantage over the epiphytic bacteria in that they are protected from adverse external environmental conditions such as those associated with temperature, osmotic potentials, and ultraviolet radiation, which are major factors limiting long-term bacterial survival (Senthilkumar *et al*., 2011). The population density of endophytic bacteria is highly variable, depending mainly on the bacterial species and host genotypes but also on the host developmental stage, inoculum density, and environmental conditions (Pillay and Nowak, 1997; Tan *et al*., 2003). Molecular analysis has shown that plant defense responses also limit bacterial populations inside plants (Rosenblueth and Martínez-Romero, 2006). Senthilkumar *et al*. (2011) alluded to the fact that organisms occupying the endosphere have most likely been selected for this niche by the plant because of the beneficial effects they offer their host and their abilities to resist the effects of plant defense products.

Of the roughly 300,000 plant species found on earth, each plant is a host to one or more species of endophytic microbes, however only a few of these plants have been completely studied in regard to their endophytic biology (Senthilkumar *et al*. 2011). As a result, the opportunity to find novel and unique endophytic microbes amid numerous plants in different ecosystems is considerable. In bananas, genera and species of endophytic bacteria identified include: *Azospirillum brasilense* and *A. amazonense* (Weber *et al*., 1999), *Bacillus* spp. (Harish, 2008; Ngamau *et al*., 2012), *Burkholderia* spp. (Weber *et al*., 1999; Ting *et. al*., 2008), *Citrobacter* spp. (Martínez *et al*., 2003), *Enterobacter* spp. (Martínez *et al*., 2003; Ngamau *et al*., 2012), *Ewingella* spp. (Ngamau *et al*., 2012), *Herbaspirillum* spp. (Weber *et al*., 1999; Weber *et al*., 2001), *Klebsiella* spp. (Martínez *et al*., 2003; Rosenblueth *et al*., 2004; Ngamau *et al*., 2012), *Pseudomonas* spp. (Harish, 2008; Ting *et. al*., 2008; Ngamau *et al*., 2012), *Rahnella* spp. (Ngamau *et al*., 2012), *Raoultella* spp. (Ngamau *et al*., 2012), *Rhizobium* spp. (Martínez *et al*., 2003) and *Serratia* spp. (Ting *et. al*., 2008; Ngamau *et al*., 2012), *Yersinia* and *Yokenella* species (Ngamau *et al*., 2012).

Potential of Endophytic Bacteria as Biofertilizer

Endophytic bacteria are known to enhance plant growth in non-leguminous crops and improve their nutrition through nitrogen fixation, phosphate solubilization or siderophore production (iron chelation) (Sturz *et al*., 2000; Iniguez *et al*., 2004; Long *et al*., 2008). Besides biofertilization, endophytic bacteria are also reported to promote plant growth and yield through production of phytostimulators such as phytohormones, the cofactor pyrrolquinolinequinone (PQQ) and the volatile acetoin; or by producing stress controllers like the enzyme 1-aminocyclopropane- 1-carboxylate (ACC) deaminase, which facilitate plant growth and development by lowering plant ethylene levels; or indirectly through biological control of plant diseases or induced resistance response (biotization) (Long *et al*., 2008; Lugtenberg and Kamilova, 2009).

<u>Biological Nitrogen fixation</u>

The banana fruit crop is widely cultivated in tropical areas where high dosages of fertilizers are commonly applied, principally nitrogen (Weber *et al*., 1999 & 2001). Nevertheless, developing countries face the problem of high costs of chemical fertilizers. Biological nitrogen fixation could be an alternative for this crop system, once the plants are able to establish association with nitrogen fixing (diazotrophic) bacteria. Non-symbiotic (associative) N-fixing bacteria can be rhizosphere-based or endophytic. It has been suggested that endophytic N-fixing bacteria may be more important than rhizospheric bacteria in promoting plant growth because they escape competition with rhizosphere microorganisms and achieve close contact with the plant tissues (Döbereiner, 1992). As such, new research efforts have focused on identification of endophytic diazotrophs that are able to supply biologically fixed nitrogen directly to their host ensuring a highly efficient nitrogen uptake by the plant.

Nitrogen-fixing endophytic bacteria have been isolated from several groups of plants (Ladha and Reddy, 2000) since the isolation of the endophytic diazotrophic bacterium *Gluconacetobacter diazotrophicus* (previously known as *Acetobacter diazotrophicus*) from a Brazilian variety of sugarcane (James and Olivares, 1997). Weber *et al*. (1999) demonstrated the association of nitrogen-fixing bacteria with banana and pineapple plants with *Azospirillum amazonense, Azospirillum brasilense*, *Azospirillum lipoferum*, *Burkholderia* sp., *Herbaspirillum seropedicae* and other unidentified

Herbaspirillum species being isolated from banana plants. Later, Weber *et al*. (2007) demonstrated the potential of endophytic diazotrophic bacteria *Herbaspirillum* and *Burkholderia* as biofertilizer and biocontrol agents. Endophytic colonization promoted growth and reduced *Fusarium* wilt severity in banana plantlets. Ting *et al*. (2008) also demonstrated that endophytes (*Serratia* and *Fusarium oxysporum*) isolated from wild bananas can promote the growth of banana plantlets and render tolerance towards *Fusarium* wilt. Earlier work by Zuraida *et al*. (2000) also showed that treatment of banana plantlets with *Agrobacteria* and *Azospirillum* resulted in 79% and 11% increase in soluble nitrogen, respectively, as compared to the control. Similarly, the activity of nitrate reductase in both plant leaves and roots and chlorophyll content were increased. Ngamau *et al*. (2012) also demonstrated capacity of endophytic bacteria associated with bananas in Kenya to fix free nitrogen having grown on nitrogen-source free medium and showed varied nitrogenase activity.

However, nitrogen-fixing endophytic bacteria seem to constitute only a small proportion of total endophytic bacteria (Ladha *et al*., 1983; Barraquio *et al*., 1997; Martínez *et al*., 2003) and increasing N_2-fixing populations in plants has been considered as a means of increasing nitrogen fixation.

<u>Phosphate solubilization</u>

Phosphorus is one of the most essential elements for the growth and development of plants. Phosphorus exists in soil as phosphate anions, which are extremely reactive. The phosphate anions are immobilized by soil cations and thus made unavailable for plants. Certain microorganisms are capable of solubilizing the unavailable form of phosphorus into available form thus promoting its uptake by plants (Gyaneshwar *et al*., 2002). Such microorganisms are called phosphate solubilizing microorganisms (PSM). Strains from the genera *Pseudomonas*, *Bacillus* and *Rhizobium* are among the most powerful phosphate solubilizers (Rodriguez and Fraga, 1999).

Ngamau *et al*. (2012) isolated 27 phosphate solubilizing microorganisms from banana plants in Kenya belonging to the genus *Pseudomonas*, *Serratia*, *Rahnella*, *Enterobacter*, *Yersinia*, *Yokenella* and *Ewingella*. *Rahnella aquatilis* showed the highest potential as phosphate solubilizer while *Pseudomonas* and *Serratia* species predominated. Reena *et al*. (2013) also isolated phosphate solubilizing microorganisms from banana rhizosphere soil and identified them as *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Micrococcus* spp., *Aspergillus niger* and *Penicillium* spp. *In-vitro* results showed *A. niger* having greater phosphate solubilization efficiency than *Bacillus subtilis.* However, the results did not match with the field trials where soil conditions may have favored the growth of *B. subtilis.* Andrade *et al*. (2014) also reported 15 out of 40 endophytic bacterial isolates obtained from banana tree roots having showed capacity to solubilize inorganic phosphate. The isolates included *Bacillus* spp., *Paenibacillus* spp. and *Lysinibacillus* spp.

Attia *et al*. (2009) studied the effect of phosphate solubilizing bacteria (PSB) and different rates of phosphorus as mineral fertilizer on growth, yield, fruit quality and mineral contents of leaf and fruit of Maghrabi banana. The results showed that the number of *Pseudomonas* spp. and *Bacillus* spp. were highest in the rhizosphere of banana plants grown in soil fertilized with 25, 50 and 75%, especially in 50% P_2O_5 compared to the recommended dose of P_2O_5 (100%). The results also showed that 25% P_2O_5 mixed with PSB enhanced plant growth, and leaf and fruit mineral content (N, P, K, Fe, Zn and Mn). The efficiency of P fertilizer was therefore raised by PSB inoculation and would thus decrease the required P rate to about 25%. PSB inoculation also enhanced fruit quality in addition to reducing environmental pollution.

Besides phosphate solubilizing bacteria, field grown bananas are also colonized by vesicular arbuscular mycorrhizal (VAM) fungi, which may also enhance uptake of nutrients and in particularly P (Harley and Smith, 1983). One of the major shortcomings of plantlets produced through tissue culture (TC) is the lack of any indigenous symbiont. The plantlets, generated *in vitro* under aseptic conditions, eliminate all microbes including natural symbionts. Therefore, the use of VAM fungi in TC technology may act as an important biological factor contributing to sustainable improvement of banana production. Thaker and Jasrai (2002) reported a significant increased growth of micropropagated banana (*Musa paradisiaca*) with vesicular arbuscular mycorrhizal (VAM) symbiont. There was a relative increase in the growth parameters of the banana plant and percentage root colonization by VAM. Increased absorptive surface area due to VAM resulted in increased nutrient uptake. The most recognized beneficial effect of mycorrhizae is improvement of P nutrition of plants (Cardoso and Kuyper, 2006).

<u>Siderophores production</u>

Although iron is the fourth most abundant element in the earth's crust, many microorganisms and plants have difficulty obtaining enough of it in nonacidic, oxygenated environments as it is usually found in the trivalent form Fe^{3+}, which forms hydrated hydroxides (such as rust) that are insoluble (Drechsel and Winkelmann, 1997). By contrast, the Fe^{2+} ion is soluble but is invariably oxidized by hydrogen peroxide in aerobic conditions. Most microorganisms can increase the concentration of extracellular soluble iron by releasing small molecules that scavenge ferric ion from ferric hydroxides and iron transport proteins. These iron scavengers are known as siderophores. Sharma and Johri, (2003) have defined siderophores (Greek: "iron carriers") as low-molecular-weight compounds with high iron (III) chelating affinity. They are responsible for the dissolution, chelation and transport of iron (III) into microbial cells. Chen *et al*., (1998) have also shown that Fe, chelated by microbial siderophores, can also be utilized by plants. Siderophore-producing bacteria would therefore improve the iron nutrition of plants. Ngamau *et al.* (2012) detected siderophore production activity in 12 *Pseudomonas* isolated from banana plants in Kenya. *Flavimonas oryzihabitans* was the most efficient in siderophore production. In addition *Enterobacter asburiae* and *Serratia proteamaculans* showed positive siderophore production activity.

<u>Microplant biotization</u>

Biotization has been defined as the metabolic response of *in-vitro* grown plant material to microbial inoculants, which promote developmental and physiological changes that enhance biotic and abiotic stress resistance in subsequent plant progeny (Sturz *et al.*, 2000; Senthilkumar *et al.*, 2011). Microplant biotization is an emerging biotechnology aimed at reducing chemical input in plant production, whilst increasing plant fitness and productivity, in the context of sustainable horticulture. Induction of stress resistance in plant propagules produced *in-vitro* before transplanting is a primary target of several researchers attempting utilization of microbial inoculants in micro-propagation (Nowak *et al*., 1995). Biotization of micro-propagated rooted banana plantlets with bacterial mixtures significantly improved their growth (Albuquerque *et al*., 2003). Rodrı´guez-Romero *et al*. (2008) also reported significant and consistent increased plant development following biotization of micro-propagated banana plants with *Pseudomonas fluorescens* strains. There is enough experimental evidence with bacteria (bacterization) and vesicular arbuscular mycorrhiza (mycorrhization) inoculations to recommend utilization of this technology in commercial micro-propagation (Nowak, 1998). Pillay and Nowak (1997) however, noted that both *in-vitro* and *ex-vitro* benefits of biotization depended on plant species, cultivar, growth conditions, and degree of endophytic colonization and that in addition, a certain threshold of bacteria concentration is required to trigger beneficial responses.

Endophytes as biological control agents

Increased environmental awareness has prompted the development of biological alternatives to chemical crop protection agents (Dimock *et al*., 1989). The use of microbes to control plant diseases is an environment-friendly approach and the intimate association of endophytic bacteria with their host plants offers a unique opportunity for their potential application as biological control agents (Senthilkumar *et al.*, 2011). The mechanisms by which endophytes act as biocontrol agents include the production of antifungal or antibacterial agents, siderophore production, competition for nutrients and niches (CNN), and indirectly through induced systemic resistance (Sturz *et al*., 2000; Sessitsch *et al*., 2002; Lugtenberg and Kamilova, 2009).

Majority of bacterial biological control agents have been selected from among the rhizobacteria (Weller, 1988; Kloepper, 1992; Beauchamp, 1993). Unfortunately, most of these biocontrol agents have not fulfilled their initial promise; their failure usually being attributed to poor rhizosphere competence and the difficulties associated with the instability of bacterial biocontrol agents in long-term culture (Schroth *et al*., 1984; Weller, 1988). However, the intimate relationship between endophytic bacteria and their hosts make them natural candidates for selection as biocontrol agents (Van Buren *et al*., 1993; Chen *et al*., 1995) and would preclude the need for selecting bacterial types with high levels of rhizosphere competence often considered necessary for successful seed or root bacterization treatments before or at planting. However, the effectiveness of endophytic bacteria as biological control agents is dependent on many factors including the host specificity, the population dynamics and pattern of host colonization, the ability to move within host tissues, and the ability to induce systemic resistance (Backman *et al*., 1997).

Endophytes potential as biological control agents is nonetheless underutilized, especially in Africa, due to limited fundamental information on them and their ecology and the high cost of product development and required regulatory approvals (Cook *et al*., 1996; Lugtenberg and Kamilova, 2009).

Challenges to the Use of Endophytes in Plant Growth Promotion

<u>Delivery Mechanisms</u>

Little research has been carried out on the delivery mechanisms of bacterial endophytes. There have been a range of delivery systems which have been tested ranging from drenching the soil with bacterial inoculum, wounding the roots before adding the inoculum to inoculating the stem stubble with a cotton swab (Gagné *et al.*, 1987). However, many researchers have focused on the seed treatment with bacterial endophytes before sowing (Misagh and Donndelinger, 1990). Soaking seeds in bacterial suspension has led to acceptable bacterial establishment (Musson *et al.*, 1995). For commercial seed coating with endophytic bacteria, sufficient shelf life of the applied organism is of major importance. At present only spore-forming Gram-positive *Bacillus* and *Paenibacillus* species give sufficient shelf life. More research is therefore needed to develop special procedures for the production of stable inoculum for Gram negative bacteria.

Stem cuttings may be another effective way to introduce beneficial endophytes to the xylem in order to buffer the plant against vascular pathogens. Introduction can be done using a cutting devise wetted with endophyte inocula. Natural colonization of the xylem may however interfere with the effectiveness of colonization by the introduced endophyte. Introduction of endophytic bacteria through this system can probably best be done with higher stem parts, which usually contain lower levels of indigenous endophytes (Van Vuurde and Elvira-Recuenco, 2000).

The most effective introduction of beneficial endophytic bacteria has been through sterile produced tissue culture material. Both Gram negative and Gram positive strains of endophytic bacteria can be applied in this system (Van Vuurde and Elvira-Recuenco, 2000). Tissue culture has been used as a delivery mechanism for fungal endophytes, based on the fact that *Fusarium oxysporum* isolates are efficient root colonizers (Gordon *et al.*, 1989). There have been several mechanisms through which fungal isolates have been inoculated into tissue culture banana plants, and colonization rates in the roots and corms determined (Paparu *et al.*, 2004 & 2006). Persistence of endophytes in inoculated tissue culture banana plants has also been evaluated (Paparu *et al.*, 2008) and it has been demonstrated that inoculated banana plants sustain inoculation up to 33 weeks after inoculation. Application of bacteria is probably most effective for endophyte-free materials, where niches can still be colonized by the introduced endophyte with relatively low competition from naturally present endophytes (Van Vuurde and Elvira-Recuenco, 2000). Ting *et al*. (2008) also recommended application of endophytes at the nursery stage on tissue-cultured clones in order to allow the establishment of the microbes prior to transplanting to the field.

<u>Inconsistent and inconclusive field results</u>

Despite the potential significant role of endophytes in sustainable agricultural production, their use is not widespread in the tropics. This could be due to the lack of consistent and conclusive results that would demonstrate the benefits of these microbial inoculants under field conditions. Reasons for this inconsistency have included limited knowledge of the plant-microbe interactions, the uncontrolled effect of plant varieties and soil types on microbial colonization and functional capacity, inaccurate strategies for screening of potential plant growth-promoting strains and lack of adequate formulation technology (Rodriguez and Fraga, 1999; Gyaneshwar *et al*., 2002).

Conclusions and Recommendations

From the current review, it is apparent that many endophytic bacteria and other microbes inhabit the tissues of banana plants and that they exhibit plant growth-promoting activities such as ability to fix free nitrogen, solubilize phosphates and produce siderophores. There is therefore potential of exploiting them as biofertilizers once conditions for their use is optimized. Application of these microbes is strongly recommended at the nursery stage on tissue-cultured clones in order to allow the establishment of endophytes prior to transplanting to the field. More research is however needed to address the intricate challenges facing the utilization of bacterial endophytes in the tropics for sustainable crop production. In particular, reliable and practical methods of inoculum delivery must be developed.

References

Albuquerque, VV, Terao, D. and Mariano, RLR. 2003. Growth-promoting and control of Fusarium wilt in micropropagated plantlets of *Musa* sp. 6th International PGPR Workshop, Kerala 2003 pp. 3-8.

Andrade LF, Dorasio de Souza GLO, Nietsche S, Xavier AA, Costa MR, Cardoso AMS, Pereira MCT, Pereira DFGS 2014. Analysis of the abilities of endophytic bacteria associated with banana tree roots to promote plant growth. *Journal of Microbiology* **52** (1):27-34.

Attia A, Ahmed MA and El-Sonbaty MR. 2009. Use of biotechnologies to increase growth, production and fruit quality of Maghrabi banana under different rates of Phosphorus. *World Journal of Agricultural Sciences* **5**(2):211-220.

Azevedo JL, Maccheroni WJ, Pereira JO, Araújo WL. 2000. Endophytic microorganisms: a review on insect control and recent advances on tropical plants. *Electronic J Biotech* **3**:40-65.

Backman, PA., Wilson, M. and Murphy, JK. 1997. Bacteria for biological control of plant diseases. In: Recheigl, N.A. and Recheigl, J.E. (Eds.), environmentally safe approaches to plant disease control, CRC/Lewis, Boca, 95-109.

Bacon CW, White JF. 2000. Microbial endophytes, Marcel Dekker, New York.

Barraquio WL, Revilla L, Ladha JK. 1997. Isolation of endophytic diazotrophic bacteria from wetland rice. *Plant Soil* **194**:15–24.

Beauchamp, CJ. 1993. Mode of action of plant growth-promoting rhizobacteria and their potential use as biological control agents. Phytoprotection **71**:19-27.

Cardoso IM, Kuyper TW. 2006. Mycorrhizas and tropical soil fertility. Agriculture, Ecosystems and Environment **116**: 72–84.

Chen, C., Bauske, EM., Musson, G., Rodriguezkabana, R. and Kloepper, JW. 1995. Biological control of Fusarium Wilt on cotton by use of endophytic bacteria. Biological Control **5**:83-91.

Chen L, Dick WA, Streeter JG, Hoitink HAJ. 1998. Fe chelates from compost microorganisms improve Fe nutrition of soybean and oat. *Plant Soil* **200**:139–147.

Cook, RJ., Bruckart, WL., Coulson, JR., Goettel, MS., Humber, RA., Lumsden, RD., Maddox, JV., Mcmanus, M., Moore, L., Meyer, SF., Quimby, PC., Stack, JP. and Vaughn, JL. 1996. Safety of Microorganisms Intended for Pest and Plant Disease Control: A Framework for Scientific Evaluation. Biological Control **7**:333–351.

Dimock, MB., Beach, RM. and Carlson, PS. 1989. Endophytic bacteria for the delivery of crop protection agents. In: Roberts, D.W. and Grandos, R.R (Eds.). Endophytic bacteria for the delivery of crop protection agents Ithaca, NY, Boyce Thompson Institute for Plant Research: 88-92.

Döbereiner J. 1992. History and new perspectives of diazotrophs in association with nonleguminous plants. *Symbiosis* **13**:1-13.

Drechsel H, Winkelmann G. 1997. Iron Chelation and Siderophores: Transition Metals and Microbial Metabolism. Harwood Academic Publishers.

FAOSTAT, 2011.The FAO statistical database. http://faostat.fao.org

Frison E, Sharrock S. 2001. The economic, social and nutritional importance of banana in the world, In: Frison and Sharrock (Eds.). Banana and food security. International symposium, Douala, Cameroon. Pp. 21-35.

Gagné S, Richard H, Rousseau H, Antouin H. 1987. Xylem-residing bacteria in alfalfa roots. *Canadian Journal of Microbiology* **33**:996-1000.

Gordon TR, Okamoto D, Jacobson DJ. 1989. Colonization of muskmelon and nonsusceptible crops by *Fusarium oxysporum* f. sp. *melonis* and other species of *Fusarium*. *Phytopathology* **79**:1095-1100.

Gyaneshwar P, Naresh Kumar G, Parekh LJ, Poole PS. 2002. Role of soil microorganisms in improving P nutrition of plants. *Plant Soil* **245**:83–93.

Hallmann J, Quadt-Hallmann A, Mahaffee WF, Kloepper JW. 1997. Bacterial endophytes in agricultural crops. *Can. J. Microbiol* **43**:895-914.

Harish S, Kavino M, Kumar N, Saravanakumar D, Soorianathasundaram K, Samiyappan R. 2008. Biohardening with Plant Growth Promoting Rhizosphere and Endophytic bacteria induces systemic resistance against *Banana bunchy top virus*. *Applied Soil Ecology* **39** (2):187-200.

Harley JL, Smith SE. 1983. Mycorrhizal symbiosis. Academic Press, London UK.

HortiNews 2013. Horticultural News, the East African Fresh Produce Journal. www.hortinews.co.ke

Iniguez AL, Dong Y, Triplett EW. 2004. Nitrogen fixation in wheat provided by *Klebsiella pneumoniae, Mol Plant Microb Interact* **17**:1078–1085.

James EK, Olivares FL. 1997. Infection and colonization of sugar cane and other graminaceous plants by endophytic diazotrophs. *Crit. Rev. Plant Sci* **17**:77-119.

Kahangi, EM. 2010. The potential of tissue culture banana (*Musa* spp.) technology in Africa and the anticipated limitations and constraints. *Acta Horticulturae* **879**:281-288.

Kloepper, JW. 1992. Plant growth promoting rhizobacteria as biological control agents. In: Metting, F.B., Jr. and Marcel Dekker, E.D (Eds.). Plant growth promoting rhizobacteria as biological control agents: pp. 255-274.

Kluepfel DA. 1993. The behavior and tracking of bacteria in the rhizosphere. *Annu Rev Phytopathol* **31**:441–472.

Ladha JK, Barraquio WL, Watanabe I. 1983. Isolation and identification of nitrogen-fixing *Enterobacter clocae* and *Klebsiella planticola* associated with rice plants. *Can. J. Microbiol*. **29**:1301–1308.

Ladha JK, Reddy PM. 2000. The quest for nitrogen fixation in rice. International Rice Research Institute. Los Banos, Laguna, Philippines.

Long HH, Schmidt DD, Baldwin IT. 2008. Native bacterial endophytes promote host growth in a species-Specific manner; phytohormone manipulations do not result in common growth responses *PLoS ONE*, **3**(7):e2702.

Lugtenberg B, Kamilova F. 2009. Plant-growth-promoting rhizobacteria. *Annu. Rev. Microbiol*., **63**:541-556.

Martinez L, Caballero-Mellado J, Orozco J, Martinez-Romero E. 2003. Diazotrophic bacteria associated with banana (*Musa* spp.). *Plant and Soil* **257**:35-47.

Misagh IJ, Donndelinger CR. 1990. Endophytic bacteria in symptom-free cotton plants. *Phytopathology* **80**:808-811.

Musson G, McInroy JA, Kloepper JW. 1995. Development of delivery systems for introducing endophytic bacteria into cotton. *Biocontrol Science and Technology* **5**:407-416.

Ngamau CN, Matiru VN, Tani A, Muthuri CW. 2012. Isolation and identification of endophytic bacteria of bananas (*Musa* spp.) in Kenya and their potential as biofertilizers for sustainable banana production. *African Journal of Microbiology Research*, **6**(34):6414-6422.

Nowak J. 1998. Benefits of *in vitro* "biotization" of plant tissue cultures with microbial inoculants. *In-Vitro Cell. Develop. Biol. Plant* **34**:122-130.

Nowak J, Asiedu SK, Lazarovits G, Pillay V, Stewart A, Smith C, Liu Z. 1995. Enhancement of *in-vitro* growth and transplant stress tolerance of potato and vegetables plantlets co-cultured with a plant growth promoting rhizobacterium. In: Chagvardieff P (Ed.). Proceedings of the international symposium on ecophysiology and photosynthetic *in-vitro* cultures. CEA, Aix-en-Provence France Pp 173–180.

Okumu MO. 2008. On-farm interaction between soil fertility factors, farmer management, pests and diseases and their effect on banana (*Musa* spp.) yields in Maragua District of Kenya. M.Sc. Thesis, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya.

Paparu P, Dubois T, Gold CS, Adipala E, Niere B, Coyne D. 2004. Inoculation, colonization and distribution of fungal endophytes in *Musa* tissue culture plants. *Uganda Journal of Agricultural Sciences* **9**:583-589.

Paparu P, Dubois T, Gold CS, Niere B, Adipala E, Coyne D. 2006. Colonisation pattern of nonpathogenic *Fusarium oxysporum*, a potential biological control agent, in roots and rhizomes of tissue cultured *Musa* plantlets. *Annals of Applied Biology*, **149**:1-8.

Paparu P, Dubois T, Gold CS, Niere B, Adipala E, Coyne D. 2008. Screenhouse and field persistence of nonpathogenic endophytic *Fusarium oxysporum* in *Musa* tissue culture plants. *Microbial ecology* **55**(3):561-568.

Pillay VK, Nowak J. 1997. Inoculum density, temperature, and genotype effects on *in vitro* growth promotion and epiphytic and endophytic colonization of tomato (*Lycopersicon esculentum* L.) seedlings inoculated with a *pseudomonad* bacterium. *Can. J. Microbiol* **43**(4):354-361.

Reena T, Dhanya H, Deepthi MS, Pravitha DL. 2013. Isolation of Phosphate Solubilizing Bacteria and Fungi from Rhizospheres soil from Banana Plants and its Effect on the Growth of *Amaranthus cruentus* L. *IOSR Journal of Pharmacy and Biological Sciences* **5**(3):6-11.

Rodriguez R, Fraga R. 1999. Phosphate solubilizing bacteria and their role in plant growth promotion *Biotechnology Advances* **17**:319-339.

Rodrı´guez-Romero AS, Badosa E, Montesinos E, Jaizme-Vega MC. 2008. Growth promotion and biological control of root-knot nematodes in micropropagated banana during the nursery stage by treatment with specific bacterial strains. *Annals of Applied Biology* **152**: 41-48.

Rosenblueth M, Martínez L, Silva J, Martínez-Romero E. 2004. *Klebsiella variicola*, a novel species with clinical and plant associated isolates. *Syst Appl Microbiol* **27**:27-35.

Rosenblueth M, Martínez-Romero E. 2006. Bacterial endophytes and their interactions with hosts. *The American Phytopathological Society* **19**(8): 827-837.

Schroth, MN., Loper, JE. and Hilderbrand, DC. 1984. Bacteria as bio-control agents of plant disease. In: Klug, M.J. and Reddy, C.A (Eds.). Bacteria as bio-control agents of plant disease Washington, D.C, American Society of Microbiology: 362-369.

Schulz B, Guske S, Dammann U, Boyle C. 1998. Endophytes host interactions II. Defining symbiosis of the endophyte-host interaction. *Symbiosis* **25**:213-227.

Schulz B, Rommert AK, Dammann U, Aust HJ. 1999. The endophyte-host interaction: a balanced antagonism? *Mycol. Res* **103**(10):1275-1283.

Scot CN, Randy CP, Kepler AK. 2006. *Musa* species (banana and plantains), species profiles for pacific island agroforestry (www.traditionaltree.org).

Senthilkumar M, Anandham R, Madhaiyan M, Venkateswaran V, Tongmin S. 2011. Endophytic bacteria: perspectives and applications in agricultural crop production, In: Maheshwari DK. (Ed.), Bacteria in Agrobiology: Crop Ecosystems, Springer Verlag, Berlin Heidelberg Pp. 61-96.

Sessitsch, A., Howieson, JG., Perret, X., Antoun, H. and Martínez- Romero, E. (2002). Advances in Rhizobium research. Critical Reviews in Plant Sciences **21**:323-378.

Sharma A, Johri BN. 2003. Growth promoting influence of siderophore-producing *Pseudomonas* strains GRP3A and PRS9 in maize (*Zea mays* L.) under iron limiting conditions. *Microbiol. Res.***158**:243-248.

Strobel G, Daisy B. 2003. Bioprospecting for microbial endophytes and their natural products. *Microbiol. Mol. Biol. Rev.* **67**(4):491-502.

Sturz AV, Christie BR, Nowak J. 2000. Bacterial endophytes: potential role in developing sustainable systems of crop production. *Critical Reviews in Plant Sciences* **19**(1):1-30.

Tan Z, Hurek T, Reinhold-Hurek B. 2003. Effect of N-fertilization, plant genotype and environmental conditions on *nifH* gene pools in roots of rice. *Environ. Microbiol* **5**:1009-1015.

Thaker MN, Jasrai YT. 2002. Increased growth of micropropagated banana (*Musa paradisiaca*) with VAM symbiont. *Plant Tissue Cult* **12**(2):147-154.

Thomas P, Soly TA. 2009. Endophytic bacteria associated with growing shoot tips of banana (*Musa* sp.) cv. Grand Naine and the affinity of endophytes to the host. *Microbial Ecology* **58**:952–964.

Ting ASY, Meon S, Kadir J, Radu S, Singh G. 2008. Endophytic microorganisms as potential growth promoters of banana, *BioControl* **53**:541-553.

Van Buren, AM., Andre, C. and Ishimaru, CA. 1993. Biological control of the bacterial ring rot pathogen by endophytic bacteria isolated from potato. Phytopathology **83**:1406.

Van Vuurde JW, Elvira-Recuenco M. 2000. Endophyte management as a tool to optimize plant quality. In: Proceedings of the Fifth International PGPR Workshop, Auburn University, Auburn.

Vanlauwe B, Giller KE. 2006. Popular myths around soil fertility management in sub-Saharan Africa, *Agric Ecosyst Environ* **116** (1-2):34–46.

Weber OB, Baldani VLD, Teixeira KRS, Kirchhof G, Baldani JI, Dobereiner J. 1999. Isolation and characterization of diazotrophs from banana and pineapple plants. *Plant and Soil* **210**:103-113.

Weber OB, Cruz LM, Baldani JI, Dobereiner J. 2001. *Herbaspirillum*-like bacteria in banana plants, *Brazilian Journal of Microbiology* **32**:201-205.

Weber OB, Muniz CR, Vitor AO, Freire FCO, Oliveira VM. 2007. Interaction of endophytic diazotrophic bacteria and *Fusarium oxysporum* f. sp. *cubense* on plantlets of banana 'Maca', *Plant Soil* **298**:47-56.

Weller, DM. 1988. Biological control of soil borne plant pathogens in the rhizosphere with bacteria. Annual Review of Phytopathology **26**:379-407.

Wilson D. 1995. Endophyte - the evolution of a term, and clarification of its use and definition. *Oikos* **73**(2):274-276.

Zuraida AR, Marziah M, Zulkifli Haji Shausuddin, Halimi S. 2000. In: Wahad Z. et al. (Eds), Proceedings of the first national banana seminar at Awana Gentig and Country resort. UPM: Serdang (MYS), 2000. Pp 343.