

PLANT GROWTH PROMOTING POTENTIAL OF BANANA (MUSA SPP.) ENDOPHYTIC BACTERIAL IN KENYA

C. N. Ngamau¹, V. N. Matiru¹, A. Tani² and C. W. Muthuri¹

¹Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya

²Institute of Plant Science and Resources, Okayama University, Kurashiki, Okayama, Japan

E-mail: cngamau@rpe.jkuat.ac.ke

Abstract

In Kenya, banana production is constrained by among others, declining soil fertility. 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 in Kenya. 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. Endophytes are diverse microbes, most commonly fungi and bacteria, which spend the entire or part of their life cycle living in internal plant tissues causing no apparent or immediate disease symptoms. Endophytes are of agronomic interest in that they can enhance plant growth in non-leguminous crops and improve their nutrition through nitrogen fixation, phosphate solubilization or iron chelation (siderophores production). This study was conducted with the aim of assessing functional potentialities of previously isolated and identified endophytic bacteria of bananas in Kenya in regard to their plant growth promoting potential that included abilities to fix free nitrogen, solubilize phosphates and produce siderophores. The 43 bacterial isolates used in this study belonged to the genera *Serratia* (17 isolates), *Pseudomonas* (12 isolates), *Rahnella* (4 isolates), *Enterobacter* (2 isolates), *Raoultella* (2 isolates), *Yokenella* (2 isolates), *Bacillus* (1 isolate), *Klebsiella* (1 isolate), *Yersinia* (1 isolate) and *Ewingella* (1 isolate). Siderophore production activity was detected with all the *Pseudomonas* isolates as determined on blue Chrome Azurol S (CAS) agar plates. Twenty seven isolates were observed to solubilize phosphates, with *Rahnella* isolates showing the highest potential as determined on NBRIP growth medium. All the isolates grew on solid nitrogen-source free medium, suggesting their ability to fix nitrogen. In conclusion, endophytic bacteria of bananas in Kenya showed plant growth promoting potential, and in particular *Rahnella* and *Pseudomonas* isolates.

Key words: *Musa* spp., endophytic bacteria, diazotrophes, phosphate-solubilizing microorganisms, siderophores

1.0 Introduction

In Kenya, 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 in Kenya. This means that the nutrients are not adequately replenished. The most obvious solution to nutrient replenishment is increased use of chemical fertilizers. However, these are expensive and out of reach for most resource-poor farmers, who constitute the vast majority of banana farmers in Kenya. Additionally, use of chemical fertilizers is not environment friendly.

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; Uribe *et al.*, 2010). Microbial inoculants based on Arbuscular mycorrhizal fungi have successfully been used by small scale farmers on bananas in some tropical countries like Colombia, Malaysia and Cuba (Uribe *et al.*, 2010). Endophytes are also increasingly gaining scientific and commercial interest because of their potential to improve plant quality and growth and their close association with internal tissues of host plants (Carroll, 1992; Schulz *et al.*, 1998; Schulz *et al.*, 1999). Endophytes are of agronomic interest in that they can enhance plant growth in non-leguminous crops and improve their nutrition through nitrogen fixation, phosphate solubilization or iron chelation (Dobereiner and Baldani, 1998; Sturzet *et al.*, 2000; Boddey *et al.*, 2003; Iniguez *et al.*, 2004; Ryan *et al.*, 2008; Uribe *et al.*, 2010). Imported microbial inoculants could be used for banana production in Kenya but because of the fitness challenge, there is need to isolate locally occurring bacteria from bananas in our practical farm fields and assess their functional potentiality as biological fertilizers. The objective of this study was to determine the isolates' capacity to fix free nitrogen, solubilize phosphates and produce siderophores *in vitro*.

2.0 Materials and Methods

2.1 Bacterial Isolates Tested

The 43 bacterial isolates used in this study belonged to the genera *Serratia* (17 isolates), *Pseudomonas* (12 isolates), *Rahnella* (4 isolates), *Enterobacter* (2 isolates), *Raoultella* (2 isolates), *Yokenella* (2 isolates), *Bacillus* (1 isolate), *Klebsiella* (1 isolate), *Yersinia* (1 isolate) and *Ewingella* (1 isolate).

2.2 Screening for Nitrogen Fixation Ability

To determine the isolates' ability to fix atmospheric nitrogen, qualitative screening of growth was done on solid N-free medium. Bacterial isolates were cultured on solid N-free medium (1 g K₂HPO₄, 0.2 g MgSO₄·7H₂O, 1 g CaCO₃, 0.2 g NaCl, 5 mg FeSO₄·7H₂O, 10 g glucose, 5 mg NaMoO₄ per litre, and 1.5% agar at pH 7.0) and their growth observed at four and ten days post inoculation. Growth on the N-free medium was used as an indication of isolates' ability to fix free nitrogen.

2.3 Screening for Phosphate Solubilization Ability

Qualitative screening for phosphate solubilizing isolates was done using the National Botanical Research Institute's phosphate (NBRIP) growth medium (Nautiyal, 1999). The medium composed of 10 g glucose, 5 g Ca₃(PO₄)₂, 5 g MgCl₂, 0.25 g MgSO₄, 0.2 g KCl, 0.1 g (NH₄)₂SO₄ per litre and 1.5% agar at pH 7. Screening of phosphate solubilizers was based on formation of visible halo zones on agar plates, which is as a result of organism's production of organic acids into the surrounding medium that dissolves inorganic phosphate resulting to a clear zone around them. The halo size was used as a measure of relative efficiency of the isolates. The halo and colony diameters were measured at 13 and 21 days post inoculation. Halo size (mm) was calculated by subtracting colony diameter from the total diameter.

2.4 Screening for Siderophore Production Ability

Siderophore production was detected using the Chrome Azurol S (CAS) agar plates as described by Schwyn and Neiland (1987). Siderophore medium used composed of three solutions. Solution A composed of 750 ml distilled water, 100 ml MM9 (x10) salts, 15 g agar, 30.24 g PIPES, 10 g glucose and 50% NaOH solution to raise pH to the pKa of PIPES (6.8). 100 ml MM9 (x10) was made up of 0.3 g KH₂PO₄, 1.0 g NH₄Cl, 0.5 g NaCl, 0.2 mM (0.05 g) MgSO₄ and 0.1 mM (0.015 g) CaCl₂. Solution B was made up of 60.5 mg CAS in 50 ml water, 10 ml 1 mM FeCl₃·6H₂O in 10 mM HCl and 72.9 mg hexadecyl trimethylammonium bromide (HDTMA) in 40 ml water while solution C was 30 ml 10% Casamino acid. The three solutions were autoclaved separately and mixed at the clean bench. Solution A was allowed to cool to 50°C, after which solution C was added. Solution B was then added to solutions A and C along the glass wall, with enough agitation to achieve mixing without generation of foam. Each plate received about 30 ml of the blue agar. Orange halos around colonies on blue agar indicated siderophore excretion. Data were taken four and seven days post inoculation.

3.0 Results

3.1 Nitrogen Fixation Ability of the Isolates

All the 43 isolates showed growth on solid N-free medium (Table 1); colony sizes ranged between 1 and 13 mm (Figure 1). However, the colony size may have also been a factor of the isolates ability to utilize glucose as a carbon source.

Table1: Qualitative screening for bacterial isolates' ability to fix free nitrogen, solubilize phosphates and produce siderophores in vitro

| Isolate ID | 16S rRNA closest relative | N-fixation | Phosphates solubilization | Siderophore production |
|--------------|---|------------|---------------------------|------------------------|
| M9V1r (30) | <i>Bacillus subtilis subsp. subtilis</i> NCIB 3610(T) | + | - | - |
| K34V2c (23) | <i>Pseudomonas protegens</i> CHA0(T) | + | + | + |
| J1V1r (31) | <i>Enterobacterasburiae</i> JCM 6051(T) | + | + | + |
| M28V2s (28) | <i>Pseudomonas koreensis</i> Ps 9-14(T) | + | + | + |
| ME10V1r (35) | <i>Serratia glossinae</i> C1(T) | + | + | - |
| K50V2s (43) | <i>Flavimonasoryzihabitans</i> IAM 1568(T) | + | + | ++ |
| K49V2s (21) | <i>Pseudomonas palleroniana</i> CFBP 4389(T) | + | + | + |
| K49V2s (22) | <i>Pseudomonas palleroniana</i> CFBP 4389(T) | + | + | + |
| E29V2c (3) | <i>Pseudomonas psychrophila</i> E-3(T) | + | + | + |
| E18V1c (16) | <i>Pseudomonas graminis</i> DSM 11363(T) | + | + | + |
| K50V2s (27) | <i>Flavimonasoryzihabitans</i> IAM 1568(T) | + | - | ++ |
| K23V1c (4) | <i>Pseudomonas protegens</i> CHA0(T) | + | + | + |
| K10V1r (12) | <i>Pseudomonas moraviensis</i> CCM 7280(T) | + | + | + |
| Isolate ID | 16S rRNA closest relative | N-fixation | Phosphates solubilization | Siderophore production |
| K39V1s (10) | <i>Pseudomonas protegens</i> CHA0(T) | + | + | + |
| K36V2c (37) | <i>Pseudomonas protegens</i> CHA0(T) | + | + | + |
| E35V1s (18) | <i>Serratia fonticola</i> DSM 4576(T) | + | + | - |

| | | | | |
|-------------|--|---|---|---|
| E17V1c (8) | <i>Serratiafonticola</i> DSM 4576(T) | + | + | - |
| E17V1c (19) | <i>Serratia glossinae</i> C1(T) | + | + | - |
| J22V1c (41) | <i>Serratia glossinae</i> C1(T) | + | + | - |
| ME7V1r (32) | <i>Serratia glossinae</i> C1(T) | + | + | - |
| E2V1r (13) | <i>Serratia plymuthica</i> DSM 4540(T) | + | - | - |
| E15V1c (15) | <i>Serratia plymuthica</i> DSM 4540(T) | + | - | - |
| E10V1r (14) | <i>Serratiafonticola</i> DSM 4576(T) | + | + | - |
| E13V2r (26) | <i>Serratia plymuthica</i> DSM 4540(T) | + | - | - |
| K30V2c (34) | <i>Serratia proteamaculans</i> DSM 4543(T) | + | - | + |
| E13V2r (20) | <i>Serratia plymuthica</i> DSM 4540(T) | + | - | - |
| M20V2c (25) | <i>Serratia proteamaculans</i> DSM 4543(T) | + | - | - |

| Isolate ID | 16S rRNA closest relative | N-fixation | Phosphates solubilization | Siderophore production |
|--------------|--|------------|---------------------------|------------------------|
| M20V2c (40) | <i>Serratia plymuthica</i> DSM 4540(T) | + | - | - |
| ME19V2c (42) | <i>Rahnella aquatilis</i> DSM 4594(T) | + | ++ | - |
| E25V2c (7) | <i>Rahnella aquatilis</i> DSM 4594(T) | + | - | - |
| ME18V2c (36) | <i>Rahnella aquatilis</i> DSM 4594(T) | + | ++ | - |
| ME19V2c (24) | <i>Rahnella aquatilis</i> DSM 4594(T) | + | ++ | - |
| E43V2 (1) | <i>Yersinia kristensenii</i> ATCC 33638(T) | + | + | - |
| K32V2c (39) | <i>Ewingella americana</i> GTC 1277(T) | + | + | - |
| ME18V2c (6) | <i>Serratia glossinae</i> C1(T) | + | + | - |

| ME8V2r (11) | <i>Serratia glossinae</i> C1(T) | + | + | - |
|-------------|---|------------|---------------------------|------------------------|
| K29V1c (5) | <i>Raoultella terrigena</i> ATCC 33257(T) | + | - | - |
| K22V1c (2) | <i>Klebsiella granulomatis</i> KH 22 | + | - | - |
| M32V1s (33) | <i>Yokenella regensburgei</i> GTC 1377(T) | + | - | - |
| K32V2c (29) | <i>Raoultella terrigena</i> ATCC 33257(T) | + | - | - |
| K24V1c (9) | <i>Serratia ureilytica</i> NiVa 51(T) | + | - | - |
| Isolate ID | 16S rRNA closest relative | N-fixation | Phosphates solubilization | Siderophore production |
| E41V2 (17) | <i>Enterobacter amnigenus</i> JCM 1237(T) | + | - | - |
| J4V1c (38) | <i>Yokenella regensburgei</i> GTC 1377(T) | + | + | - |

Key: positive (+); strongly positive (++); negative (-).

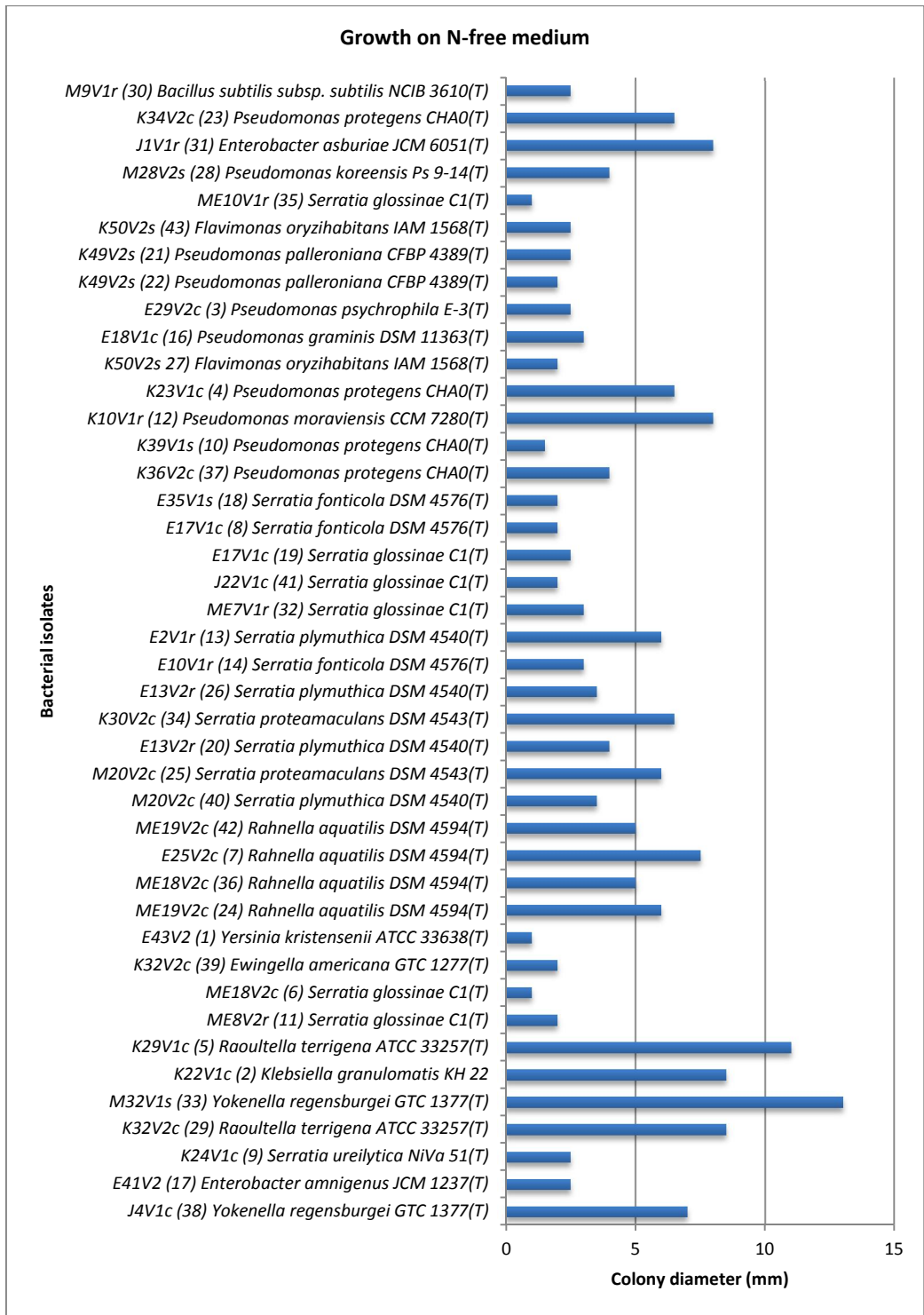


Figure1: Growth of banana endophytic bacterial isolates on solid N-free medium 10 days after inoculation. All the 43 strains grew on N-free medium and colony sizes ranged between 1 and 13 mm

3.2 Phosphate Solubilization Ability of the Isolates

All the 43 isolates grew on NBRIP medium albeit some colonies being very small (1.5 mm diameter). However, not all the isolates were observed to form visible dissolution halos on the NBRIP medium agar plates. Screening of isolates' phosphate solubilizing ability is based on formation of visible halo zones on agar plates. At 13 days post inoculation (DPI) some strains had started forming visible dissolution halos but most (63%) started forming visible dissolution halos at 21 DPI (**Figure 2**). The halo sizes ranged between 1.5 and 17 mm. The strains that showed positive activity included *Pseudomonas* (11 isolates), *Serratia*(9 isolates), *Rahnella*(3 isolates), *Enterobacter*(1 isolate), *Yersinia* (1 isolate), *Yokenella*(1 isolate) and *Ewingella*(1 isolate). Isolates ME19V2c (42), ME19V2c (24), and ME18V2c (36) all *Rahnella*aquatilis had the largest halo size of 17 mm, 16 mm and 12 mm, respectively (Plate 1). A qualitative summary of the isolates' phosphate solubilization ability is shown on Table 1.

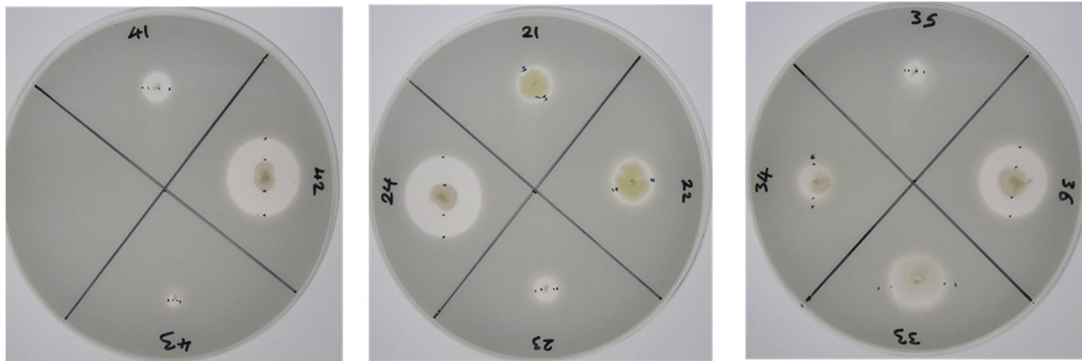


Plate 1: Qualitative screening for phosphate solubilizing isolates on National Botanical Research Institute's phosphate (NBRIP) growth medium agar plates (Nautiyal, 1999), 21 days post inoculation

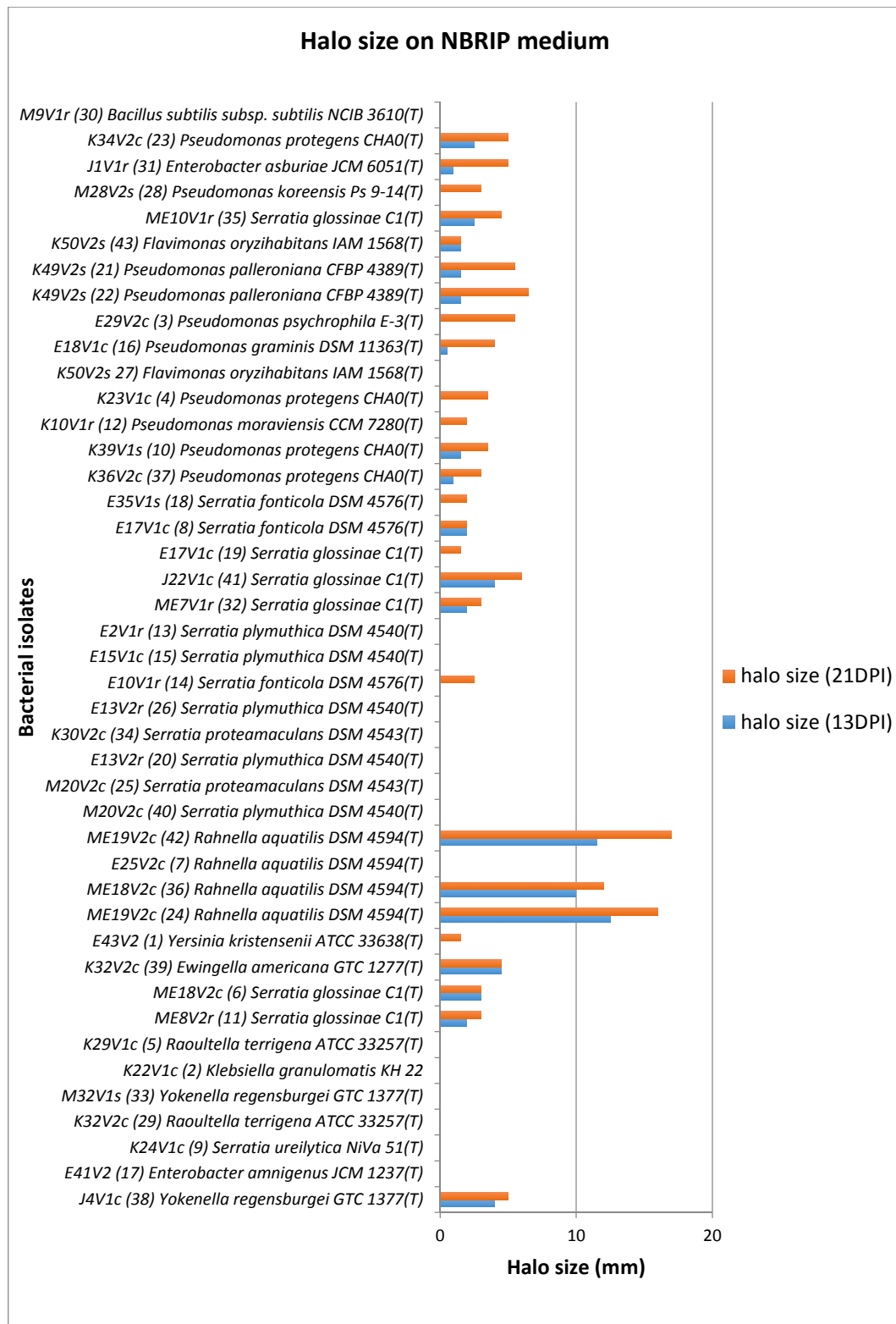


Figure 2: Qualitative screening of 43 isolates for phosphate solubilization on NBRIP medium agar plates (Nautiyal, 1999). Halo and colony diameters were measured at 13 & 21 days post inoculation (DPI)

3.3 Siderophore Production Ability of the Isolates

All the 43 isolates except M9V1r (30) - *Bacillus subtilis* subsp. *subtilis* grew on CAS agar plates, however not all had orange halos around their colonies (Plate 2). Distinct orange halos were observed with all the 12 *Pseudomonas* isolates with isolates K50V2s (43) and K50V2s (27) both identified as *Flavimonas oryzihabitans* having the largest orange halos, that is strongly positive for siderophore production (Plate 2 & Table 1). In

addition *Enterobacterasburiae* (J1V1r) and *Serratia*proteamaculans (K30V2c) showed positive siderophore production activity (Table 1).

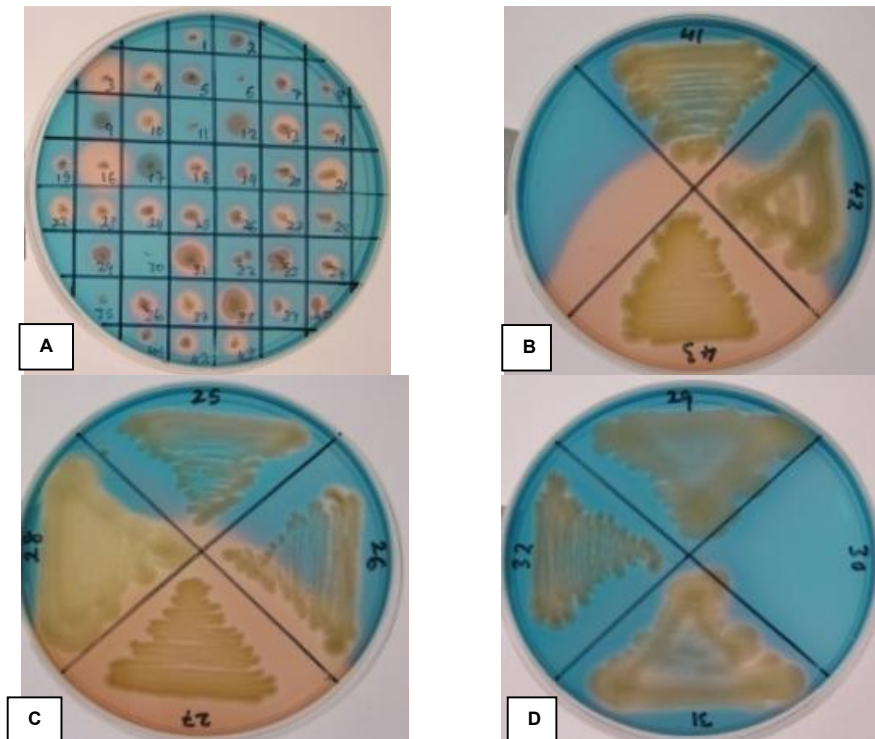


Plate 2: Qualitative screening for siderophore production on Chrome Azurol S (CAS) agar plates. A: All the 43 strains on one plate at one DPI. B and C: Isolates K50V2s (43) and K50V2s (27) with the largest orange halos at 7 DPI, respectively. D: Isolate M9V1r (30) - *Bacillus* spp., which did not grow on CAS agar plate

4.0 Discussion

Screening for nitrogen fixation ability was qualitatively done on solid N-free medium and all the isolates tested showed growth on the medium. This may be explained by the fact that two of the isolation media used were nitrogen free (LGI and NFb). Growth of the bacterial isolates on N-free medium was suggestive of the isolates ability to fix atmospheric nitrogen. 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* from a Brazilian variety of sugarcane (James and Olivares, 1997). The commonly reported endophytic diazotrophic bacteria associated with bananas include *Azospirillum*, *Burkholderia*, *Citrobacter*, *Enterobacter*, *Herbaspirillum*, *Klebsiella* and *Rhizobium* species (Weber *et al.*, 1999; Weber *et al.*, 2001; Martinez *et al.*, 2003; Weber *et al.*, 2007). Biological nitrogen fixation (BNF) accounts for 65% of the nitrogen utilized in agriculture (Dakora and Keya, 1997). Since BNF is not limited to legumes only, increasing the amount of biologically fixed N in non-legume crops like bananas is of paramount importance for sustainable production.

Rahnella aquatilis (ME19V2c and ME18V2c) formed the largest visible dissolution halos and were therefore considered the most efficient phosphate solubilizers. Screening of phosphate solubilizers on NBRIP medium agar plates is based on formation of visible halo zones on the agar plates as a result of organism's production of organic acids into the surrounding medium (Nautiyal, 1999). The organic acids dissolve inorganic phosphate resulting to clear zones around them. The halo size is used as a measure of relative efficiency of the isolates. The findings of this study are consistent with those of Kim *et al.* (1998) who reported *Rahnella aquatilis* having genes (pyrroloquinolinequinone) that are necessary for mineral phosphate solubilization. Vyas *et al.* (2010) also identified a phosphate-solubilizing bacterial strain from *Hippophae rhamnoides* rhizosphere as *Rahnella* spp. The use of phosphate solubilizing bacteria as inoculants increases P uptake by the plant and the crop yield as well (Rodriguez and Fraga, 1999). Utilization of identified *Rahnella* species as microbial inoculants in banana production in Kenya would therefore enhance P uptake and hence banana productivity.

Distinct orange halos were observed with all the 12 *Pseudomonas* isolates with *Flavimonasoryzihabitans* (K50V2s) having the largest orange halos. Orange halos around bacterial colonies on blue Chrome Azurol S (CAS) agar are indicative of siderophore excretion (Schwyn and Neiland, 1987). When a strong chelator like siderophore removes iron from the highly coloured iron dye complex of chrome azurol S, iron (III), its colour turns from blue to orange. The *Pseudomonas* isolates and especially *Flavimonasoryzihabitans* (K50V2s) could therefore be considered high siderophore producers. These findings are similar to those of Gangwar and Kaur (2009) who reported *Pseudomonas* spp. isolated from ryegrass as high siderophore producer. Siderophores are responsible for the dissolution, chelation and transport of iron (III) into microbial cells (Sharma and Johri, 2003). It has also been shown that Fe chelated by microbial siderophores can also be utilized by plants (Chen *et al.*, 1998). Siderophore-producing bacteria would therefore improve the iron nutrition of plants. Siderophores can also promote plant growth indirectly by reducing or preventing harm caused by plant-pathogenic microorganisms (Leong, 1986).

Isolate M9V1r (30), identified as *Bacillus subtilis subsp. subtilis* did not show growth on CAS agar plates. This could be explained by the fact that *Bacillus subtilis subsp. subtilis* Gram-positive and Gram-positive bacteria are reported to be sensitive to HDTMA detergent used in the siderophore medium (Schwyn and Neilands, 1987). HDTMA may therefore have become toxic to *Bacillus subtilis subsp. subtilis* (M9V1r) causing it not to grow.

5.0 Conclusions

From this study, it is apparent that many diazotrophic microbes inhabit the tissues of banana plants and there is the potential of exploiting them once conditions for their use is optimized. *Rahnella aquatilis* (ME19V2c and ME18V2c) and *Flavimonasoryzihabitans* (K50V2s) having showed ability to solubilize phosphate and produce siderophore, respectively and also ability to fix free nitrogen could be proposed as potential biofertilizers for sustainable banana production in Kenya.

Acknowledgements

The authors wish to thank the Research, Production and Extension Division of JKUAT and the JSPS-AASPP program, Japan, for sponsoring this study. Special appreciation also goes to the banana farmers in Juja, Maragua, Embu, Meru and Kisii for allowing us access to their fields for sampling. Accessing the farmers would have been difficult without the assistance of the respective agricultural officers, we are therefore very grateful to them. The authors also acknowledge departments of Botany, Horticulture and Food Science and Technology, and the Institute of Biotechnology Research at JKUAT and the Institute of Plant Science and Resources (IPSR), Okayama University for availing their lab facilities for this study. We also thank the project's research assistant Mr. Julius Mugweru for his persistent dedication to the success of this study. We are also grateful to Ms. Fujitani (IPSR) and Mr. Joseph Muthanga (JKUAT) for their immeasurable technical assistance.

References

- Boddey, R.M., Urquiaga, S., Alves, B.J.R. and Reis V. (2003). Endophytic nitrogen fixation in sugarcane: present knowledge and future applications. *Plant Soil*, **252**, pp 139-149.
- Carroll, C.G. (1992). Fungal mutualism. In: Carroll, C.G. and Wicklow, D.T. Book Fungal mutualism. New York, Dekker 327-254.
- Chen, L., Dick, W.A., Streeter, J.G. and Hoitink, H.A.J. (1998). Fe chelates from compost microorganisms improve Fe nutrition of soybean and oat. *Plant Soil*, **200**, pp 139-147.
- Dakora, F.D. and Keya, S.O. (1997). Contribution of legume nitrogen fixation to sustainable agriculture in Sub-Saharan Africa. *Soil Biol. Biochem.*, **29**, pp 809-817.
- Döbereiner, J. and Baldani, V. L. D. (1998). Biological nitrogen fixation by endophytic diazotrophs in nonleguminous crops in the tropics. In: Malik K.A., Mirza M. S., Ladha J. K. (Eds.) Nitrogen fixation with non-legumes, Vol 1. Kluwer Academic Publishers, Dordrecht, 3-7.
- Gangwar, M. and Kaur, G. (2009). Isolation and characterization of endophytic bacteria from endorhizosphere of sugarcane and ryegrass. *The Internet Journal of Microbiology*, **7** (1).
- Iniguez, A.L., Dong, Y. and Triplett, E.W. (2004). Nitrogen fixation in wheat provided by *Klebsiella pneumoniae*, *Molecular Plant-Microbe Interactions*, **17**, pp 1078-1085.
- James, E.K. and Olivares, F. L. (1997). Infection and colonization of sugar cane and other graminaceous plants by endophytic diazotrophs. *Critical Reviews in Plant Sciences*, **17**, pp 77-119.
- Kim, K.Y., Jordan, D. and Krishnan, H.B. (1998). Expression of genes from *Rahnella aquatilis* that are necessary for mineral phosphate solubilization in *Escherichia coli*, *FEMS Microbiology Letters*, **159**, pp 121-127.
- Ladha, J. K. and Reddy, P. M. (2000). The Quest for Nitrogen Fixation in Rice. International Rice Research Institute: Los Banos, Laguna, Philippines.
- Leong, J. (1986). Siderophores: their biochemistry and possible role in the biocontrol of plant pathogens. *Annual Review of Phytopathology*, **24**, pp 187-208.
- Martinez, L., Caballero-Mellado, J., Orozco, J. and Martinez-Romero, E. (2003). Diazotrophic bacteria associated with banana (*Musa* spp.). *Plant and Soil*, **257**, pp 35-47.
- Nautiyal, C.S. (1999). An efficient microbiological growth medium for screening phosphate solubilizing microorganisms, *FEMS Microbiology Letters*, **170**, pp 265-270.
- Okumu, M.O. (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.
- Rodriguez, R. and Fraga, R. (1999). Phosphate solubilizing bacteria and their role in plant growth promotion, *Biotechnology Advances*, **17**, pp 319-339.
- Ryan R.P., Germaine K., Franks A., Ryan D. and Dowling D. (2008). Bacterial endophytes: recent developments and applications. *FEMS Microbiology Letters*, **278**, pp 1-9.
- Schulz, B., Guske, S., Dammann, U. and Boyle, C. (1998). Endophytes host interactions II. Defining symbiosis of the endophyte-host interaction. *Symbiosis*, **25**, pp 213-227.

- Schulz, B., Rommert, A.K., Dammann, U. and Aust, H.J. (1999). The endophyte-host interaction: a balanced antagonism? *Mycological Research*, **103**(10), pp 1275-1283.
- Schwyn, B. and Neilands, J.B. (1987). Universal Chemical Assay for the Detection and Determination of Siderophores, *Analytical Biochemistry*, **160**: pp 47-56.
- Sharma, A. and Johri, B. N. (2003). Growth promoting influence of siderophore-producing *Pseudomonas* strains GRP3A and PRS9 in maize (*Zea mays* L.) under iron limiting conditions. *Microbiological Research*, **158**, pp 243-248.
- Sturz, A. V., Christie, B. R. and Nowak, J. (2000). Bacterial Endophytes: Potential Role in Developing Sustainable Systems of Crop Production. *Critical Reviews in Plant Sciences*, **19**(1), pp 1-30.
- Thomas P. and Soly, T.A. (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**, pp 952–964.
- Uribe, D., Sa´nchez-Nieves, J. and Vanegas, J. (2010). Role of Microbial Biofertilizers in the Development of a Sustainable Agriculture in the Tropics, In: Dion P. (Ed.), *Soil Biology and Agriculture in the Tropics*, Soil Biology 21, Springer-Verlag, Berlin Heidelberg, 235-250.
- Vanlauwe, B. and Giller, K.E. (2006). Popular myths around soil fertility management in sub-Saharan Africa. *Agriculture, Ecosystems and Environment*, **116** (1-2), pp 34–46.
- Vyas, P., Joshi, R., Sharma, K.C., Rahi, P., Gulati, A. and Gulati, V. (2010). Cold-Adapted and Rhizosphere-Competent Strain of *Rahnella* sp. with Broad-Spectrum Plant Growth-Promotion Potential, *The Journal of Microbiology and Biotechnology*, **20**(12), pp 1724-1734.
- Weber, O.B., Baldani, V.L.D., Teixeira, K.R.S., Kirchhof, G., Baldani, J.I. and Dobereiner, J. (1999). Isolation and characterization of diazotrophs from banana and pineapple plants. *Plant and Soil*, **210**, pp 103-113.
- Weber, O.B., Cruz, L.M., Baldani, J.I. and Dobereiner, J. (2001). *Herbaspirillum*-like bacteria in banana plants, *Brazilian Journal of Microbiology*, **32**, pp 201-205.
- Weber, O.B., Muniz, C.R., Vitor, A.O., Freire, F.C.O. and Oliveira, V.M. (2007). Interaction of endophytic diazotrophic bacteria and *Fusarium oxysporum* f. sp. *ubense* on plantlets of banana ‘Maca’, *Plant Soil*, **298**, pp 47-56.