2-2009

Evaluating *Pseudomonas aeruginosa* as Plant Growth–Promoting Rhizobacteria in West Africa

Anthony O. Adesemoye
Adekunle Ajasin University and Auburn University, tony.adesemoye@unl.edu

Esther O. Ugoji
University of Lagos

Published in *Archives of Phytopathology and Plant Protection* 42:2 (February 2009), pp. 188–200;
doi: 10.1080/03235400601014791.

Submitted December 22, 2005; published online January 12, 2009.

Evaluating *Pseudomonas aeruginosa* as Plant Growth–Promoting Rhizobacteria in West Africa

Anthony O. Adesemoye[1,3] and Esther O. Ugoji[2]

1. Department of Microbiology, Adekunle Ajasin University, Nigeria
2. Department of Botany and Microbiology, University of Lagos, Akoka, Nigeria
3. Department of Entomology and Plant Pathology, Auburn University, Auburn, USA

Corresponding author – Anthony O. Adesemoye, Department of Entomology and Plant Pathology, 209 Life Science Building, Auburn University, Auburn AL 36849, USA, telephone 1-334-844-1982, email adeseao@auburn.edu

Abstract

Some parameters of growth were examined in three test crops as indices of plant growth–promoting rhizobacteria (PGPR) ability of *Pseudomonas aeruginosa*. Crops include *Abelmoschus esculentus* L. (okra), *Lycopersicon esculentum* L. (tomato), and *Amaranthus* sp. (African spinach). This study aimed to examine the effectiveness of PGPR in West Africa and determine whether the inoculation method has an impact on PGPR's effectiveness. Bacterium was isolated from topsoil in the Botanical Garden, University of Lagos, Nigeria. Inoculation with bacteria was done by soaking seeds in 10^6 cfu/ml of bacterial suspension, and coating was done using 10% starch (w/v) as seed adhesive with 10^6 cfu/ml of bacterial suspension. The third treatment involved soaking seeds in distilled water and later applying NPK 15:15:15 fertilizer. Control seeds were soaked in distilled water. Two methods of bacterial inoculation (soaking and coating) produced statistically similar results to plants grown with fertilizer but performed better than the control, suggesting a high potential of *P. aeruginosa* as PGPR.

Keywords: PGPR, *Pseudomonas aeruginosa*, seed inoculation, plant growth, chemical fertilizer, agricultural pollution

Introduction

Limited agricultural land, soil pollution, environmental degradation, and crop diseases are among the many factors hindering agriculture in the modern day and which have brought about the challenge to develop new methods of improvement in crop yield and disease control. The usual chemical methods of improving plant growth and plants disease control have been reported to have environmental pollution effects (Utkhede 1992; Akhtar 1998). Public concern over pollution, as well as pathogen resistance to some pesticides, have intensified the need to find alternative methods for stimulating plant growth and for protection against crop diseases (Dal-Bello et al. 2002). Environmentally friendly microorganisms have proved useful in plant growth promotion, disease control, and pest management (da Luz et al. 1998; Weller 1988; Kloepper et al. 2001; Kravchenko et al. 2002; Mathiyazhagan et al. 2004), and they represent a promising solution in overcoming the problems of disequilibrium in natural balance usually associated with chemical methods (da Luz 2001). Although bacterial cultures (both live and dead) have been widely studied and used in developed countries with evidence of good support to agriculture (Ryder et al. 1999; Timmusk et al. 1999), nothing similar has been reported on the west coast of Africa.

The diversity of microorganisms in the soil, especially in the rhizosphere is important in good soil health. Certain species contribute to soil fertility and crop yield (Leung et al. 1994; Borneman et al. 1996). Beneficial microorganisms include mycorrhizal fungi, bacteria with biopesticide activity (such as *Pseudomonas* spp.) and nitrogen-fixing bacteria (*Azospirillium*, *Rhizobium*) among others (Kennedy & Smith 1995). Microbial communities that reside on the rhizoplane and in the rhizosphere of plants considerably influence plants' growth and development. The rhizosphere is a narrow zone of soil subject to the influence of living roots as manifested by the leakage or exudation of substances that affect microbial activity and comprises the inhabitants that are able to colonize roots. Root colonization reflects the capacity of bacteria to multiply and keep pace with the growing root in field soil (Kloepper et al. 1989). Although the physiology of plants may have a major impact on colonization and the microbial population associated with their roots, the characteristics of the soil is another very important factor for instance, the survival of introduced strains varies with different soil texture (Latour et al. 1996).

The genus *Pseudomonas* has been studied showing usefulness in plant growth promotion and biological control. Pseudomonad is a diverse group of Gram-negative, aerobic heterotrophic bacteria found in soil, some are aquatic, some can be found in animals (primarily in wounds, burns, and urinary tract infections), as well as in the leaves of tobacco plants (Wackett 2000). Many fluorescent *Pseudomonas* strains (e.g., *Pseudomonas aeruginosa*), which colonize the rhizosphere, exert a protective effect on the roots through the production of in situ antibiotic compounds that promote growth and prevent microbial infections (Jenni et al. 1989; Wackett 2000). There is a need for in-depth studies on the effectiveness of *Pseudomonas* spp. as PGPR across the globe.

Plant growth–promoting rhizobacteria (PGPR) are beneficial, naturally occurring, free-living bacteria that colonize the plant rhizosphere, and they are different from legume-rhizobia symbiosis, where the bacteria microsymbionts exist within discrete root structures (Kloepper & Schroth 1978; Kloepper et al. 2001; Bullied et al. 2002). PGPR enhance the

adaptive potential and nutrient uptake or solubilization of their host through a number of proposed mechanisms. Such mechanisms include the fixation of molecular nitrogen, the mobilization of recalcitrant soil nutrients, the control of phytopathogens, and the synthesis of phyto-hormones and vitamins (da Luz 2001, citing Kloepper 1991; Kravchenko 2002). Some bacteria strains directly regulate plant physiology by mimicking the synthesis of plant hormones, whereas others increase mineral and nitrogen availability in the soil (Ryu et al. 2003). Soil mineralization, hydrocyanic acid, siderophores, and induction of resistance, have been suspected as having roles in plant growth-promotion (da Luz 2001; Polyanskaya et al. 2002). Production of metabolites (auxins, cytokinins, and gibberellins), enhance the productivity of agricultural crops but could also improve crop quality by increasing the proteins, essential amino acids, and vitamins (Campbell 1985). The contribution of a particular mechanism to the overall effect of introduced bacteria may depend on the physiology of plants and the agronomic conditions of the cultivation (Polyanskaya et al. 2002).

Despite the enormous importance of microorganisms in the process of nutrient absorption, they are often not well considered when experimental results on nutrient uptake by plants are interpreted. The interaction of some bacteria in the soil with the roots of plants could result in the stable insertion of part of the genome of the bacterium into the plant genome (Miki & Iyer 1998). This natural ability of bacteria to genetically transform the plant tissue is the basis of gene transfer technology. Although it is possible to incorporate transgenic plants into breeding programs for crop improvements, reports have shown that PGPR are possibly better alternatives. The growth-promoting effect of some organisms appears to occur only in specific environmental situations and with specific host species or cultivars, and the effects have been observed to be crop specific (Ryu et al. 2003). The objective of this study was to:

1) examine the effectiveness of PGPR in the West African sub-region using three test crops and
2) determine whether the method of seed inoculation has any impact on the effectiveness of PGPR.

Materials and methods

Collection and analyses of soil samples

Soil samples were collected from the Botanical Garden, University of Lagos, Lagos, Nigeria, at a depth of 5 cm with a disinfected trowel. The soil was transferred to sterilized Bijou bottles and taken to the laboratory within 10 min of collection for physico-chemical analyses and isolation of microorganisms. Part of the soil sample was stored in the refrigerator at 4°C for future use until the end of the analyses and isolation (Atlas & Bartha 1998, Barakate et al. 2002). Analyses were carried out to determine soil composition and percentage of the constituent parts. The pH value of the test soil was determined from 1:1 soil-water suspension (Barakate et al. 2002) with a pH meter (Unicam 9450, Orion model No. 91-02), water-holding capacity was determined by slightly modifying the method of Pramer & Schmidt (1964, cited by Silvana & Claudio 2001) with the use of a perforated

milk tin in place of Hilgard cup. Soil moisture and the total nitrogen content of the test soil were determined by Kjeldahl digestion method (El-Sayed et al. 2002). Organic matter content was determined by using the Walkey-Black method (Mallarino & Bermudez 2002) while soil texture was determined by modifying hydrometer method (Avendano et al. 2004).

Isolation and characterization of Pseudomonas aeruginosa

Isolation from soil

Centrimide agar (Schleicher & Schuell) and Nutrient agar (International Diagnostic Group) were prepared according to the manufacturer's specifications and used in the isolation of the test organism. Centrimide agar is a selective medium for *Pseudomonas aeruginosa*. Ten g of the soil sample was added to a flask containing 90 ml of sterile distilled water, followed by serial dilutions to obtain 10^{-6} dilution (Atlas & Bartha 1998; Barakate et al. 2002). Sterile pipettes were used to draw approximately 1 ml of 10^{-6} dilution aseptically onto one sterile centrimide agar plate and a hockey stick was used to spread the inoculum on the plate. The hockey stick was sterilized by dipping it in 70% (v/v) alcohol and passing through the Bunsen burner (Adesemoye & Adedire 2005). This was incubated aerobically for 18–24 h in the incubator at 30°C.

Identification and biochemical tests

Identification was done at the Federal Institute of Industrial Research, Oshodi (FIIRO) Lagos, Nigeria by carrying out biochemical tests (Polyanskaya et al. 2002). Tests carried out included: Gram stain, oxidase, indole production, motility, urease activity, citrate utilization, nitrate reduction, hydrogen sulphide production, and carbohydrate utilization. Other tests performed were: starch hydrolysis, gelatin hydrolysis, catalase, spore staining, and methyl-red Voges-Proskauer. Following the characterization of *Pseudomonas aeruginosa*, the pure isolate was maintained on nutrient agar slants and stored in the refrigerator at 4°C (Jenni et al. 1989; Wackett 2000).

Seeds of test plants: source and disinfection

Seeds of three test crops—*Abelmoschus esculentus* L. (okra), *Lycopersicon esculentum* L. (tomato), and *Amaranthus* spp. (African spinach)—were purchased from the seedling unit of Ondo State Agricultural Development Programme (ADP), Akure, Ondo State, Nigeria. The seeds were packaged in sealed waterproof bags. Before planting, the seeds were surface-disinfected by soaking in 70% (v/v) ethanol for 2 min followed by 0.2% (w/v) sodium hypochlorite and rinsed five times in sterile, distilled water, a modification of the method used by Ryu et al. (2003). The seeds were then air-dried at 31°C by placing on sterilized filter papers inside Petri dishes and kept in the laminar flow chamber for 5 min before bacterial inoculation (bacterization).

Bacterial inoculum and bacterization of test seeds

Two methods were used for the bacterization of test seeds: soaking and coating. Five loopfuls of the bacterial isolate were added to a conical flask containing 250 ml of Tryptone soy

broth (TSB) and incubated. Optical density (OD) was measured with the spectrophotometer at 600 nm during incubation at 0, 24, and 48 h, to monitor microbial growth. The microbial population of the inoculum used was 10^6 cfu/ml (Kravchenko et al. 2002; Waldrop et al. 2003; Kuklinsky-Sobral et al. 2005). The bacterial suspension was then used for bacterization of seeds through soaking and coating. Bacterization by soaking was done by adopting the methods of da Luz (2001) with modifications. This was done by soaking some tomato seeds in a bacterial suspension containing 10^6 cfu/ml for 5 min; the set was labeled BS to mean seeds bacterized by soaking. Another set of tomato seeds was bacterized by coating with bacterial suspension containing 10^6 cfu/ml that was mixed with 10% starch w/v. This set was labeled BC representing seeds bacterized by coating. The starch was to enhance inoculant adhesion to the seed (Bullied et al. 2002). The control tomato seeds were soaked in sterile distilled water only for 5 min and labeled DW. These treatment patterns were followed for okra and spinach. After the treatments, the seeds were allowed to air-dry at room temperature (31°C) on sterilized filter papers placed in Petri dishes. The seeds were then planted in plastic cans as listed below.

Planting design and maintenance of crops

Monitoring seeds for plumule emergence in the lab

Ten seeds for each type of treatment were placed on wet filter paper in Petri dishes and kept in the laboratory at room temperature. Seeds started germinating on the third day with the plumule emerging. The number of seeds that developed a plumule in each set of ten was counted and recorded as the basis for estimating plumule emergence. The process was repeated three times and the percentages were then calculated.

Setup of seed treatment

Thirty-six new plastic pots were used in this study. Before use, the pots were kept in the oven at 45°C for 16 h, after which 2 kg of field soil was weighed into each of them. After treatments, seeds were planted in the plastic pots according to the setup detailed below, and they were allowed to germinate in the glasshouse at the University of Lagos's Botanical Garden.

The setup for each of the test crops (okra, tomato, and spinach) in the plastic pots included plants growing from the following:

1) Seeds bacterized by soaking (BS)
2) Seeds bacterized by coating (BC)
3) Seeds soaked in distilled water and later supplemented with NPK 15:15:15 fertilizer (WF)
4) Seeds soaked in distilled water (DW) (control).

Maintaining test crops in the glasshouse

Each of the four treatments above was conducted in triplicate, totaling 12 pots for each of the three test crops. Initially, three seeds were planted in each of the pots but the plant stand was pruned to one in each pot at 14 days after planting (DAP). After sowing, the

pots were watered every two days with tap water using a watering can (Dal-Bello et al. 2002) throughout the growth period of the crops (65 days). Weeding was done once a week.

Fertilizer application

Fertilizer used in this experiment was NPK 15:15:15, which was obtained from Ondo State Agricultural Input Supply Company, Akure, Nigeria. Fertilizer suspension was prepared by dissolving 100 g into 1000 ml of distilled water. Then 30 ml of suspension (containing approximately 3 g of fertilizer) was added to the pots that were planned for fertilizer application (one of the four treatments in each of okra, tomato, and spinach). Thus, fertilizer was not applied to any of the plants growing from bacterized seeds. Fertilizer application was done only once (at 14 DAP), to correspond to one time inoculation of bacteria to other seeds.

Collection of plant samples

Experiments ended at 65 DAP by collecting all plant samples. Each pot was flooded with water to loosen soil from the plant roots. Rhizosphere and rhizoplane soil adhering to the roots were washed in slow-running water. Care was taken not to damage any part of the root system. Plants were kept on a lab table for 2 hr at room temperature to allow excess water to drain off; fresh weights were recorded. Each plant sample was put into a paper envelope, properly labeled, and transferred to the oven. The biomass dry weights were determined after drying at 70°C for 48 h.

Data collection and statistical analysis

The time of emergence of each plant was recorded. Shoot height of plants were measured using a meter rule at 10-day intervals until 60 DAP. The day on which each plant developed the first fruit was recorded. Fresh and dry weights of each plant sample were recorded. Data obtained was analyzed using SAS 9.1 software (SAS Institute, Cary, North Carolina) and treatment means were separated by least significant differences at 95% confidence limit ($p < 0.05$).

Results

Composition of soil sample

Using the soil textural triangle (Brandy & Weil 1999), the soil sample was classified as loamy sand. The soil contained 82.05% sand, 8.43% clay, and 9.52% silt. It contained 0.85% organic matter, 0.5% organic carbon, and its water-holding capacity was 0.23%. The soil was slightly acidic (pH 5.7); however, it was within a range suitable for many microorganisms and plant roots (Table I).

Table I. Soil sample composition

Parameters	Composition
Water-holding capacity (ml/g)	0.23
pH	5.7
Moisture content	3.1%
Organic carbon	0.50%
Organic matter	0.85%
Nitrogen	0.21%
Silt	9.52%
Clay	8.43%
Sand	82.05%

The soil sample was classified as loamy sand due to this analysis.

Plumule emergence

The percentage emergence of okra seeds bacterized by soaking was 80%, while 70% of the seeds bacterized by coating emerged. These were increases of 14% and 0% respectively over the control seeds. For tomato, 90% each of seeds bacterized by soaking and by coating emerged, translating to a 28% increase over the 70% emergence of seeds soaked in distilled water. In African spinach, the emergence rates were 70% and 60% for seeds bacterized by soaking and coating respectively, an increase of 40% and 20% over the emergence rate recorded for control spinach seeds (Table II).

Table II. Percentage of plumule emergence third day after seed bacterization and planting

	Percentage of emergence in crops		
Seed treatment	Tomato	Okra	Spinach
Seed bacterized by soaking (BS)	90% (28%)	80% (14%)	70% (40%)
Seed bacterized by coating (BC)	90% (28%)	70% (0%)	60% (20%)
Seed soaked in distilled water (DW)	70%	70%	50%

All values are the mean of three replicates. Values in parentheses indicate percent increases over the control.

Growth of test crops

Growth (plant height) of the three test crops over 60 days measured at 10-day intervals is presented in Figures 1, 2, and 3 for tomato, okra, and African spinach, respectively. At about 50 days after planting (DAP), tomato plants were no longer able to stand erect so had to be supported, but okra and spinach remained erect throughout the study period. All the three test crops showed differences in their response to treatments, and the examples at 40 and 60 DAP are shown (Table III). For the tomato plants at 60 DAP, there was no significant difference among plants from seeds bacterized by soaking (BS treatment), by coating (BC treatment), and plants that received fertilizer application without bacterial inoculation (WF treatment) (62.0 ± 1.30 cm, 60.5 ± 0.86 cm, and 61.5 ± 1.45 cm, respectively). The control plants (DW treatment) were significantly different from the other three treatments, throughout the study period, among the tomato plants. The trend was similar at 10, 20, 30, 40, 50, and 60 DAP (Figure 1).

Table III. Growth of three test crops under four treatments at 40 and 60 days after planting (DAP)

	Okra		Tomato		African spinach	
Treatment	40 DAP	60 DAP	40 DAP	60 DAP	40 DAP	60 DAP
BS	28.9a	62.0a	25.3b	41.3b	5.8a	16.3a
BC	28.1a	60.5a	28.4a	46.0a	4.8b	16.0a
WF	26.8b	61.5a	26.6b	41.7ab	5.8a	16.5a
DW	18.3c	46.0b	20.8c	30.5c	3.8c	11.4b
LSD (0.05)	1.09	4.1	1.3	4.53	0.2	0.9

All values are the mean of three replicates. Means with different letters are significantly different at p = 0.05. BS: plants from seeds bacterized by soaking. BC: plants from seeds bacterized by coating. WF: plants from seeds soaked in distilled water plus NPK fertilizer application. DW: plants from seeds soaked in distilled water (control).

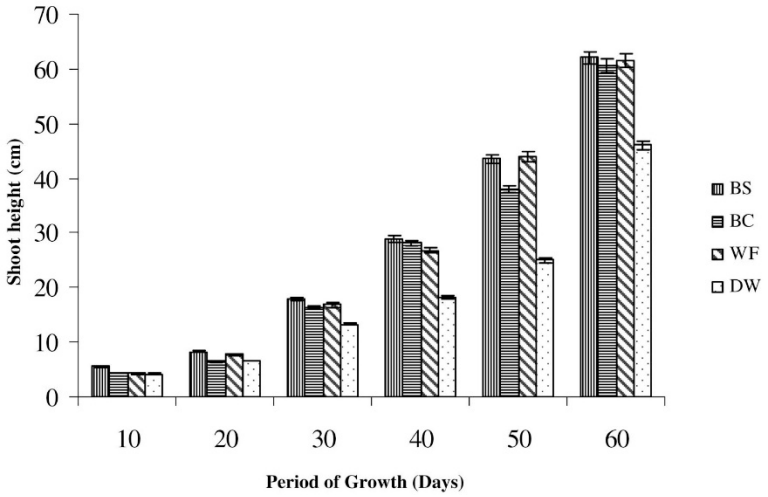

Figure 1. Growth of tomato under different seed treatments. BS: plants from seeds bacterized by soaking; BC: plants from seeds bacterized by coating. WF: plants from seeds soaked in distilled water plus NPK fertilizer application; DW: plants from seeds soaked in distilled water only (control).

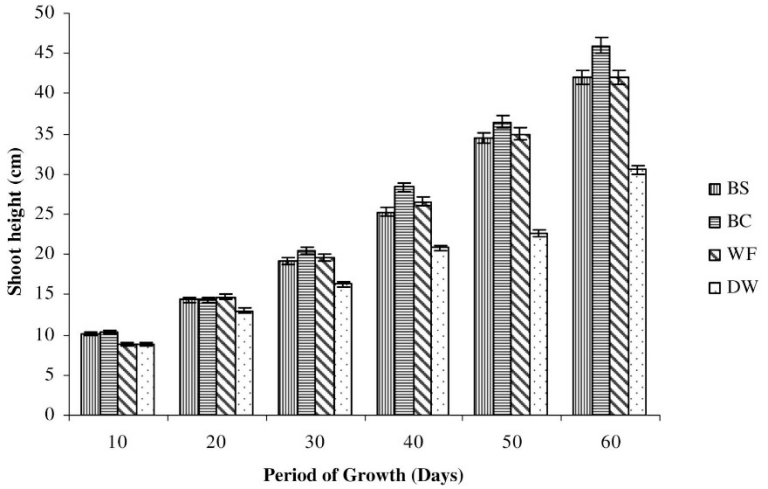

Figure 2. Growth of okra under different seed treatments. BS: plants from seeds bacterized by soaking; BC: plants from seeds bacterized by coating. WF: plants from seeds soaked in distilled water plus NPK fertilizer application; DW: plants from seeds soaked in distilled water only (control).

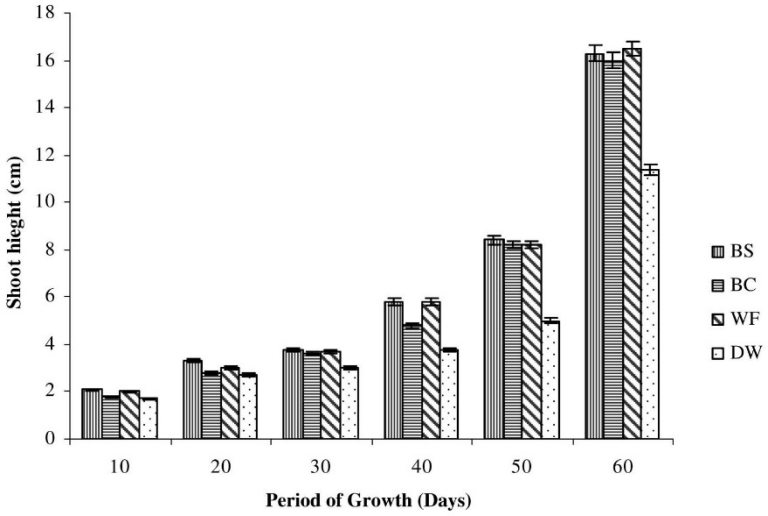

Figure 3. Growth of spinach under different seed treatments. BS: plants from seeds bacterized by soaking; BC: plants from seeds bacterized by coating. WF: plants from seeds soaked in distilled water plus NPK fertilizer application; DW: plants from seeds soaked in distilled water only (control).

Okra plants from BC treatment showed the highest mean shoot height among all the okra plants (46.0 ± 0.36 cm) at 60 DAP. Although this was significantly different from the mean shoot height of okra BS treatment at $p < 0.05$, there was no significant difference between BS and WF treatments. The mean height of each of the three treatments (BS, BC, and WF) for okra was statistically higher than okra plants growing from seeds soaked in distilled water (control). The trend of growth for the four treatments at 10-day intervals over a period of 60 DAP is shown in Figure 2. Also, spinach showed a similar trend through the six sampling times (Figure 3). At 40 DAP, mean shoot height for BS and WF treatments were the same but significantly different from BC treatment. In turn, BC treatment was significantly different from the control. At 60 DAP, all three treatments (BS, BC, and WF) were significantly the same but higher than the control crops (Table III).

Fruiting of plants

On average, of the three replicates, the first set of plants to fruit was okra, whose seeds were bacterized by soaking (45 days) followed by those bacterized by coating (46 days) (Table IV). The okra plants with fertilizer application were next (48 days) while the control took 53 days. In tomato, fruit production started on the 55th day after planting (DAP) in those seeds that were bacterized by coating, 56 DAP in the set of plants from seeds bacterized by soaking but 59 DAP in plants to which fertilizer was applied while control was 65 DAP (Table IV).

Table IV. Days for production of first fruit/flower after planting

	Days of first fruit/flower	
Seed treatment	Tomato	Okra
Seed bacterized by soaking (BS)	56	45
Seed bacterized by coating (BC)	55	46
Seeds soaked in distilled water + NPK Fertilizer application to plants (WF)	59	48
Seed soaked in distilled water (DW)	65	53

All values are the mean of three replicates. Values in parentheses indicate percent increases over the control.

Dry biomass

The dry biomass (shoot and root) of the plants from treated seeds varied at 65 DAP depending on the treatment (Table V). There was no statistical difference among the dry biomass for the three treatments (BS, BC, and WF) for tomato. However, they were greater than the control (DW treatment) at $p < 0.05$ level of significance. Separation of means showed a value of 0.11 for least significant difference (LSD). For okra, the trend was the same with the three treatments but they differ significantly from the control. Results for spinach were slightly different. The mean dry biomass for BS and BC treatments were statistically the same but were greater than the mean value for WF treatment. On the other hand, WF treatment was statistically greater than the control (DW). The comparison of the four treatments for the three test crops is shown in Figure 4.

Table V. Dry biomass of three crops under four treatments at 65 days after planting (DAP)

Treatment	Tomato	Okra	African spinach
BS	3.03a	3.60a	1.63a
BC	3.00a	3.62a	1.62a
WF	2.98a	3.40a	1.51b
DW	2.00b	2.80b	0.88c
LSD (0.05)	0.11	0.25	0.05

All values are the mean of three replicates. Means with different letters are significantly different at p = 0.05. BS: plants from seeds bacterized by soaking. BC: plants from seeds bacterized by coating. WF: plants from seeds soaked in distilled water plus NPK fertilizer application. DW: plants from seeds soaked in distilled water (control).

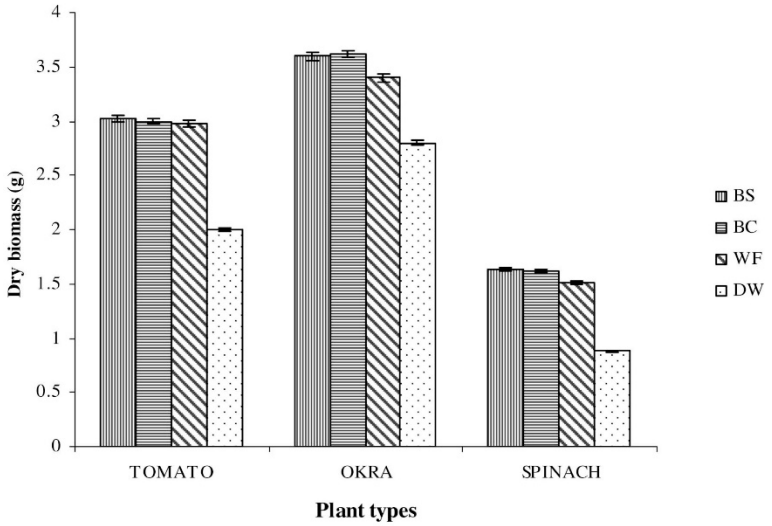

Figure 4. Dry biomass of plants at 65 days of growth. BS: plants from seeds bacterized by soaking; BC: plants from seeds bacterized by coating. WF: plants from seeds soaked in distilled water plus NPK fertilizer application; DW: plants from seeds soaked in distilled water only (control).

Discussion

The field soil used for this study was classified as loamy sand using the soil textural triangle (Brandy & Weil 1999). The soil was a suitable environment for the cultivation of the chosen crops used in this study. This type of soil was chosen with the thinking that the performance of rhizosphere-competent fluorescent pseudomonads would be dependent on the type of host plant as well as soil characteristics. The capacity of microorganisms to stimulate the availability of nutrients and affect the composition of root exudates (Latour et al. 1996) is affected by environmental conditions. Seeds were surface-disinfected before planting so that epiphytic microorganisms and other microorganisms that can interfere

with the result of the study were removed or killed because plants naturally provide surfaces amenable to colonization (or sometimes invasion) and supply exudates rich in carbon source (Lindow et al. 1996; Wackett 2000). Subsequent observations regarding the change in the growth pattern of the treated seeds were thus believed to be due to the treatment, rather than to either the microorganisms previously resident on the seeds or autochthonous microbes in the soil. Previous studies on grain legumes (soyabean and common bean) reported increases of 33% to 40% in seedling emergence in the field, but some other studies in different years did not demonstrate any improvements in seedling emergence (Bullied et al. 2002). In this study, increases in emergence rate over the control differ from one crop to another but range from 14% to 40% (Table II). This is an indication that bacterization could increase the percentage of seeds that will germinate as well as reduce the length of time for plumule emergence. However, it was not clear why there was no increase in emergence rate for okra seeds bacterized by coating relative to control.

The growth of crops (shoot height) was enhanced by the treatments in comparison with the control plants. The mean shoot height of tomato bacterized by soaking or by coating performed at an equal level to plants with fertilizer application but higher than the control experiments (Figure 1). A similar trend was recorded for all the test crops in this study (Table III). In some instances, plants from bacterized seeds performed better than those which received fertilizer application. For instance, at 40 DAP, tomato plants from seeds bacterized by soaking or coating were significantly higher than those to which fertilizer had been applied (Table III). In okra, the mean shoot height of seeds bacterized by coating and those bacterized by soaking recorded increases over seeds soaked in distilled water (control) but were statistically the same compared to plants where fertilizer was used. The pattern of growth mediated by bacterization through soaking was similar to those mediated by bacterization through coating. Improvement in growth observed in crops treated with *Pseudomonas aeruginosa* as PGPR, compared to the control might have been facilitated by a better root system, better soil water uptake, and better nutrient uptake similar to the findings of El-Sayed et al. (2002).

Other parameters used for comparison of growth in all the test crops were dry biomass and date for production of first fruit. Dry biomass in tomato and okra were significantly the same for all crops from seeds inoculated with *P. aeruginosa* and crops to which fertilizer was applied. In spinach, plants from inoculated seeds were even better than plants with fertilizer. Generally, all the crops inoculated by coating or by soaking, started fruit production on about the same day (Table IV) indicating that the two methods of bacterial inoculation had similar impact. Plants treated by seed soaking or coating produced their first fruits earlier than those treated with fertilizer and the control experiments. From this study, it could be concluded that the method of bacterial inoculation (either by soaking or by coating) does not affect the effectiveness of PGPR since both methods produced the same results in all the parameters tested among the three test crops.

The ultimate aim of fertilizer application to any crop plant is to improve its overall growth performance and consequently increase its yield (Adeyemi 2001). In this study, there was a clear improvement in overall growth after NPK 15:15:15 application compared to the control experiments; however, it is evident from our results (see Figures 1–4) that PGPR treatments are as effective as chemical fertilizers in plant growth promotion and

could sometimes perform better. Increased growth, early fruiting and increased dry biomass supported by PGPR in this study is similar to the observations of Polyankaya (2002), da Luz (2001) and Kloepper et al. (1989). *Pseudomonas aeruginosa* has proved suitable as PGPR with these results, which further strengthens its usefulness both in the environment and agriculture. Other reports have shown the development of interesting strategies of chemical warfare by *Pseudomonas* spp. against a range of plant pathogens, mostly fungi (Pinkart et al. 1996; Thomashow 1996; Alonso et al. 1999; Wackett 2000; Yakovleva et al. 2002).

Conclusion

Chemical fertilizers, such as NPK, have been very useful for plant growth promotion. However, given the growing concern about the effect of such fertilizers on the environment, efforts at developing PGPR to promote growth in plants need greater attention, particularly in developing countries of West Africa where little is known about PGPR. Also, the ability of PGPR to combine growth promotion with plant disease control as presented in other reports is a plus. However, we envisage some problems with attempts to substitute chemical fertilizers fully with PGPR, especially in large-scale farming. A combination of PGPR with reduced fertilizer rates will be the best option. We therefore recommend future studies on the appropriate combinations of PGPR and chemical fertilizers.

References

Adesemoye AO, Adedire CO. 2005. Use of cereals as basal medium for the formulation of alternative culture media for fungi. *World J Microb Biot* 21:329–336.

Adeyemi AA, Wahua AT, Ayeni AO. 2001. Effects of cropping patterns and NPK (15-15-15) fertilizer application on N, P and K uptake of component crops in cassava/maize/melon intercropping. *Nigerian J Botany* 14:49–62.

Akhtar M. 1998. Biological control of plant parasitic nematodes by neem products in agricultural soils. *App Soil Ecol* 71:219–223.

Alonso A, Rojo F, Martinez JL. 1999. Environmental and clinical isolates of *Pseudomonas aeruginosa* show pathogenic and biodegradative properties irrespective of their origin. *Environ Microbiol* 1:421–430.

Atlas RM, Bartha R. 1998. *Microbial Ecology: Fundamentals and Applications*. 4th ed. California, USA: Benjamin/Cummings Publishing, Co., pp 219–222; 386–389.

Avendano F, Pierce FJ, Schabenberger O, Melakeberhan H. 2004. The spatial distribution of soyabean cyst nematode in relation to soil texture and soil map unit. *Agron J* 96:181–194.

Barakate M, Ouhdouch Y, Oufdou K, Beaulieu C. 2002. Characterization of rhizospheric soil streptomycetes from Moroccan habitats and their antimicrobial activities. *World J Microb Biot* 18:11–15.

Brandy NC, Weil RR. 1999. *The Nature and Properties of Soils*. 12th ed. New Jersey, USA: Prentice-Hall Inc., pp 125–126.

Borneman J, Skroch PW, O'Sullivan KM, Palus JA, Rumjanek NG, Jansen JI, Nienhuis J, Triplett EW. 1996. Molecular microbial diversity of an agricultural soil in Wisconsin. *App Environ Microb* 62:1935–1943.

Bullied WJ, Buss TJ, Vessey JK. 2002. *Bacillus cereus* UW85 inoculation effects on growth, nodulation, and N accumulation in grain legumes: Field studies. *Can J Plant Sci* 82:291–298.

Campbell R. 1985. *Plant Microbiology*. 1st ed. London: Edward Arnold Publishers Ltd., pp 42–47.

Dal-Bello GM, Monaco CI, Simon MR. 2002. Biological control of seedling blight of wheat caused by *Fusarium graminaerum* with beneficial rhizosphere microorganisms. *World J Microb Biot* 18:627–636.

da Luz WC. 2001. Evaluation of plant growth–promoting and bioprotecting rhizobacteria on wheat crop. *Fitopatologia Brasileira* 26:1–8.

da Luz WC, Bergstrom GC, Stockwell CA. 1998. Seed applied bioprotectants for control of seedborne *Pyrenophora tritici-repentis* and agronomic enhancement of wheat. *Can J Plant Pathol* 20:384–386.

Elkarim AK, Usta S. 2001. Evaluation of some chemical extraction methods used as indices of soil nitrogen availability in polatly state farm soil in Ankara province. *Turkish J Agric Forestry* 25:337–345.

El-Sayed El-SA, El-Didamony G, El-Sayed EF. 2002. Effects of mycorrhizae and chitin-hydrolysing microbes on *Vicia faba*. *World J Microb Biot* 18:505–515.

Jenni B, Isch C, Aragno M. 1989. Nitrogen fixation by new strains of *Pseudomonas pseudoflava* and related bacteria. *J Gen Microbiol* 135:461–467.

Kennedy AC, Smith KI. 1995. Soil microbial diversity and the sustainability of agricultural soils. *Plant Soil* 170:75–86.

Kloepper JW, Scroth MN. 1978. Plant growth–promoting rhizobacteria on radishes, pp 879–882. In: Station de pathologie vegetale et phyto-bacteriologie (ed.), proceedings of the 4th International Conference on Plant Pathogenic Bacteria, Vol. II. Gilbert-Clarey, Tours, France.

Kloepper JW, Lifshitz R, Zablotowicz RM. 1989. Free-living bacterial inocula for enhancing crop productivity. *Trends in Biotechnol* 7:39–44.

Kloepper JW, Rodriguesz-Ubana R, Zehnder GW, Murphy JF, Sikora E, Fernandez C. 2001. Plant root-bacterial interactions in biological control of soilborne diseases and potential extension to systemic and foliar diseases. *Australian Plant Pathol* 28:21–26.

Kravchenko LV, Makarova NM, Azarova TS, Provorov NA, Tikhonovich IA. 2002. Isolation and phenotypic characterization of plant growth–promoting rhizobacteria with high antiphytopathogenic activity and root-colonizing ability. *Microbiology* 71:444–448.

Kuklinsky-Sobral J, Araujo WL, Mendes R, Pizzirani-Kleiner AP, Azevedo JL. 2005. Isolation and characterization of endophytic bacteria from soybean (Glycine max) grown in soil treated with glyphosphate herbicide. *Plant Soil* 273:91–99.

Latour X, Corberand T, Laguerre G, Allard F, Lemanceau P. 1996. The composition of fluorescent psuedomonad populations associated with roots is influenced by plants and soil type. *Appl Environ Microb* 62:2449–2456.

Leung K, England LS, Cassidy MB, Trevors JT, Weir S. 1994. Microbial diversity in soil: effect of releasing genetically engineered microorganisms. *Molec Ecol* 3:413–422.

Lindow SE, McGourty G, Elkins R. 1996. Interactions of antibiotics with *Pseudomonas fluorescens* strain A506 in the control of fire blight and frost injury to pear. *Phytopathology* 86:841–848.

Mallarino AP, Bermudez M. 2002. Yield and early growth responses to starter fertilizer in No-till corn assessed with precision agriculture technologies. *Agronomy* J 94:1024–1033.

Mathiyazhagan S, Kavitha K, Nakkeeran S, Chandrasekar G, Manian K, Renukadevi P, Krishnamoorthy AS, Fernando WGD. 2004. PGPR mediated management of stem blight of *Phyllanthus amarus* (schum and thonn) caused by *Corynespora cassiicola* (Berk and curt) wei. *Arch Phytopathol Plant Protec* 37:183–199.

Miki BL, Iyer VN. 1998. Fundamentals of gene transfer in plants. In: Dennis DT, Turpin DH, Lefebvre DD, Layzell DB, editors. *Plant Metabolism*, 2nd ed. UK: Pearson Educational, pp 561–577.

Pinkart HC, Wolfram JW, Rogers R, White DC. 1996. Cell envelope changes in solvent-tolerant and solvent-sensitive *Pseudomonas putida* strains following exposure to o-Xylene. *Appl Environ Microbiol* 62:1129–1132.

Polyanskaya LM, Vedina OT, Lysak LV, Zvyagintsev DG. 2002. The growth-promoting effect of *Beijerinka mobilis* and *Clostridium* sp. cultures on some agricultural crops. *Microbiology* 71:109–115.

Ryder MH, Yan Z, Terrace TE, Rovira AD, Tang WH, Correl RL. 1999. Use of strains of *Bacillus* isolated in China to suppress take-all and Rhizoctonia root rot and promote seedling growth of glasshouse grown wheat in Australian soils. *Soil Biol Biochem* 31:19–29.

Ryu C, Farag MA, Hu C, Reddy MS, Wei H, Pare PW, Kloepper JW. 2003. Bacterial volatiles promote growth in arabidopsis. *J P Nat Acad Sci USA* (www.pnas.org) 100:4927–4932.

Silvana AM, Claudio A. 2001. Application of calorimetry to microbial biodegradation studies of agrochemicals in oxisols. *J Environ Qual* 30:954–959.

Thomashow LS. 1996. Biological control of plant root pathogens. *Curr Opin Biotechnol* 7:343–347.

Timmusk S, Nicander B, Granhall U, Tillberg E. 1999. Cytokinin production by *Paenibacillus polymyxa*. *Soil Biol Biochem* 31:1847–1852.

Utkhede RS. 1992. Biological control of soil-borne pathogens of fruit trees and grapevines. *C J Plant Pathol* 14:100–105.

Wackett LP. 2000. Pseudomonas entering the post-genomic era. *Environ Microbiol* 2:348–354.

Waldrop MP, McColl JG, Powers RF. 2003. Effects of forest post-harvest management practices on enzyme activities in decomposing litter. *Soil Sci Soc Am* 67:1250–1256.

Weller DM. 1988. Biological control of soil-borne plant pathogen in the rhizosphere with bacteria. *Ann Rev Phytopathol* 26:379–407.

Yakovleva LM, Zdorovenvenko GM, Gvozdyak RI. 2002. Some characteristics of *Pseudomonas syringae* pv. *Maculicola* dissociants. *Microbiology* 71:205–210.