International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
DOI: 10.22192/ijarbs Coden: IJARQG(USA) Volume 5, Issue 2 - 2018

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2018.05.02.014

Studies on Indole Acetic Acid (IAA) Production by Rhizobacteria and Growth promoting potentials

Mike-Anosike, E.E., Braide, W and Adeleye, S.A
Department of Microbiology, Federal University of Technology, Owerri, Imo State, Nigeria

Abstract

This study investigated the production of indole acetic acid by soil bacteria. The isolation and characterization of the soil rhizospheric bacteria was done using standard methods. Screening for potential for IAA production by rhizospheric bacteria identified six species of bacteria with varied potentials. The identities of the bacteria are *Staphylococcus* sp (RSS1), *Micrococcus* sp I (RSS2), *Micrococcus* sp II (RSS3), *Bacillus* sp I (RSS4), *Bacillus* sp II (RSS5) and *Pseudomonas* sp (RSS6). The concentrations of IAA produced by the bacterial isolates ranges from 4.0mg/L by *Pseudomonas* sp to 10.0mg/L by *Micrococcus* sp I (RSS2). Hydroponic studies determining the effects of inoculation on the various bacterial isolates on maize seeds for germination and growth promotion were examined. Bacterisation of maize seeds did not only enhance germination but also promote root and shoot elongation. The results obtained showed that inoculation with high IAA producing bacteria lead to increase in number of lateral roots.

Keywords: Indole Acetic Acid (IAA), Rhizospheric soil, maize seeds, Rhizobacteria

Introduction

The rhizosphere is the narrow region of soil which is directly influenced by root secretions and associated soil microorganisms (Kloepper *et al*., 1993). Many bacteria that feed on slough of plant cells, protein and sugars released by root are found in the rhizosphere (Bashan *et al*., 2004).

Plant growth promoting rhizobacteria (PGPR) are soil bacteria that colonize the roots of plants following inoculation onto the seeds that enhance plant growth (Biswas *et al*., 2000; Husen, 2003; Jeon *et al*., 2003). PGPR enhanced plant growth by direct and indirect means with its specific mechanisms is not fully understood (Thakuria *et al*., 2004; Belimov *et al*., 2004). Indole-3-acetic acid (IAA) is a heterocyclic compound containing carboxymethyl group (acetic acid) that belongs to the most studied phytohormone, and is involved innumerous mechanisms in plant physiology. They are responsible for division, extension and differentiation of plant cells and tissues (Husen, 2003; Nemhauser *et al*., 2006). IAA alters metabolism and morphology of plants, leading to better absorption of minerals and water, giving rise to larger and healthier plants (Biswas *et al*., 2000; Dey *et al*., 2004; De-Bashan *et al*., 2008). The involvement of tryptophan, pH of the culture medium and vitamins has significant effectonthe amount of IAA produced (Ona *et al*., 2003; Zakharova *et al*., 2000; Won, 2011).

Generally, auxin governs the form and shape of plant body, direction and strength of growth of all organs and their mutual interaction (Patten and Glick, 2002). It induces shoot epical dominance, encourages growth and development as well as play minor role in the initiation of flowering and development of reproductive organs and delay fruit senescence

(Nemhauser *et al*., 2006). The best effect of PGPR on plantgrowth was reduction in environmental stresses by the bacteria, providing the plant with a more favourable.

Inoculation sometimes permits plant growth in soil that normally did not allow plant growth as a result of environmental stressors, which include drought, salinity, heavy metal toxicity, toxicity by other substances and suboptimal levels of nitrogen (Bacilio *et al*., 2003).

This study reports on the production of indole acetic acid by soil rhizobacteria and their growth promoting capabilities.

Materials and Methods

The rhizospheric soil of *Panicum maximum* was collected at the premises of the Federal University of Technology, Owerri, Imo State, Nigeria. Composite soil samples randomly collected into a sterile polyethylene bag was analysed within one hour of collection.

Isolation of PGPR Bacteria from soil sample

Soil sample was analysed microbiologically by serial dilution in a sterile physiological saline(Cheesbrough, 2002). Ten grams of soil sample was dissolved in ninety milliliters of physiological saline in a 200 ml Uniscope conical flask to obtain 10^1dilutions. Further dilutions were done decimally until 10^5were obtained. One-tenth milliliter (0.1 ml) of the various dilutions was dispensed onto freshly prepared surface dried nutrient agar medium and spread evenly with a sterile hockey-stick like glass spreader before incubating at ambient temperature (28 ± 02^0C) for 48h.

Characterization and identification of soil PGPR

Pure cultures of the isolates were characterized on the basis of colonial, microscopic and biochemical methods (Cheesbrough, 2002). The identities of the isolates was confirmed with reference to standardidentification manual (Buchanan and Gibbon, 2000).

Standardization of inoculums

A loopful (6 mm) of the isolates were picked from the slants and inoculated differently into 200 mls conical flask containing 30 mls of sterile tryptone soy broth. The cultures were incubated on a rotary shaker at 150 rpm at room temperature for 24 h. The cultures centrifuged at 3000 rpm for 15 mins, was washed in physiological saline and re-suspended in sterile distilled water while optical density (O.D) of the suspension was adjusted to 0.6 at 600 nm.

Screening for IAA production

Screening for IAA production was done using Jeon's medium as described by Jeons *et al*. (2003). Pure cultures of the bacterial isolates were inoculated into 5 mls of Jeon's medium in 20ml test tubes. The tubes wee incubated at room temperature for 72h, thereafter centrifuged at 3000rpm for 10 mins and the supernatant used for the screening.

Two milliliters (2mls) of the each supernatant was mixed with 2 mls of Salkowski's reagent (2% of 0.5 $FeCl_2$ in 35% perchloric acid) and incubated at room temperature in the dark for 30 mins. The presence of IAA was determined by the development of pink colour and the IAAconcentrationwasmeasured spectrophotometrically at 530 nm and quantifiedinIAA standard curve. The absorbance was calculated with the formula:

% inhibition/stimulation=

$$\frac{\text{Abs in control} - \text{Abs in treated sample} \times 100}{\text{Abs in control}}$$

Root and Shoot Elongation Assay

Root and shoot elongation assay was carried out by sterilization of the maize seed in 1% sodium hypochloride (hypo bleach). The seeds were washed repeatedly with distilled water to remove any trace of the bleach on the maize seeds. The sterilized maize seeds were inoculated with 0.1ml of 24 h old suspension of the isolates, followed by seed germination in a petri dish containing moist cotton wool.A control was set up without inoculants. The seeds bearing radicles were suspended with the aid of a needle in water contained in a beaker. Elongation of shoots and roots and number of lateral root was observed and measurement taken weekly for a period of three weeks.

Results

Identification of isolates

Tables 1 and 2shows the colonial and microscopic characteristics of rhizospheric bacteria isolated from soil of *Panicum maximum*. The biochemical characteristics and cell morphology of the bacteria isolated is shown in Table 3.The bacterial isolates identified include species of *Micrococcus, Staphylococcus, Bacillus* and *Pseudomonas*.

Table 1 Colonial characteristics of Bacteria isolated from the soil of *Panicum maximum*on Nutrient Agar medium

Colony code	Colonial characteristics	Probable identity
RSS1	Small circular, low convex and entire golden yellow colonies	Staphylococcus sp
RSS2	Small circular low convex and entire yellow colonies	*Micrococcus* sp
RSS3	Large circular raised and entire orange colonies	*Micrococcus* sp
RSS4	Large serrated dull and dry flat cream colonies	*Bacillus* sp
RSS5	Large mucoid and slimy cream colonies	*Bacillus* sp
RSS6	Irregular bluish green spreading colonies	*Pseudomonas sp*

Table 2 Microscopic and cell morphology of Bacterial isolates

Colony code	Cell morphology	Motility	Grams stain	Spore	Flagellum	Capsule	Probable Identity
RSS1	Cocci predominantly in clusters, few in pairs and tetrads	-	G+	-	-	-	*Staphylococcus*sp
RSS2	Cocci predominantly in tetrads, few in pairs	-	G+	-	-	-	*Micrococcus*sp
RSS3	Cocci predominantly in tetrads, few in pairs	-	G+	-	-	-	*Micrococcus*sp
RSS4	Large short rods with central spores in chains	+	G+	+	+	-	*Bacillus*sp
RSS5	Large Rods in chains and pairs	+	G+	+	+	-	*Bacillus*sp
RSS6	Small slender rods predominantly in singles and short chains	+	G-	-	+	-	*Pseudomonas* sp

Table 3 Biochemical and sugar fermentation

Colony code	Cat	Oxi	IN	MR	VP	Cit	Glu	Suc	Lac	Mann	Mal	Xyl	Ara	Coag	Identity of Bacterial Isolates
RSS1	+	-	-	-	+	-	+	+	+	+	+	-	-	+	*Staphylococcus aureus*
RSS2	+	-	-	+	-	+	-	-	-	-	-	-	-	-	*Micrococcus luteus*
RSS3	+	-	-	+	-	+	+	+	-	-	-	-	-	-	*Micrococcus roseus*
RSS4	+	-	-	-	+	+	+	-	-	-	-	-	-	-	*Bacillus cereus*
RSS5	+	-	-	-	+	+	+	-	-	-	-	+	+	-	*Bacillus subtilis*
RSS6	+	+	-	+	-	+	+	-	-	+	-	+	+	-	*Pseudomonas aeruginosa*

Cat, catalase; Oxi, oxidase; IN, indole; MR; methyl red; VP; VogesProskaeur; Cit, citrate; Glu, glucose; Suc, sucrose; Lac, lactose; Mann, mannitol; Mal, maltose; Xyl, xylose; Ara, arabinose; Coag, coagulase.

Indole Acetic Acid (IAA) Production by Bacterial isolates

Table 4 shows the concentration of IAA produced by the bacteria. All the bacterial isolates exhibited positive IAA production capabilities at different concentrations in the presence of L-Tryptophan.

Table 4 Indole Acetic Acid (IAA) Production by Bacterial isolates

Bacterial isolates	Colony code	IAA (mg/L)
Staphylococcus aureus	RSS1	6.0 ±0.05
Micrococcus luteus	RSS2	10.0 ±0.01
Micrococcus roseus	RSS3	7.0 ±0.02
Bacillus cereus	RSS4	6.2 ±0.04
Bacillus subtilis	RSS5	5.8 ±0.05
Pseudomonas aeruginosa	RSS6	4.0 ±0.01

RSS: Rhizospheric Soil Sample

Growth Performance by PGPR Bacteria

The growth performance of PGPR bacteria in respect to shoot and root elongation and number of lateral roots produced is shown in Tables 5.1-5.7. Growth performance was positive for all the PGPR bacteria compared to the control without the presence of any bacteria. Lateral root was delayed in the control until after 96hrs (fourth day). Higher shoot and root elongation was observed on maize seeds inoculated with *Bacillus cereus* and *Micrococcus luteus*, although this was not significant compared to seeds inoculated with the other isolates. The maximum number of lateral roots was observed with*Bacillus cereus*, *Pseudomonas aeruginosa*and *Micrococcus luteus*.

Table 5.1 Shoot and Root Elongation by Plant Growth Promoting Rhizospheric Bacteria (Day 1)

PGPR Bacteria	*Staphylococcus aureus*	*Micrococcus luteus*	*Micrococcus roseus*	*Bacillus cereus*	*Bacillus subtilis*	*Pseudomonas aeruginosa*	Control
Shoot Length (cm)	1.1	1.1	1.0	1.2	1.2	1.2	1.0
Root length (cm)	1.2	1.3	1.2	1.3	1.3	1.2	1.2
Lateral Root number	-	-	-	-	-	-	-

Table 5.2 Shoot and Root Elongation by Plant Growth Promoting Rhizospheric Bacteria (Day 2)

PG=PR Bacteria	*Staphylococcus aureus*	*Micrococcus luteus*	*Micrococcus roseus*	*Bacillus cereus*	*Bacillus subtilis*	*Pseudomonas aeruginosa*	Control
Shoot Length (cm)	1.9	2.0	2.1	2.4	2.2	1.6	1.5
Root length (cm)	2.3	2.5	2.3	2.4	2.5	2.2	2.5
Lateral Root number	1	1	-	3	-	1	-

Table 5.3 Shoot and Root Elongation by Plant Growth Promoting Rhizospheric Bacteria (Day 3)

PGPR Bacteria	*Staphylococcus aureus*	*Micrococcus luteus*	*Micrococcus roseus*	*Bacillus cereus*	*Bacillus subtilis*	*Pseudomonas aeruginosa*	Control
Shoot Length (cm)	3.0	3.2	2.9	3.5	3.0	2.1	2.0
Root length (cm)	3.5	4.0	3.5	5.5	3.1	3.5	3.0
Lateral Root number	2	2	1	5	1.	2	-

Table 5.4 Shoot and Root Elongation by Plant Growth Promoting Rhizospheric Bacteria (Day 4)

PGPR Bacteria	*Staphylococcus aureus*	*Micrococcus luteus*	*Micrococcus roseus*	*Bacillus cereus*	*Bacillus subtilis*	*Pseudomonas aeruginosa*	Control
Shoot Length (cm)	3.9	4.0	3.5	5.1	3.5	3.5	2.5
Root length (cm)	4.8	5.8	4.6	7.5	4.3	4.8	3.5
Lateral Root number	3	3	2	7	2	5	-

Table 5.5 Shoot and Root Elongation by Plant Growth Promoting Rhizospheric Bacteria (Day 5)

PGPR Bacteria	*Staphylococcus aureus*	*Micrococcus luteus*	*Micrococcus roseus*	*Bacillus cereus*	*Bacillus subtilis*	*Pseudomonas aeruginosa*	Control
Shoot Length (cm)	4.5	4.8	4.6	6.5	4.6	4.2	2.6
Root length (cm)	6.5	7.1	7.8	9.0	5.5	5.5	4.0
Lateral Root number	6	5	4	10	3	8	3

Table 5.6 Shoot and Root Elongation by Plant Growth Promoting Rhizospheric Bacteria (Day 6)

PGPR Bacteria	*Staphylococcus aureus*	*Micrococcus luteus*	*Micrococcus roseus*	*Bacillus cereus*	*Bacillus subtilis*	*Pseudomonas aeruginosa*	Control
Shoot Length (cm)	5.0	5.9	5.8	7.0	5.1	5.0	2.7
Root length (cm)	10.1	7.5	8.5	10.0	6.3	6.2	4.6
Lateral Root number	10	10	5	13	5	12	3

Table 5.7 Shoot and Root Elongation by Plant Growth Promoting Rhizospheric Bacteria (Day 7)

PGPR Bacteria	*Staphylococcus aureus*	*Micrococcus luteus*	*Micrococcus roseus*	*Bacillus cereus*	*Bacillus subtilis*	*Pseudomonas aeruginosa*	Control
Shoot Length (cm)	5.9	7.1	6.5	7.8	6.2	6.1	2.8
Root length (cm)	12.5	7.8	11.6	11.3	7.5	7.9	5.5
Lateral Rootnumber	14	17	7	14	8	14	3

Discussion

Microorganism living in the soil exhibit many different types of association or interaction. Some of the associations are neutral; some are beneficial or positive; others are detrimental or negative.The bacterial species isolated from the rhizospheric soil of*Pannicum maximum* belong to the genera *Staphylococcus*, *Pseudomonas*, *Bacillus* and *Micrococcus*. They are soil borne organisms with potentials to increase soil yield by releasing beneficial exudates and sometimes antimicrobials (Alexander, 2000). All the isolates exhibited positive IAA producing abilities at different concentrations. High IAA producers caused increased in lateral root number of the seedlings. Kloepper*et al*. (1991) and Mashigushi (2011) reported that high concentration of IAA has low inhibition on the roots of monocotyledon. The abilities of all the isolates to germinate maize seedlings also proved their potentials to produce gibberellins (Cassan*et al*., 2009b). Patten and Glick (2002) reported that low level of IAA stimulates root elongation while high level of bacterial IAA production resulted to the formation of lateral and adventitious roots as was evident in the seedlings inoculated with *Bacillus* and *Micrococcus*.

The different level of indolepyruvic decarboxylase (IPdc) gene are presumably the principal enzyme which determines IAA biosynthesis in the isolates (Zimmer *et al*., 1998; Mashigushi, 2011). Patten and Glick (2002) also suggested that the interaction between plant roots and bacterial IAA may be due to direct stimulation of plant cell elongation or cell division and indirectly by influencing bacterial amino-cyclopropane-carboxylate (ACC) deaminase activity. ACC deaminase hydrolyzes plant ACC (the immediate precursor of the phytohormoneethylene),thereby, prevention the inhibition ofplant growth by thelevelof ethylene produced (Achard*et al*., 2006).

The results suggested that plant growth promoting rhizobacteria (PGPR) have the potentials to produce indole acetic acid (IAA) thereby improving the growth of plants (Bai*et al*., 2003; Ahmad *et al*., 2006; Zhao, 2010). This could therefore add to knowledge that, the use of PGPR as an inoculant biofertilizer is an efficient approach to replace chemical fertilize application for sustainable cultivation of maize plant.

Investigation into the mechanism of PGPR as biocontrol under greenhouse and field condition could open up window to clarify the role of PGPR as biofertilizer and pesticides that exert beneficial effects on plant growth and development (Bloembery *et al*., 2001; Benizri *et al*., 2001; Jetiyanon and Kloepper, 2002).

References

Achard, P., Cheng, H., De Grauwe, L., Decat,J., Schoutteten, H., Moritc, T., Van derstraeten, D., Peng, J and Herberd, N.P. (2006). Integration of plant responses to environmentally activated phytohormones. *Singnals Science*, **311**:91-94.

Ahmad, F., Ahmad, I and Khan, M.S. (2006).Screening of free living rhizospheric bacteria for their multiple growth promoting activities. *Microbial. Res*., **36**:1-9.

Alexander, M. (2000). *Soil Microbiology*. Eleventh edition. Wiley and Harcourt, Texas, USA. pp 58-60.

Bacilio, M., Vazquez, P and Bashan, Y, (2003). Alleviation of obnoxious effects of cattle ranch compost on wheat seed germination by inoculation with *Azospirillium* spp. *Boil. Fertile. Soils*, **38**:26-266.

Bai, Y., Zhou, X and Mith, D.L. (2003). Enhanced soybean plant growth resulting from coinoculation of *Bacillus* strain with *Bradyrhizobium japonicum*. *Crop Sci*.,**43**:1774-1781.

Bashan, Y., Holguin, and de-Bashan, L.E. (2004). *Azospirillum*- plant relationships: physiological, molecular, agricultural and environmental advances. *Can. J. Microbiol*., **50**:521-577.

Belimov, A.A., Kunakova, A.M., Safronova, V.I., Stepanok, V.V., Yudkin, L.Y., Alekseev, Y.V and Kozhemyakov,A.P.(2004). Employment of rhizobacteria for inoculation of barley plantscultivated in contaminated lead and cadmium. *Microbiology*, **73**:99-106.

Benizri, E., Baudoin, E and Guckert, A. (2001). Root colonization by inoculated plant growth promoting rhizobacteria. *Biocontrol Technol*., **11**:557-574.

Biswas, J.C., Ladha, L.K and Dazzo, F.B.(2000). Rhizobia inoculation improves nutrient nutrient uptake and growth of low land rice. *J. Soil Sci*., **64**:1644-1650.

Bloembery, G.V and Lugtenberg, B.J.J. (2001). Molecular basisof plant growth promotion and biocontrol by rhizobacteria. *Curr. Opin. Plant Biol*., 4:343-350.

Buchanan, R. E. and Gibbon, N. E. (2000). *Bergey's Manual of Determinative Bacteriology.* 10th edition. The Williams and Wilkin's Co. Baltimore. pp 1246-1249.

Cassan, F., Perrig, S.V., Masciarelli, O., Penna, C and Luna, V. (2009b). *Azospirilliumbrasilense* Az39 and *Bradyrhizobiumjaponicum* E 109, inoculated singly or in combination, promote seedgermination and early seedling growth in *Zea mays* and *Glycine max*. *Eur. J. Soil Biol*. **45**:28-35.

Cheesbrough, M. (2002). *District Laboratory Practice in Tropical Countries Part Two*. Low price edition, Cambridge University Press, UK. pp 117.

DE-Bashan, L.E and Bachan, Y. (2008). Joint immobilization of plant growth promoting bacteria and green microalgae in alginate beads as experimental model for studying plant- bacterium interaction. *Applied Environmental Microbiology*, **74**:6798-6802.

Dey, R., Pal, K.K., Bhatt, D.M and Chauhan, S.M. (2004). Growth promoting and yield enhancement of peanut (*Arachis hypogaea*) by application of growth-promoting rhizobacteria. *Microbial. Res*., **159**:371-394.

Husen, E (2003). Screening of soil bacteria for plant growth promoting activities *in vitro*. *J.Agric. Sci*., **4**:27-31.

Jeons, L.S., Kim, H., Ahn, T and Song, H. (2003). Plant growth promotion in soil by some inoculated microorganism. *Journal of Microbiology*, **41**(4):27-276.

Jetiyanon, K and Kloepper, J.W. (2002). Mixtures of plant growth promoting rhizobacteria for induction of systemic resistance against multiple plant diseases. *Biol. contr*., **24**:285-298.

Kloepper, J.W. (1993). *Plant Growth Promoting Rhizobacteria as Biological Control Agents in Soil Microbial Ecology: Application in Agricultural and Environmental* Management F.B. metting,Jr., edition,Marcel DekkerInc. New York,USA.pp 255-274.

Kloepper, J.W., Zablotowies, R.M., Tipping, E.M and Lifshits, R. (19910. *Plant growth promoting mediated by a bacterial rhizosphere colonizer*. In: keister, D.L., Cregain, P.B (editions), the rhizosphere and plant growth. Kluwere Academic Publishing, Dordrecht, pp 315-326.

Mashiguchi, K. (2011). The main auxin biosynthesis pathway in Arabidopsis. *Proc. Natl. Acad. Sci. USA*,**108** (45):18512-18517.

Nemhauser, J.L., Hong, F and Chory, J (2006). Different plant hormones regulate similar processes through largely non overlapping transcriptional responses. *Cell*, **126**: 467-475.

Ona, O., Van,I. J., Prinsen, E and Vanderleyden, J. (2005). Growth andindole-3-acetic acid biosynthesis of *Azospirilliumbrasilense*Sp245 is environmentally controlled. *FEMS Microbiol. Lett*.,**246**:125-132.

Patten, C.L and Glick, B.R. (1996). Bacterial biosynthesis of indole-3-acetic acid. *Canadian Journal of Microbiology*. **42**:207-220.

Thakuria, D., Talukdar, N.C., Goswami, C., Hazarika, S., Boro, R.C and Khan, M.R. (2004). Characterization and screening of bacteria from rhizosphere of rile grown in acidic soils of Assam. *Curr. Sci*., **86**:978-985.

Won, C. (2011). Conversion of tryptophan to indole-3-acetic acid by tryptophan aminotransferase of Arabidpsis and Yuccas in Arabidopsis. *Proc. Natl. Acad. Sci.USA*, **103**(45):18518-18523.

Zakharova, E.A., Losipenko, A.D and Ignatov, V.V. (2000). Effects of water soluble vitamins on the production indole-3-acetic acid by *Azospirillium brasilense*. *Microbiol. Res*., **155**:209-214.

Zhao, Y. (2010). Auxin biosynthesis and its role in plant development. *Annual Review Plant Biology*.**61**:49-64.

Zimmer, W., Wesche, M and Timmermans, L. (1998). Identification and isolation of the indole- 3-pyruvic decarboxylase gene from *Azospirillium brasilense*Sp7: sequencing and functional analysis of the gene locus. *Curr. Microbiol*., **36**:327-331.

Access this Article in Online

Website: www.ijarbs.com

Subject: Agricultural Sciences

Quick Response Code

DOI:10.22192/ijarbs.2018.05.02.014

How to cite this article:
Mike-Anosike, E.E., Braide, W and Adeleye, S.A. (2018). Studies on Indole Acetic Acid (IAA) Production by Rhizobacteria and Growth promoting potentials. Int. J. Adv. Res. Biol. Sci. 5(2): 133-140.
DOI: http://dx.doi.org/10.22192/ijarbs.2018.05.02.014