

Tomato plant growth promotion potential of *Janibacter* sp strain RC18

Osaro-Matthew, R. C*¹., Ire, F. S. ², and Frank-Peterside, N.²

1. Department of Microbiology, Michael Okpara University of Agriculture Umudike. Nigeria
Department of Microbiology, University of Port Harcourt, Choba. Nigeria

Abstract

Actinomycetes are indispensable in the search for novel, potent and eco friendly fertilizers. The aim of this study was to investigate the ability of *Janibacter* sp. RC18 to enhance tomato plant growth. *Janibacter* sp. RC18 isolated from turmeric rhizosphere was screened for *in vitro* and *in vivo* plant growth promoting traits. The *in vitro* plant growth promotion traits exhibited by the strain were phosphate solubilization and ammonia production. The strain RC18 inoculated tomato plant recorded significant ($P < 0.05$) increase in plant height ($45.67\text{cm} \pm 1.5$), number of leaves (69.67 ± 3.5), weight ($19.40 \text{ g} \pm 0.53$), and high soil phosphate solubilizing bacterial count ($7.2 \times 10^5 \text{ CFU/g}$) compared to the uninoculated control. High endogenous plant nutrient content N, P, K, (4.4%, 0.8 % and 1.1%) was also obtained with inoculation of *Janibacter*. Significant ($p < 0.01$) positive correlation was recorded with endogenous plant nutrient content and all the plant growth parameters except with the number of flowers. Findings from this study indicate that *Janibacter* sp RC18 is a good candidate for bio-inoculant formulation.

Keywords: *Janibacter*, Tomato, plant growth promotion, nutrient uptake, correlation.

Correspondence: osaromatthewruth@gmail.com: +2347035385253.

Introduction

The goal number 2 of the sustainable development goals of United Nations General Assembly 2015 is to achieve zero hunger by the year 2030 which involves: promoting sustainable agricultural practices (United Nations, 2015). Therefore, it is expected that by 2030 agricultural productivity should be doubled, also that eco-friendly agricultural practices that help preserve ecosystems and progressively improve soil health should be adopted.

Actinomycetes due to their filamentous nature are considered as transitional groups between bacteria and fungi, they are Gram-positive bacteria that are saprophytic in nature (Barka et al., 2016; Olarenwaju & Babalola, 2019).

They are widely distributed in terrestrial ecosystems and constitute a large component of soil microbial population, especially plant rhizosphere soil. Actinomycetes have the attention of agroindustries as a source of novel agro agents, as plant growth promoters and as biocontrol tools (Anwar et al., 2016; Arumugam et al., 2017). Actinomycetes are unlimited producers of various agro active compounds such as antifungals, antibacterial, indole acetic acids, siderophores, chitinases and phytohormones and have phosphate and potassium solubilizing abilities (Anwar et al., 2016; Olarenwaju et al., 2017; Chitraselvi, 2018).

Actinomycetes play a major role in plant growth, therefore have been frequently screened for potential agroactive agents for sustainable agriculture (Xue, et al., 2013; Wang, et al., 2015).

Inoculation of plants with actinomycetes by several studies have demonstrated it to be a promising agricultural approach that enhances soil health, increases crop production and controls plant disease (Gopalakrishnan et al., 2015; Jog et al., 2016; Radha, 2016; Bhatti et al., 2017; Htwe, et al., 2019). Significant increase in agronomic properties of agricultural important crops in response to inoculation with actinomycetes have been repeatedly reported; Gopalakrishnan et al. (2015) on cowpea, Robles-Hernández et al. (2015) on pepper and Toumatia et al. (2016) on wheat.

Over the years there has been an enormous research interest towards actinomycetes, but its agricultural application has been poorly investigated in Nigeria. Hence the objective of this study was to evaluate the effect of actinomycetes inoculation on tomato plants.

Materials and methods

Ammonia Production

The selected Isolate was inoculated in triplicates into 5 ml of peptone water and incubated at 28 °C for 10 days in a rotary incubator at 120 rpm. After incubation, 0.5 ml of prepared Nessler's reagent was added to each culture. Ammonia production was indicated by the appearance of yellow that changed to brown (Cappuccino & Sherman, 2002).

Hydrogen Cyanide Production

Isolate was streaked on an actinomycetes isolation agar modified with 4.4 glycine / l to assay its hydrogen cyanide production ability. A whatman filter paper No. 1 dipped in 2% sodium carbonate in 0.5% picric acid for a minute was placed beneath the petri dish lids. The lids were stuck to the plates with paraffin and incubated at 28 °C for 10 days. Orange to red color change of filter paper was HCN production positive (Lorck, 1948).

In-vivo Screening for Plant Growth Promotion Activities

Soil and Seedling Preparation

Plastic pots and bags were surface sterilized with 1% sodium hypochlorite and filled with 1kg of garden soil sterilized at 121 °C for 20 min. Tomato (Roma VF) seeds sterilized in

Experimental actinomycete

In a previous study by Osaro-Matthew et al. (2020) a rare actinomycetes genus with name and accession number *Janibacter* sp strain RC18 MK473882, was isolated from rhizosphere soil of turmeric.

Preliminary screening of Janibacter sp RC18 for Plant Growth Promotion Activities

Phosphate Solubilization

Pikovskaya's agar (Pikovskaya, 1948) containing tri-calcium phosphate (TCP), was used for evaluating the selected actinomycetes isolates for phosphate solubilisation ability. Selected strain was inoculated onto a Pikovskaya's agar plate and incubated for 5 days at 28 °C, solubilization of phosphate was observed by a clear zone around the isolate.

70% ethanol were planted in sterile soil using a broadcast method for 15 days.

Preparation of Janibacter sp strain RC18 Inoculum and Organic Fertilizers

The optimized starch soybean broth was used in the cultivation of *Janibacter* sp strain RC18 which involved a 7-day fermentation process carried out on a rotary-incubator at 30 °C and 150 rpm. *Janibacter* sp strain RC18 cells were harvested after incubation by centrifugation at 10,000 rpm for 20 min. Thereafter, pellet cells were washed twice with distilled water and re-suspended into 0.01M phosphate buffer (pH 7). The optical density (OD) of the prepared inoculum was adjusted to 0.1 at 600 nm using a spectrophotometer to obtain cell concentration of 10⁸ CFU/ml (Aka & Babalola, 2016). The positive control used was Nutriplant liquid organic fertilizer at a ratio of 0.01:1 (1ml of liquid organic fertilizer to 100ml of water), whereas sterile water was used as negative control.

Pot Experiment

On the 15th day, the tomato plants were transplanted into sterilized plastic bags. The treatments were applied to the plant in triplicates after one week of transplant which constitutes 10ml each of both *Janibacter* and the organic fertilizer preparations. Subsequently the liquid organic fertilizer was

added weekly till the 42nd day, then the plants were uprooted and measurements of the length (height), number of leaves, branches and flowers were taken, and plants were *Plant Mineral Content Analysis*

The plant roots were dried and ground to allow it to pass 0.5 mm sieve. The calcium and magnesium contents were determined using an atomic absorption spectrophotometer. Micro-kjeldahl was used for N content analysis, colorimetric method was adopted for the estimation of the phosphorus content and the flame photometer method was adopted for the determination of the potassium content (Carpenter & Hendricks, 2003).

Total phosphate solubilizing Bacterial count

The after-planting soil samples from the pot experiments were evaluated for the presence of phosphate solubilizing bacteria. One gram of each sample was prepared and inoculated into Pikovskaya's agar, incubated at 28 °C for 24 hours after which the total viable counts of the phosphate solubilizers were taken.

Statistical analysis

All experiments were carried out in triplicates and statistically analyzed using SPSS (statistical package for social science) version

($p > 0.05$) difference in the number of flowers observed on the tomato plants treated with *Janibacter*, organic fertilizer and the untreated control (UFUI). However, tomato plants in pots treated with *Janibacter* numerically had a higher number of flowers (1 ± 1) compared to the fertilized and negative control plants (0.00 ± 0).

weighed (Gopalakrishnan et al., 2015; Nnabude et al., 2015).

27. Data obtained were compared using analysis of variance (ANOVA), the means of the various groups were compared with the Duncan multiple range test and correlation analysis was carried out on appropriate data.

Results

In -vivo and in vitro Screening for Plant Growth Promotion Activities

Strain R₁03 was screened for *in vivo* plant growth promotion traits as shown in Table 1. The result obtained showed that strain was a phosphate solubilizer and an ammonia producer.

As represented in Table 2, soil treatment with *Janibacter* sp. Strain RC18 significantly ($P < 0.05$) enhanced the growth of tomato (Roma VF) based on the following results obtained from the plant: height (45.67 ± 1.5 cm), fresh plant weight (19.40 ± 0.53 g), number of leaves (69.67 ± 3.5) and number of branches (9.00 ± 1), compared to the data obtained from positive control (fertilized) and negative control (unfertilized-uninoculated). There was no significant

Total Phosphate Solubilising Bacteria (PSB) Count

The after-harvest phosphate solubilizing bacterial count from soil samples Fig. 1, indicates that the highest PSB count was obtained from soil treated with *Janibacter* sp. Strain RC18 (7.2×10^5 CFU/g) and the lowest PSB count was found in UFUI (unfertilized - uninoculated) soil (4.1×10^5 CFU/g).

Table 1: Preliminary screening of *Janibacter* sp RC18 for plant growth promotion trait

Phosphate solubilization	Hydrogen cyanide	Ammonia production
+	-	+

Key: + = positive, - = negative

Table 2: Effect of *Janibacter* Sp Strain RC 18 on Tomato (Roma VF) Growth At 8 Weeks After Planting

Treatment	Height (cm)	Number of leaves	Number of flowers	Number of branches	Fresh weight (g)
I	45.67±1.5 ^c	69.67±3.5 ^c	1.00±1 ^a	9.00±1 ^c	19.40±0.53 ^c
F	34.00±4 ^b	37.33±3.5 ^b	0.00±0 ^a	6.67±0.58 ^b	12.70±0.36 ^b
UFUI	13.33±3.5 ^a	12.67±3.7 ^a	0.00±0 ^a	3.33±0.58 ^a	5.00±0.1 ^a

Values show means of triplicates ±SD. Values with different superscript on the same column are significantly different at P≤0.05.

Key: I= Inoculated, F= fertilized and UFUI= Unfertilized - uninoculated

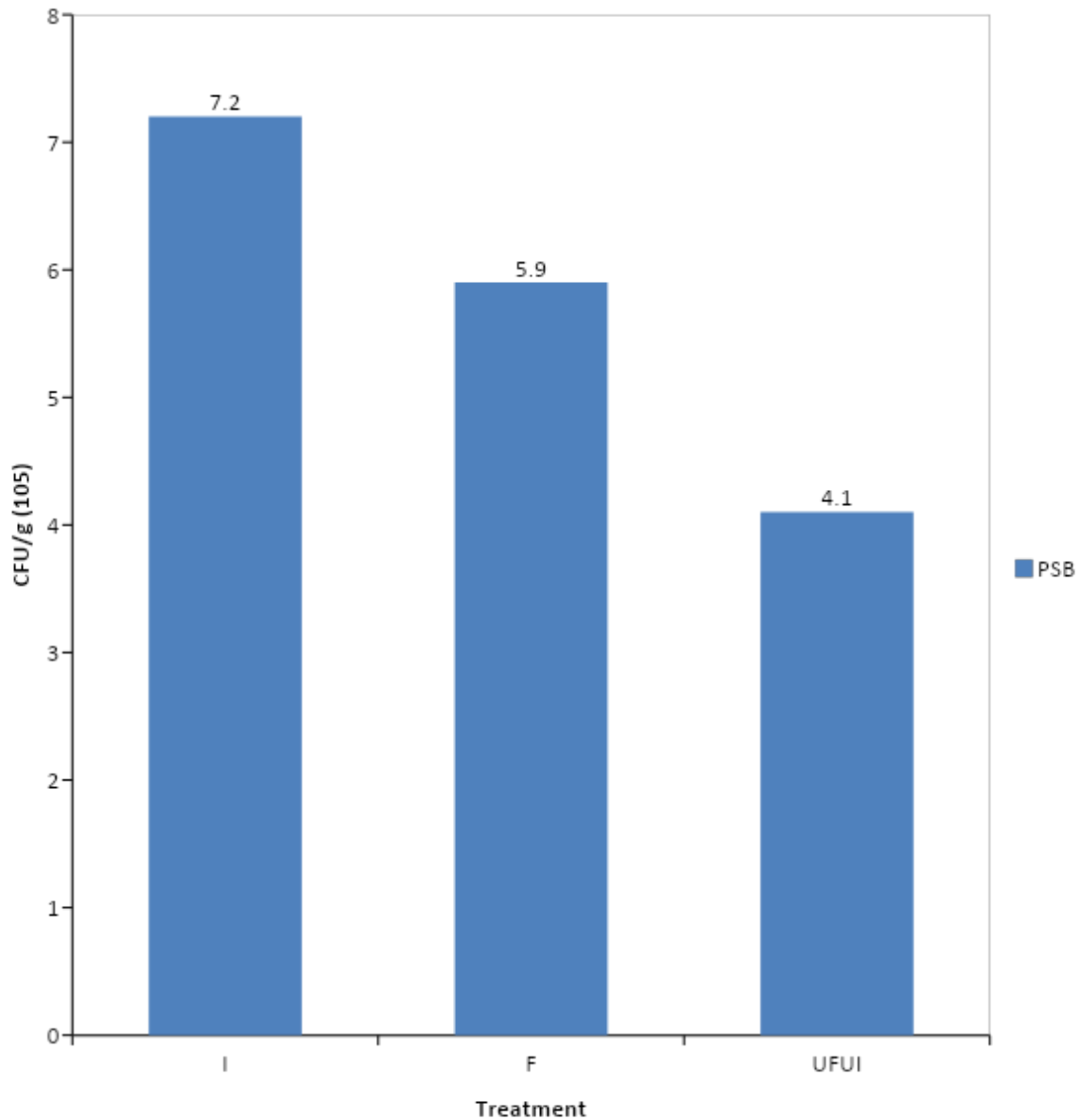


Fig. 1. Total phosphate solubilizing bacterial count

Key: I= Inoculated, F= fertilized, UFUI= Unfertilized, Uninoculated, PSB= phosphate solubilizing bacteria.

The Effect of Janibacter sp. RC 18 inoculation on plant mineral content

As presented in Fig. 2, significantly ($p < 0.05$) higher content of primary nutrient N, P, K,

(4.4%, 0.8 % and 1.1%) respectively, and secondary nutrient Ca (2.53%) and Mg (1.66%) were observed in the inoculated tomato plants. This was followed by the

fertilized plants and the least nutrient uptake was observed in the negative control plant (UFUI)

Relationship between Plant Growth and Plant Nutrient Uptake

The result of correlation relationships between plant nutrient uptake and plant growth parameters as represented in Table 3 revealed that plant N, P, K uptake correlated positively and significantly ($p < 0.01$) with plant height, number of leaves, number of branches and fresh weight.

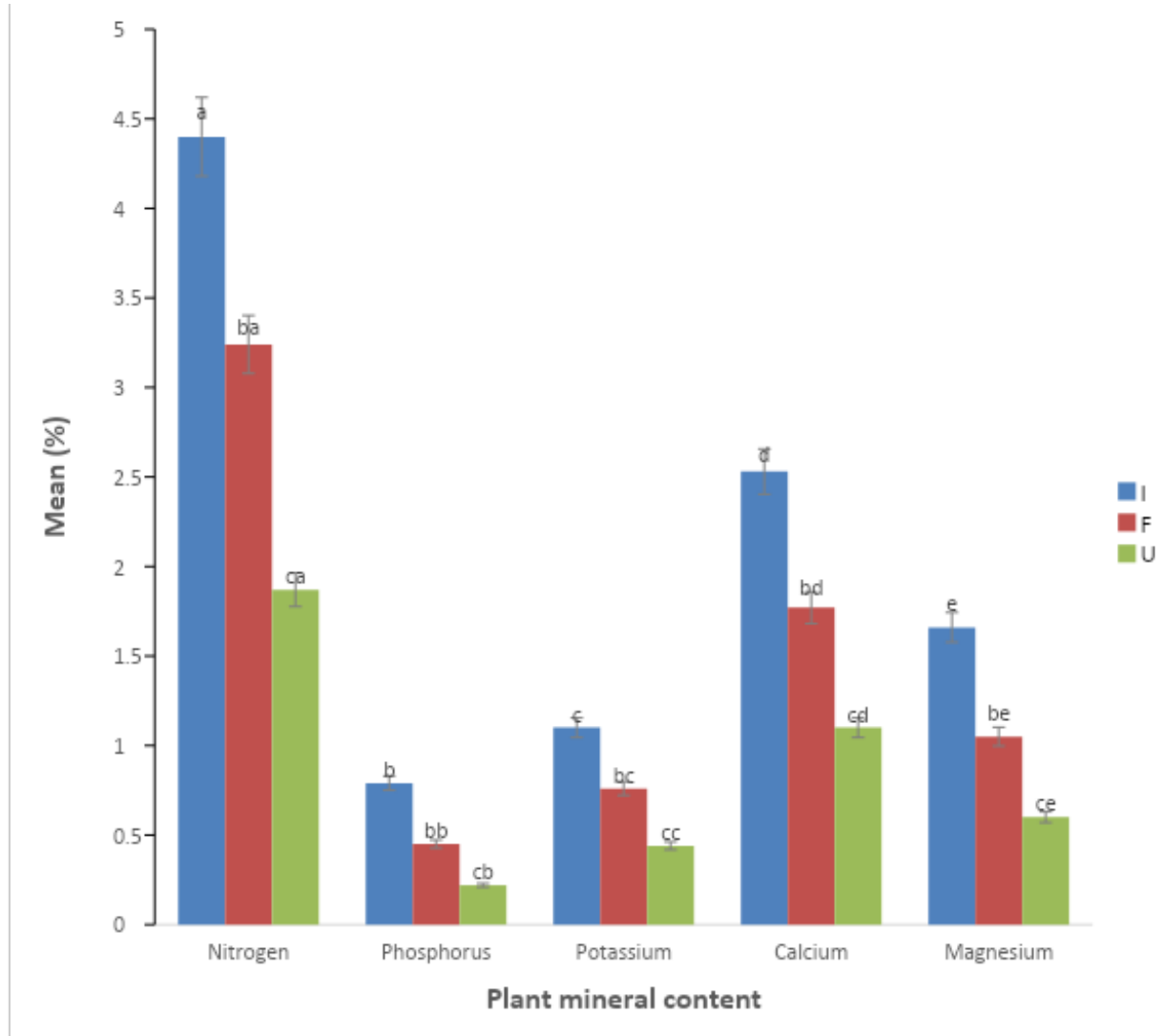


Fig. 2: Effect of *Janibacter* Sp Strain RC 18 on Tomato (Roma VF) mineral content

Key: I= inoculated, F= fertilized, U= Unfertilized- uninoculated.

Table 3: Correlation coefficient between plant growth and plant nutrient uptake

Growth parameters	Endogenous plant mineral content				
	N (%)	P (%)	K (%)	Ca (%)	Mg (%)
Plant height	0.949**	0.943**	0.916**	0.943**	0.908**
Number of leaves	0.952**	0.984**	0.920**	0.979**	0.947**
Number of flowers	0.453	0.666	0.699*	0.653	0.625
Number of branches	0.886**	0.946**	0.940**	0.945**	0.898**
Fresh weight	0.966**	0.985**	0.942**	0.961**	0.961**

*Means significant at $p < 0.05$, ** means significant at $p < 0.01$

Discussion

In vitro Plant Growth Promotion Activities

The agronomic potential of *Janibacter* sp. strain RC18 was revealed by the result obtained from *In vitro* screening for selected plant growth promotion traits viz phosphate solubilization, ammonia production and hydrogen cyanide production. Strain RC18 was a phosphate solubilizing actinomycetes, an ammonia producer but a non-producer of hydrogen cyanide. This result is in accordance with the report of Radha (2016) who reported that 41 actinomycetes exhibited some plant growth promoting potential but were all negative for hydrogen cyanide production. Olarenwaju & Babalola (2019) attributed the increase in available soil phosphate to the presence of phosphate solubilizing actinomycetes. Rodriguez et al. (2016) stated that the ability of microorganisms to produce ammonia is an indication that it can increase the soil available nitrogen. The exhibition of these plant growth promoting traits makes strain RC18 a good biofertilizer candidate.

In vivo Plant Growth Promotion Activities

Janibacter sp RC18 being an actinomycetes of rhizosphere origin was believed to have ability to colonize and enhance plant growth as expected from most rhizobacteria. *Janibacter* sp strain RC18 showed that it can promote tomato plant growth *In vivo* in a pot experiment. Using organic fertilizer as a positive control, strain RC18 significantly ($P < 0.05$) increased plant height (25.6%), number of leaves (46.42%), number of branches (25.9%) and fresh weight (34.54%). This result agrees with some studies: Gopalakrishnan et al. (2015) inoculated chickpea with *Streptomyces* sp and obtained a significant increase in growth and yield. In 2016 actinomycetes were inoculated into wheat plants and increase in growth and yield were recorded (Anwar et al., 2016). Htwe et al. (2019) also observed an increase in agronomic properties of three economically important leguminous crops (soybean, cowpea, and mungbean), enhanced nodulation and nitrogen fixation by *Bradyrhizobium* because of inoculation with

Streptomyces griseoflavus. Based on this result it can be emphatically stated that strain RC18 had high tomato plant root colonization competence compared to the experimental soil microflora.

Phosphate solubilizing bacterial (PSB) population

improved the soil fertility status. The fact that synergistic interaction between microorganisms can provide nutrients and stimulate each other physically and biochemically (Schoebitz et al., 2016), can explain the presence of high PSB population in the *Janibacter* treated soil. This result is in concordance with the result obtained by Golpalakrishnan et al. (2015) who reported increased soil microbial biomass because of inoculation of actinomycetes.

Plant Mineral content

In agreement with this result are the observations of Htwe et al. (2018) where *Streptomyces griseoflavus* P4 improved nutrient content of soybean. Htwe et al. (2019) observed that *Streptomyces griseoflavus* enhanced nutrient uptake in Mung bean, cowpea, and soybean. Also, Golpalakrishnan et al. (2013) recorded an increase in plant nutrient content in rice because of inoculation with *Streptomyces*.

The correlation analysis of plant growth parameters, and nutrient content, showed positive correlation between plant growth parameters (plant height, number of flowers, number of leaves, and fresh weight) and uptake of nutrients (N, Ca, P, K and Mg). The negative correlation between uptake of N and number of flowers might be linked to nitrogen

References

Aeron, A., Kumar, S., Pandey, P. & Maheshwari, D.K. (2011). Emerging role of plant growth promoting rhizobacteria in agrobiolgy. In D.K. Maheshwari (Ed.). Bact. Agrobiol. Crop Ecosyst. Springer Berlin Heidelberg., Pp. 1-36.

Aka, J. R. & Babalola, O. O. (2016). Effect of bacterial inoculation of strains of *Pseudomonas aeruginosa*, *Alcaligenes faecalis* and *Bacillus subtilis* on germination, growth, and heavy metal (Cd, Cr, and Ni) uptake on

The effect of Strain RC18 on the population of soil beneficial microorganisms was estimated using phosphate solubilizing bacterial population as an index. The abundance of PSB in actinomycetes treated soil is presumptive evidence that inoculation with *Janibacter*

There was an increase in nutrient content in plants treated with strain RC18 compared with un-inoculated positive and negative control. Several functionalities associated with actinomycetes, especially production of indole acetic acid (IAA) might explain the increase in the nutrient content of the tomato plant. Although strain RC18 was not assayed for production of IAA which enhances uptake of nutrient by increasing the size and surface area of the root (Aeron et al., 2011), some researchers have shown that most actinomycetes can produce IAA (Anwar et al., 2016; Bhai et al., 2016).

toxicity to flowering stage (Tilley, 2020). This implies that plant growth and development largely depend on adequate concentration of mineral nutrients in the soil which was achieved with the inoculation of RC18. Rosenani et al. (2016) has shown that there is a correlation between nutrient uptake and plant growth.

Conclusion

Sustainable agriculture assures food security for the growing population. The *In vitro* and *In vivo* plant growth promotion traits of *Janibacter* sp. RC18 viz phosphate solubilization, ammonia production and increase in tomato plant agronomic properties is evidence that it is a promising microbial resource for green and sustainable agriculture.

Brassica juncea. Int. J. Phytoremediation. 18 (2): 200 – 209.

Anwar, S., Ali, B. & Sajid, I. (2016). Screening of actinomycetes for various *in vitro* and *in vivo* plant growth promotion (PGP) traits for agroactive compounds. Front. Microbiol. 7:198.

Arumugam, S., Rajendran, V. & Subramaniam, G. (2017). Plant growth-promoting actinobacteria: a new

strategy for enhancing sustainable production and protection of grain legumes. *3 Biotech.* **7**:102.

Barka, E.A., Vatsa, P., Sanchez, L., Gaveau-Vaillant, N., Jacquard, C., Klenk, H.-P., Clément, C., Ouhdouch, Y. & Van-Wezel, G.P. (2016). Taxonomy, physiology, and natural products of Actinobacteria. *Microbiol. Mol. Biol. Rev.* **80**: 1–43.

Bhai, R.S., Lijina, A., Prameek, T.P., Krishna, P.B. & Anushreet (2016). Biocontrol and growth promotion potential of *Streptomyces* spp. In black pepper (*Piper nigrum*). *J. Biol. Control.* **30**: 177-189.

Bhatti, A. A., Haq, S. & Bhat, R. A. (2017). Actinomycetes benefit from soil and plant health. *Microb. Pathog.* **111**:458-487.

Cappuccino, J. G. & Sherman, N. (2002). *Microbiology: A laboratory manual*. 6th edition, Pearson Education Inc. San Francisco. Pp. 15 -224.

Carpenter, C. E. & Hendricks, D. C. (2003). *Food analysis*. 3rd ed. S. S. Nelson ed. Springer LLC. New York. Pp. 198 – 206.

Chitraselvi, P. E. R. (2018). Actinomycetes: dependable tool for agriculture. *Cur. Invest. Agric. Cur. Res.* **1**(5): 128-130.

Gopalakrishnan, S., Vadlamudi, S., Bandifinda, P., Sathya, A., Vijayabharathi, R., Rupela, O., Kudapa, H., Katta, K. & Varshney, R. K. (2013). Evaluation of *Streptomyces* strains isolated from herbal vermicompost for their plant growth promotion. *Microbiol. Res.* **169** (1): 40 – 48. Doi:10.1016/j.micres.2013.09.008.

Gopalakrishnan, S., Srinivas, V., Alekhya, G., Prakash, B., Kudapa, H., Rathore, A. & Varshney, R.K. (2015). The extent of grain yield and plant growth enhancement by plant growth-promoting broad-spectrum Osaro- Matthew, R. C., Ire, F. S. & Frank-Peterside, N. (2020). Screening of actinomycetes from turmeric (*Curcuma longa* L.) and ginger (*Zingiber officinale*) rhizosphere for antifungal activity. *J. Adv. Microbiol.* **20**(2): 18 – 28. doi: 10.9734/JAMB/2020/v20i230214.

Pikovskaya, R.I. (1948). Mobilization of phosphates in soil in connection with the

Streptomyces sp. in chickpea. *Springer Plus.* **4**(1): 1–10.

Htwe, A.Z., Moh, S.M., Moe, K. & Takeo. Y. (2018). Effect of co-inoculation of *Bradyrhizobium elkanii* BLY3-8 and *Streptomyces griseoflavus* P4 on Rj4 Soybean varieties. *Soil Sci. Plant Nutr.* **64**(4): 449-454.

Htwe, A. Z., Moh, S. M., Soe, K. M., Moe, K. & Takeo, Y., (2019). Effects of biofertilizers produced from *Bradyrhizobium* and *Streptomyces gresioflavus* on plant growth nodulation, nitrogen fixation, nutrient uptake, and seed yield of mung bean, cowpea and soybean. *Agron.* **9**(2): 77- 85.

Jog, R., Nareshkumar, G.I. & Rajkumar, S. (2016). Enhancing soil health and plant growth promotion by actinomycetes. In: Gopalakrishnan S, Sathya A, Vijayabharathi R (eds) *Plant growth promoting actinobacteria*. Springer, Singapore., Pp. 33–45.

Lorck, H. (1948). Production of hydrocyanic acid by bacteria. *Physiol. Plant.* **1**:142-146.

Nnabude, P. C., Nweke, I. A. & Nsoanya, C. N. (2015). Response of three varieties of tomatoes *Lycopersicon esculentum* to liquid organic fertilizer, (Alfa life) and inorganic fertilizer (NPK 20:10:10) and for soil improvement. *Eur. J. Phys. Agric. Sci.* **3**(2): 28-37.

Olanrewaju, O.S., Glick, B.R. & Babalola, O.O. (2017). Mechanisms of action of plant growth promoting bacteria. *World J. Microbiol. Biotechnol.* **33**(11): 197-205.

Olanrewaju, O.S. & Babalola. O.O. (2019). *Streptomyces*: implication and interaction in plant growth promotion. *Appl. Microbiol. Biotechnol.* **103**(3): 1179-1188.

vital activities of some microbial species. *Mikrobiologia.* **17**: 362-370.

Radha. T. K. (2016). *Diversity of actinomycetes in crop rhizosphere of arid, semi-arid and humid region (AER 3 and AER 6) and their effect on Maize (zea mays. L.) and Chickpea (Cicere ariertinum)* . Ph.D dissertation. Department of Agricultural

Microbiology. University of Agricultural Sciences GKV Bengaluru.

Robles-Hernández, L., Hernández-Huerta, J., González-Franco, A., Hernández-Rodríguez, O., Núñez-Barrios, A. & Pérez-Leal, R. (2015). *Streptomyces* PRIO41 as plant growth promoter of jalapeño pepper plants and as biocontrol agent of *Fusarium*. *Phyton. (Buenos Aires)*.84(2):253–261.

Rodriguez, A. A., Forzani, M. V., Soares, R, S., Sibov, S. T. & Vieira, J. D. G. (2016) Isolation and selection of plant growth promoting bacteria associated with sugarcane. *Agropec Trop. Gio.* 46(2): 149-158.

Rosenani, A. B., Rovica, R., Cheah, P.M. & Lim, C.T. (2016). Growth performance and nutrient uptake of oil palm seedling in prenursery stage as influenced by oil palm waste compost in growth media. *Int. J. Agro.* Article ID: 6930735.

Schoebitz, M., Lopez, M. D., Serri, H., Martinez, O. & Eric, Z. (2016) Combined application of microbial consortium and humid substance to improve the growth performance of the seedlings. *J. Soil. Sci. Plant Nutri.* 16(4): 1010-1023.

Xue, L., Xue, Q., Chen, Q., Lin, C. & Shen, G. (2013). Isolation and evaluation of rhizosphere actinomycetes with potential application for biocontrol of *Verticillium* wilt of cotton. *Crop Prot.* 43: 231-240.

Tilley, N. (2020). *Gardening Know how*: Understanding Nitrogen requirements for plants. Accessed from <http://www.gardeningknowhow.com>

Toumatia, O., Compant, S., Yekkour, A., Goudjal, Y., Sabaou, N., Mathieu, F., Sessitsch, A. & Zitouni, A. (2016). Biocontrol and plant growth promoting properties of *Streptomyces mutabilis* strain IA1 isolated from a Saharan soil on wheat seedlings and visualization of its niches of colonization. *S. Afr. J. Bot.* **105**: 234–239.

United Nations. (2015). *Transforming our world: the 2030 agenda for sustainable development*. United Nations treaty series. Retrieved from <http://www.un.org>.

Wang, G. L., Que, F., Xu, Z.S., Wang, F. & Xiong, A. S. (2015) Exogenous gibberellins altered morphology, anatomic and transcriptional regulatory networks of hormones in carrot root and shoot. *BMC Plant Biol.* 15: 290.