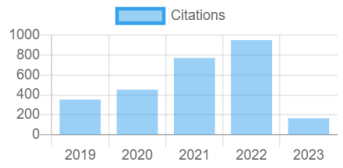
Research Journal of Microbiology

Research Journal of Microbiology is a peer-reviewed, open-access journal that publishes original research articles and reviews articles on microorganisms and their interaction with hosts and the environment. The journal covers all aspects of basic, medical and applied research inclusive of but not limited to microbes, bacteria, fungi, viruses, archaea, and protozoa.

Types of Articles:

Original Article, Review, Mini Review, Systematic Review, Methodological Article, Short Communication, Case Report, and Editorial.

Citations



Journal Metrics

Acceptance	Citations	APC
52 %	6884	325 $

Indexed In

- ASCI-Database
- Asian Digital Library
- FSTA
- Google Scholar
- ProQuest

Editorial Board

Research Journal of Microbiology
Science International
Dubai Silicon Oasis Headquarters Building 4th Floor, C & D Wing,
P.O. Box 126208 Dubai Sillicon Oasis, Dubai, UAE

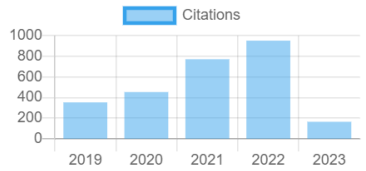
ISSN: 1816-4935

SCIONE™
A Product of The ACSE

Research Journal of Microbiology

Publisher: Science International

Citations



Most Cited

Antifungal Activity of Silver and Copper Nanoparticles on Two Plant Pathogens, *Alternaria alternata* and *Botrytis cinerea*

★ 272 Citations

Biosurfactants-Types, Sources and Applications

★ 207 Citations

Bacterial Lipases as Potential Industrial Biocatalysts: An Overview

★ 182 Citations

Indexed In

- ASCI-Database
- Asian Digital Library
- FSTA
- Google Scholar
- ProQuest

Volume 11, Number 2-3, 2016

Isolation and Biochemical Characterization of Microorganisms Associated with the Fermentation of Kersting's Groundnut (*Macrotyloma geocarpum*)

C. Abiola and V.O. Oyetayo

Research Journal of Microbiology Volume 11, Number 2-3, 47-55, 2016

Abstract PDF Fulltext Citations XML References

Effect of Varying Storage Temperatures on the Microbiological Quality of Street Sold Water in the Kumasi Metropolis, Ghana

M.G. Addo, C.A. Woods, L.A. Andoh and K. Obiri-Danso

Research Journal of Microbiology Volume 11, Number 2-3, 56-63, 2016

Abstract PDF Fulltext Citations XML References

Variation of Microbial Load of Sudanese Nubian Goat' Milk as Affected by Lactoperoxidase Enzyme System, Stage of Lactation and Storage Temperature

H.M.I. Mohamed, I.E.M. El Zubeir and A.A. Fadlelmoula

Research Journal of Microbiology Volume 11, Number 2-3, 64-69, 2016

Abstract PDF Fulltext Citations XML References

In planta Screening of Rhizobacterial Community for Promoting Maize (*Zea mays* cv. BISI 2) Growth in Dryland Agriculture

E. Hidayati, A.T. Wahyudi, A. Suwanto and R. Widyastuti

Research Journal of Microbiology Volume 11, Number 2-3, 70-79, 2016

Abstract PDF Fulltext Citations XML References

Bacteriological Monitoring for the Northern Khors of Lake Nasser, Egypt

Sayeda M. Ali, Amal A. Othman and Mohammed T. Abbas

Research Journal of Microbiology Volume 11, Number 2-3, 80-92, 2016

Abstract PDF Fulltext Citations XML References

Alternative Antibiotics Source from Symbiont of Lactid Acid Bacteria Inside Stomach of Honeybees (*Apis mellifera* and *Apis dorsata*) Against Multiresistant Antibiotics Pathogenic Bacteria

Novra Arya Sandi and Siti Isrina Oktavia Salasia

Research Journal of Microbiology Volume 11, Number 2-3, 93-100, 2016

Research Journal of Microbiology

ISSN 1816-4935

Academic Journals Inc.

www.academicjournals.com

OPEN ACCESS Research Journal of Microbiology

ISSN 1816-4935
DOI: 10.3923/jm.2016.70.79

Research Article

In planta Screening of Rhizobacterial Community for Promoting Maize (*Zea mays* cv. BISI 2) Growth in Dryland Agriculture

[1]E. Hidayati, [2]A.T. Wahyudi, [2]A. Suwanto and [3]R. Widyastuti

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Mataram University, Lombok 83125, Indonesia
[2]Department of Biology, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University, Bogor 16680, Indonesia
[3]Department of Soil Science and Land Resources, Faculty of Agriculture, Bogor Agricultural University, Bogor 16680, Indonesia

Abstract

In this study, screening of rhizobacterial community structure was performed to obtain potential rhizosphere bacterial community for plant growth promotion in dryland agriculture. Rhizosphere soil samples were collected from better growth performance of maize planted in dryland farm. Eleven rhizosphere soil samples were selected based on their effect on the growth of maize in a greenhouse. The water holding capacity of maize growth medium was adjusted in 100, 75 and 50%. Rhizobacterial communities were analyzed using Terminal Restriction Fragment Length Polymorphism (RFLP) based on metagenomic and cultivation-dependent approaches. The TR D is the best maize rhizosphere soil sample obtained from *in planta* screening. In the 50% water holding capacity, TR D treatment can increase the vegetative growth of maize. The TR D treatmen generally showed increased fresh weight of upper biomass (47.1%), dry weight of upper biomass (45.2%) and root dry weight (33.4%). The bacterial community structure of TR D included Burkholderiales, Pseudomonas, Bacillus, Candidatus, Alphaproteobacteria, Betaproteobacteria, Rhizobiales, Sinobacteraceae and Acidobacteria. Burkholderiales is the dominant group in metagenomic and *Pseudomonas* sp. is the dominant group in cultivation-dependent approach. In the other soil samples, such as TR A and TR K1, Burkholderiales and *Bacillus* sp. were the dominant. This study indicated that *in planta* screening can use to obtain potential rhizosphere bacterial community for growth promoting of maize in dryland.

Key words: *In planta*, bacterial community, dryland, rhizosphere, maize, metagenomic, cultivation-dependent

Received: November 15, 2015 **Accepted:** January 21, 2016 **Published:** February 15, 2016

Citation: E. Hidayati, A.T. Wahyudi, A. Suwanto and R. Widyastuti, 2016. *In planta* screening of rhizobacterial community for promoting maize (*Zea mays* cv. BISI 2) growth in dryland agriculture. Res. J. Microbiol., 11: 70-79.

Corresponding Author: A.T. Wahyudi, Department of Biology, Bogor Agricultural University, Bogor 16680, Indonesia Tel: +62-251-8622833

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Availability of agricultural land is critical to fulfil global food demand. The high rate of degradation and conversion of fertile agricultural land (Djaenudin, 2008) resulted in the expansion of agricultural activities leads to dryland. Based on data from Millennium Ecosystem Assessment (MEA., 2005), dryland cover more than 40% of the world terrestrial land. Dryland area is likely to become more widespread in line with the widespread of drought as a result of global climate change. Dryland areas in Indonesia are part of the potential world drylands. The efforts to raise productivity of dryland in Indonesia has been done actively, mainly for many commodities such as maize.

Several innovations have been made to increase the productivity of dryland, among other with involving the role of microbes. The involvement of microbes as part of the effort to improve development of dryland is an appropriate step because they are playing important roles in soil ecosystem and plants growth. This is because almost 90% of important process in the soil mediated by microbes (Nannipieri *et al*., 2003; Sengupta and Dick, 2015). Rhizosphere bacterial community is the group of soil microbes that live within the plants root zone. Many rhizosphere bacterial species have been developed as plant growth promoter (Glick, 2012).

Biological agents, such as microbial agents, are potential and prospect alternative compare with chemical fertilizers because of its eco-friendly and environmentally low-cost. The development of microbial agents often faced some problems. However, not all types of biological agents can be effective and give consistent effect during application. Many report of successful application of microbial agents in line with the failures application (Bashan and Dubrovsky, 1996; Malusa *et al*., 2012; Mazid and Khan, 2014). Some reasons cause the application failure of microbial agents, among others, reduced of effectiveness of microbial agents, inability to compete with indigenous microbes and incompatibility with environmental conditions (Subba-Rao *et al*., 1993; Bashan *et al*., 2014; Mazid and Khan, 2014; Geetha and Joshi, 2013).

At present, microbial agents have been developed still based on a single strains and multiple strains. According to Bashan *et al.* (2014), the development of bacterial inoculant involves a number of procedures. Briefly, in the research stage, bacteria from various agricultural or environmental rhizosphere samples were isolated and screened. Furthermore, the bacterial isolates were identified and characterized of their plant growth promotor traits. Selected isolates then applied to the target plants in a greenhouse. This is a commonly used procedure by researchers (Khalid *et al*., 2004; Husen *et al*., 2011; Thijs *et al*., 2014). Selection method of strains based on *in vitro* screening for plant growth promoting traits may give unexpected result when tested in target plant. Therefore, the inoculum selection techniques based on *in planta* screening is necessary to propose. *In planta* screening is likely to get the effective inoculum appropriate to the plants target.

Soil ecosystem and plant growth are highly complex and dynamic, especially in dryland ecosystems (D'Odorico and Bhattachan, 2012). Dryland ecosystems are different from wetland ecosystem because the growth of plants in drylands ecosystem have more constraints, particularly by the limitations of water and low soil fertility (Abdurachman *et al*., 2008). In principle, microbes in nature are together in a community to perform its function well in the complexity and dynamic ecosystems. Therefore, it is necessary to find more appropriate inoculum that can face the complexity and dynamic processes in dryland ecosystems associated with plant growth. Plant growth promoting rhizobacteria based on community should be considered to be developed.

The aims of this study were (a) To obtain the sample of microbial community originated from maize soil rhizosphere of dryland farm based on *in planta* screening and (b) To assess the culturable and unculturable forms of the rhizobacterial community structure and diversity. The rhizobacterial communities selected based on *in planta* screening are more likely to be inhabited by an effective bacterial consortium of plant growth promotor. Moreover, the rhizobacterial communities selected based on *in planta* screening may play their role better when applied back to the target plant and ecosystem. This study is the basic research for assessing the *in planta* screening to find good rhizobacterial community. Information about the rhizobacterial communities fingerprint, diversity and dominant bacteria can be use as comparison basic data for next researches. Other than that, selected rhizobacterial community can develop as inoculum for drought farming condition or other environmental condition and plant target.

MATERIAL AND METHODS

Rhizosphere soil collection and *in planta* screening in greenhouse: Rhizosphere soil samples were used in this study was defined as the soil adhering to fine root of maize originated from Lombok dryland farm, West Nusa Tenggara, Indonesia. Eleven rhizosphere soil samples (TR A, TR B, TR C, TR D, TR E, TR F, TR G, TR H, TR I, TR J and TR K) were collected from rhizosphere of better growth performance of two

months age maize (*Zea mays* cv. BISI 2) planted in dryland farm located at West Lombok (S 08°31'39.3'', E 116° 37'49.3'') and North Lombok (S 08°13'42.4'', E116°21'24.4''). The soil samples were collected by the method of Zhao *et al.* (2010).

In the first planting, all of soil samples were selected in a greenhouse based on its effect on the growth of maize. Sand river was used as a maize growth medium in pots. Sand river was sieved using a 3.35 mm mesh sieve, homogenized and sterilized for 1 h. Three kilograms of sand river were placed in plastic pots (polybag). Each pot was dampened to 100% of water holding capacity before planting. A 3 cm deep hole was opened in the center of pot. A total of 100 g rhizosphere soil sample was placed in the hole. Maize seed (*Zea mays* cv. BISI 2) was used in this study. Maize seeds were surface desinfected with 0.5% NaOCl for 2 min and 75% ethanol for 1 min and washed three times with sterile water. Seeds were placed in moist sterile cotton for germinating. After 2 days, a uniform germinating seeds, which have radicle 5-7 mm long, were chosen. One maize germinating seed was introduced into the hole of growth medium and then the hole was covered with sand river. Fertilizers (urea: 225 mg, phosphate: 150 mg, kalium: 150 mg and rock phosphate: 200 mg) were added after 5 old day maize after planting. The water holding capacity of growth medium was adjusted in 75 and 50%. Control 1 (TR K1: sterilized rhizosphere soil sample) and control 2 (urea: 300 mg; phosphate: 200 mg, kalium: 200 mg, and rock phosphate: 250 mg) were used as comparison treatment. Pots were set in 50×50 cm distance and carried out following the completely randomized design. After 30 days of growth, various growth variables were recorded (leaf number, 7th leaf width, 7th leaf length, plant length, plant height, fresh weight of upper biomass, dry weight of upper biomass and root dry weight). Rhizosphere soil samples from each plant were collected. In the second planting, five soil samples collected from rhizosphere of better growth performance of maize from the first planting. A greenhouse screening was used to selected all of soil samples based on its effect on the growth of maize. NPK fertilizer (234 mg) was added after 5-old-day maize after planting. The water holding capacity of growth medium was adjusted in 100, 75 and 50%. The TR K1 (sterilized rhizosphere soil sample) was used as a comparison treatment. The experiment was carried out following the randomized block design. Briefly, the methods for the second planting is the same as in the first planting. In the third planting, the soil sample collected from rhizosphere of the best growth performance of maize from the second planting was tested the effect on the growth of maize in 50% of water holding capacity and various doses of NPK fertilizer. Control 1 (TR K1: sterilized rhizosphere soil sample) and control 2 (no rhizosphere soil sample) were used as a comparison treatment. The experiment was carried out following the randomized block design. The methods for the third planting is the same as before.

Statistical analysis: Each treatment comprised 3 replicates. Data obtained was analyzed using analysis of variance (ANOVA) using Microsoft software. Treatments showed significant differences were tested further by Tukey's test at 5% level using a data processing program Minitab16.

Preparation of culture media: Rhizobacteria were cultivated using four culture media: (1) Commercial medium Soil Extract Agar (SEA, HIMEDIA, Mumbai, India), (2) Nutrient agar with low nutrients consisted of 1% (0.08 g L^{-1}) Nutrient Broth (Criterion, Santa Maria, CA) and 18 g L^{-1} agar powder (NA/n), (3) NAln supplemented with 50% soil extract (Naln-SE) and (4) NAln supplemented with 25% maize root extract (NAln-SE). On each medium, 50 μg mL^{-1} of antifungal nystatin was added. Firstly, soil extract and root extract prepared. One part of dryland natural soil was mixed with 2 parts of sterile water (w/v). Soil slurry was sterilized for 1 h (O'Neill *et al.*, 2009) and then allowed 24 h at room temperature. The supernatant was filtered with thick layer sterile cotton and stored at 6-7°C. Maize root extract was prepared from maize root mass (*Zea mays* ev. BISI 2). One part of the root mixed with 2 parts of sterile water (w/v). Root slurry was sterilized for 20 min and then allowed 24 h at room temperature. The supernatant was filtered with thick layer sterile cotton and stored at 6-7°C.

Rhizobacterial community analysis: Rhizobacterial communities were analysed using Terminal Restriction Fragment Length Polymorphism (T-RFLP) based on cultivation-independent (metagenomic) and cultivation-dependent approaches. Rhizobacterial communities were analyzed at this step are the communities inhabitant of the rhizosphere soil collected from maize plant in the second planting. Soil sample collected from uncropped soil at the same location in dryland farm at North Lombok (K0) was used as a comparison.

The DNA extraction for metagenomic preparation as described below. Ten grams of soil samples were suspended in 90 mL of sterile physiological saline solution and shaken at 125 rpm for 15 min. The suspension was transferred into a 50 mL falcon tube and centrifuged at 10000×g for 30 min. Soil pellet obtained was dried at 60°C for 30 min. DNA was extracted from 0.25 g dry soil pellet using power soil DNA extraction kit (Mo Bio Laboratories, Inc.). Quality and quantity of DNA were assessed by the ratio of $A_{260/280}$ and $A_{260/230}$ using NanoDrop (Thermo Scientific, Inc.). DNA extraction for cultivation-dependent preparation as described below.

Ten grams of soil samples were suspended in 90 mL of sterile physiological saline solution and shaken at 125 rpm for 15 min. From dilutions 10^{-3}, 10^{-4}, 10^{-5}, 100 µL aliquot of suspensions were spreaded on the surface of 3 replicated agar plates of all of four culture media (SEA, NAln, NAln-SE and NAln-RE) and incubated for 7 days at room temperature. Bacterial colonies growing on each culture medium was taken and dried at 60°C for 30 min. DNA was extracted from 0.25 g dry weight cell using Power Soil DNA extraction kit (Mo Bio Laboratories, Inc.). Quality and quantity of DNA were assessed. The 16S rDNA communities from metagenomic and cultivation-dependent preparation were amplified using fluorescently labeled primer 27F 6-FAM carboxyfluorescein (5'-AGAGTTTGATCCTGGCTCAG-'3) and unlabeled 1492R (5'-TACGGTTACCTTGTTACGACT-'3). The reactions were conducted using 50 µL mixtures contained 25 µL GoTaq(R) Green (Promega), 1 µL (10 pmol) forward and reverse primers, 5 µL (approximately 200 ng) DNA template and 18 µL Nuclease Free Water (NFW). Reactions were done in triplicate. Conditions for PCR were as follows: an initial denaturation step of 98°C for 45 sec; 30 amplification cycles of denaturation (98°C for 45 sec); annealing (55°C for 45 sec) and elongation (72°C for 45 sec); and a final extention step of 72°C for 7 min. 16S rDNA products were detected using gel electrophoresis with 1% agarose gel for 45 min and observed and documented with Geldoc 1000 (BIO RAD). The PCR products were purified to remove unincorparated nucleotides and primers by the QIAquick PCR purification kit (Qiagen, Germany). For culture-dependent preparation, PCR product from all culture media from the same sample were pooled. Purified PCR products were digested using *Msp*I endonuclease restriction enzyme (Fermentas) was as follows: 5 µL purified DNA, 1 µL enzime, 2 µL 10x Buffer Tanggo and 12 µL NFW. Reaction was done at 37°C for 3 h and then inactivated by incubation at 65°C for 20 min. All reaction were done in triplicate. Restriction products were delivered to 1st Base Malaysia for T-RFLP analysis. The fluorecently labeled 5'-terminal restriction fragments were detected and analyzed by GeneMapper v4.0.

Data analysis of T-RFLP profiles: Terminal Restriction Fragment (T-RF) sequence target in the range 50-500 bp were used for the T-RFLP analysis. The T-RFLP numerical data was exported to Exel (Microsoft) for further analysis. Percentage value of each T-RF peak area of the total peak area of each sample was calculated. The threshold value of peak area was set as 1% of total peak area. The T-RFs with relative peak area less than 1% of total peak area were remove from data set to eliminate possible background noise (Zhang *et al*., 2008). The T-RFs value that differed by less than 0.5 bp was considered identical (Dunbar *et al*., 2001; Smith *et al*., 2005). Numerical value of T-RF rounded to the nearest ten. A binary data for presence and absence of the T-RF size and patterns of peak area were calcutaed for further analysis. The T-RF size observed in the community compared with T-RF size within the Ribosomal Database Project (RDP) Soil database based on RDP r12u10 by RuLi and (R10, U27)700.829 Good Quality (>1200 Bacterial) using Microbial Community Analysis III (MICA3) Phylogenetic Assignment Tools (PAT+) (http://mica.ibest.uidaho.edu/digest.php). The same observed T-RF size to the T-RF size in the database is assignment to have the same phylogenetic affiliation.

Comparison of rhizobacterial community diversity inhabitant of each soil sample was calculated using Shannon diversity index (H'):

$$H' = -\Sigma(Pi \times \log Pi)$$

where, $Pi = n_i N^{-1}$, n_i is the peak area and N is the sum of the total peak areas.

RESULTS

Comparison of the vegetative growth values of maize in the first planting as shown in Table 1. Adjustment of water holding capacity of growth medium at 75 and 50% were statistically significantly different (data not shown), while the treatment with rhizosphere soils were not statistically significantly different compared with control 1 (TR K1) and control 2. However, there was a tendency that the treatment with certain kinds of rhizosphere soil generated the higher vegetative growth values compared with control 1 and control 2 (Table 1). Five rhizosphere soils (TR B, TR D, TR F, TR G and TR K) that give better growth performance then reselected in the second planting. The TR A and TR K1 that give lower growth performance were used as a comparison treatment.

In the second planting, adjustment of water holding capacity of growth medium at 100, 75 and 50% were not statistically significantly different, while the treatment of certain kinds rhizosphere soils were statistically significantly different compared with TR A and TR K1 (data not shown). Comparison of the vegetative growth values of maize as shown in the form of value scale in Fig. 1. Treatment with TR D give highest vegetative growth values in some variables, except for leaf width and leaf length. Width distance of value with TR D treatment showed in plant length, fresh weight of upper biomass, dry weight of upper biomass and root dry weight. Based on the scale, TR D categorized as a rhizosphere

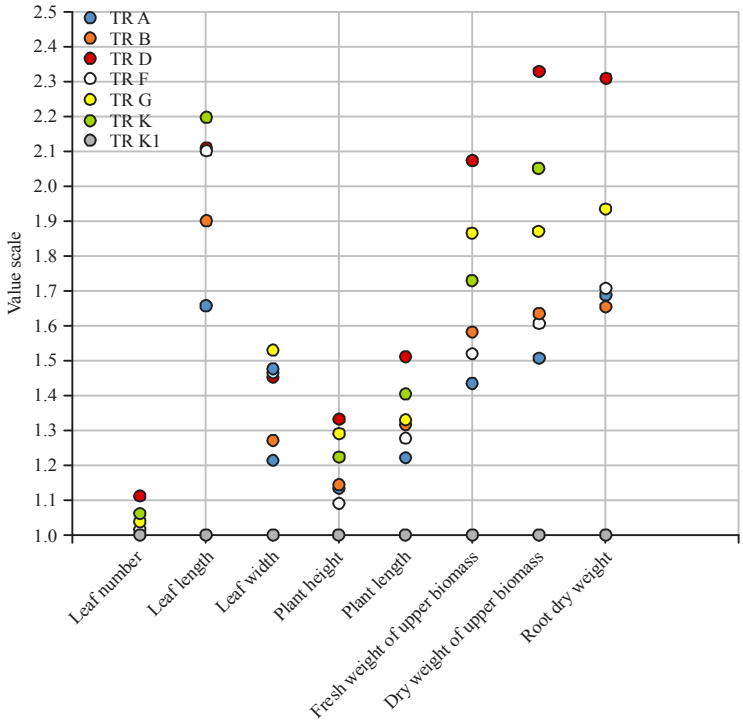


Fig. 1: Value scale of 30-old-day maize growth variables were treated using maize rhizosphere in the second planting

Table 1: Average value of 30 old day maize growth variables were treated using maize rhizosphere soils in the first planting

	Growth variables							
Rhizosphere soils	Leaf number (sheet)	Leaf length (cm)	Leaf width (cm)	Plant heigth (cm)	Plant length (cm)	Fresh weight fo upper biomass (g)	Dry weight of upper biomass (g)	Root dry weight (g)
TR A	10.50^{a}	23.67^{cde}	2.68^{abc}	29.88^{abc}	41.80^{abc}	18.01^{a}	2.75^{a}	1.59^{ab}
TR B	10.33^{a}	28.55^{a}	2.98^{a}	35.22^{ab}	48.28^{a}	19.77^{a}	3.47^{a}	2.14^{a}
TR C	10.66^{a}	24.25^{bcde}	2.88^{abc}	31.57^{abc}	42.62^{abc}	20.23^{a}	3.08^{a}	1.92^{ab}
TR D	10.83^{a}	24.98^{abcde}	2.68^{abc}	30.73^{abc}	44.00^{abc}	21.08^{a}	3.13^{a}	1.93^{ab}
TR E	10.66^{a}	26.17^{abcd}	2.83^{abc}	34.03^{abc}	45.88^{abc}	19.86^{a}	3.39^{a}	2.05^{a}
TR F	10.00^{a}	28.02^{ab}	2.93^{ab}	36.37^{a}	48.53^{a}	19.61^{a}	3.35^{a}	2.05^{a}
TR G	10.33^{a}	27.23^{abc}	2.93^{ab}	34.05^{abc}	46.88^{abc}	20.11^{a}	3.32^{a}	1.84^{ab}
TR H	10.66^{a}	21.22^{e}	2.5b^{c}	30.43^{abc}	42.12^{abc}	19.58^{a}	2.79^{a}	1.54^{ab}
TR I	10.33^{a}	25.72^{abcd}	2.77^{abc}	35.10^{ab}	47.70^{ab}	20.71^{a}	3.28^{a}	1.98^{ab}
TR J	10.33^{a}	25.03^{abcde}	2.80^{abc}	33.95^{abc}	46.30^{abc}	18.08^{a}	3.05^{a}	1.69^{ab}
TR K	10.83^{a}	24.60^{abcde}	2.92^{ab}	32.42^{abc}	44.62^{abc}	20.54^{a}	3.44^{a}	2.09^{a}
Control 1 (TR K1)	10.00^{a}	22.52^{de}	2.52^{c}	27.18^{c}	40.00^{c}	13.76^{a}	2.40^{a}	1.32^{b}
Control 2	10.00^{a}	22.73^{de}	2.72^{abc}	28.87^{bc}	41.03^{bc}	15.89^{a}	2.62^{a}	1.47^{ab}

The numbers followed by the same letter within a column are not significantly different at 5% level by Tukey's test

soil which give better growth performance of maize. Treatment with TR A and TR K1 give not good of growth performance of maize where the growth variables lower than TR D treatment, while treatment with TR G give medium (between TR D, TR A and TR K1) growth performance of maize. In the third planting, TR D then checked for the effect on the growth of maize at 50% water holding capacity of growth medium.

Comparison of the vegetative growth values of maize in the third planting as shown in Table 2. In the condition of water holding capacity of growth medium at 50%, treatment with TR D give significantly different vegetative growth values compare with control 1 and control 2. The results showed that TR D can enhance the growth of maize in all dose of fertilizer, especially when the plants grown under 0% fertilizer (no fertilizer). Based on average data of all treatment combinations, addition of 100 g of TR D to the growth medium can increase the fresh weight of upper biomass (47.1%), dry weight of upper biomass (45.2%) and root dry weight (33.4%).

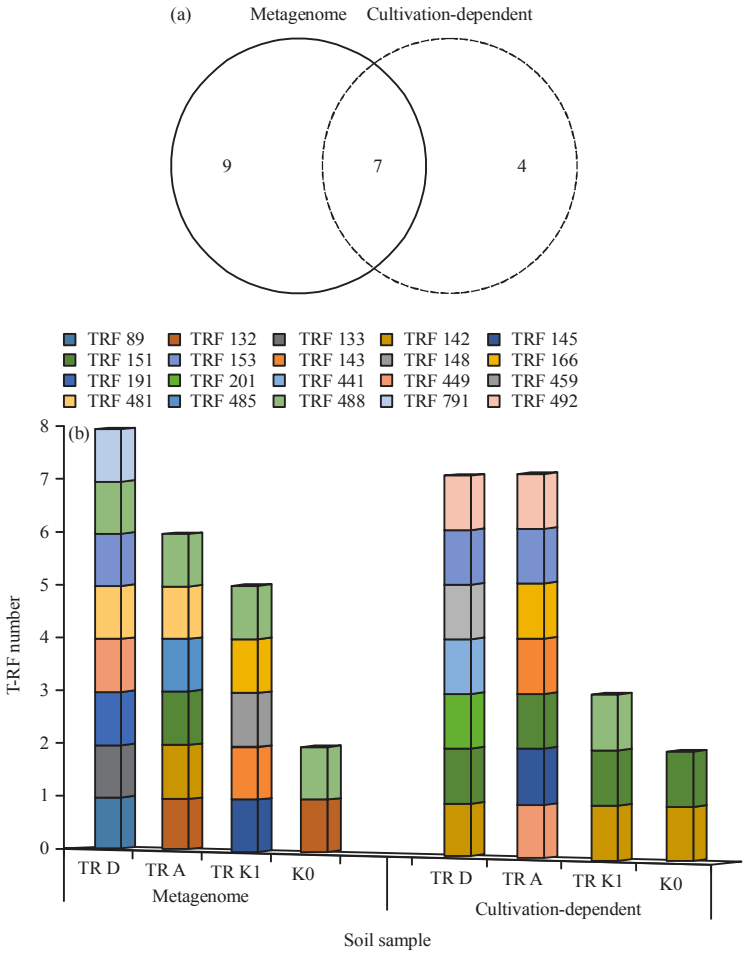


Fig. 2(a-b): (a) Comparison of T-RF total number of bacterial community from soil samples in metagenomic and cultivation-dependent approaches and (b) T-RF number and size of bacterial community inhabitant of each soil sample

Table 2: Average value of 30-old-day maize growth variables were treated using maize rhizosphere soils in the third planting

Treatments		Growth variables							
Fertilizer doses (%)	Soil samples	Leaf number (sheet)	Leaf length (cm)	Leaf width (cm)	Plant height (cm)	Plant length (cm)	Fresh weight of upper biomass (g)	Dry weight of upper biomass (g)	Root dry weight (g)
100	TR D	12.33^{a}	39.63^{a}	4.07^{a}	45.50^{a}	62.90^{a}	32.53^{a}	3.77^{a}	2.25^{a}
	Control 1 (TR K1)	11.33^{a}	34.07^{a}	3.63^{a}	37.63^{a}	52.17^{b}	21.14^{ab}	2.79^{ab}	1.77^{ab}
	Control 2	11.33^{a}	34.77^{a}	3.60^{a}	35.83^{a}	51.43^{b}	19.00^{b}	2.38^{b}	1.56^{b}
75	TR D	12.33^{a}	41.77^{a}	4.20^{a}	42.00^{a}	59.07^{a}	27.20^{a}	3.66^{a}	2.29^{a}
	Control 1 (TR K1)	10.67^{a}	33.93^{a}	3.33^{a}	31.67^{b}	49.97^{a}	16.93^{a}	2.09^{ab}	1.83^{ab}
	Control 2	10.00^{a}	37.73^{a}	3.70^{a}	34.20^{b}	53.13^{a}	13.43^{a}	1.92^{b}	1.48^{b}
50	TR D	12.00^{a}	38.03^{a}	4.23^{a}	40.87^{a}	53.37^{a}	26.23^{a}	3.22^{a}	2.21^{a}
	Control 1 (TR K1)	10.33^{b}	38.77^{a}	3.30^{b}	32.20^{a}	53.20^{a}	11.33^{a}	1.98^{b}	1.23^{b}
	Control 2	10.33^{b}	30.83^{a}	3.17^{b}	32.93^{a}	46.93^{a}	16.01^{a}	1.81^{b}	1.75^{ab}
0	TR D	12.00^{a}	36.10^{a}	3.87^{a}	33.30^{a}	49.73^{a}	24.51^{a}	3.06^{a}	2.27^{a}
	Control 1 (TR K1)	10.67^{a}	24.93^{a}	3.00^{b}	30.97^{a}	46.43^{a}	10.42^{b}	1.67^{ab}	1.07^{b}
	Control 2	10.00^{a}	23.40^{a}	2.87^{b}	30.60^{a}	38.47^{a}	10.50^{b}	1.45^{b}	1.21^{b}

The numbers followed by the same letter within a column are not significantly different at 5% level by Tukey's test

The T-RFLP analysis of rhizobacterial communities showed that total of 20 T-RFs obtained from communities in all of four rhizosphere soil samples (TR D, TR A, TR K1 and K0). Sixteen T-RFs obtained from metagenomic and 11 T-RFs from cultivation-dependent approach, only found 7 T-RFs matching in both approaches (Fig. 2a). The T-RF number and size of bacterial community inhabitant of each soil sample showed in Fig. 2b. The number of TRF in metagenomic approach

Table 3: Diversity index of bacterial community of each soil sample

	Metagenomic		Cultivation-dependent	
Bacterail community	T-RF No.	H'	T-RF No.	H'
TR D	8	1.602	7	1.575
TR A	6	1.372	7	1.648
TR K1	5	1.440	3	0.854
K0	2	0.646	2	0.391

was higher in TR D (8 T-RFs) and lower in TR A (6 T-RFs), TR K1 (5 T-RFs) and K0 (2 T-RFs). The number of T-RF in cultivation-dependent approach was higher in TR D and TR A (7 T-RFs) and lower in TR K1 (3 T-RFs) and K0 (2 T-RFs). Based on T-RFLP fingerprint patterns were obtained (data not shown), there is a differences profile patterns between bacterial community of TR D compared with bacterial communities in the other soil samples. Generally, in the metagenomic and cultivation-dependent approaches, there are two groups of T-RF size (100 and 400 bp range size) were obtained in all of four communities. In TR D community, in addition to the two groups T-RF size also appears T-RF 89 in metagenomic and T-RF 201 in cultivation-dependent approach.

Comparison of rhizobacterial composition and abundance showed in Fig. 3. By metagenomic approach, T-RF 488 belonging to Burkholderiales was detected as a group of bacteria that composed the communities in all soil samples were observed. In the community of TR D, Burkholderiales and *Pseudomonas* sp. (T-RF 485) are the dominant bacterial groups detected by metagenomic and *Pseudomonas* sp. detected dominant by cultivation-dependent approach. *Bacillus* sp. (T-RF 145 and T-RF 153) was detected as a group of bacteria that composed the communities of TR D, TR A and TR K1 by metagenomic approach. This group also detected dominant in the community of TR K1 by metagenomic and in the community of TR A by cultivation-dependent approach. Comparison of bacterial communities diversity of four soil samples by metagenomic and cultivation-dependent approaches as shown in Table 3. Diversity index by metagenomic showed that the bacterial community inhabitant of TR D was higher (1.60) than communities of TR A (1.37), TR K1 (1.44) and K0 (0.65). Diversity index by culture-dependent showed that the bacterial community inhabitant of TR A was higher (1.65) than communities of TR D (1.58), TR K1 (0.85) and K0 (0.4).

DISCUSSION

In this study, it is showed that growth performance of maize was different in response to rhizosphere soils treatment. The TR D is the best rhizosphere soil sample obtained using twice *in planta* screening. In the first planting, the rhizosphere soil treatment effect is not seen clearly. It may because of water treatments or variation of soil samples it self. Based on the data shown in Table 1, there was a tendency that although the plants fertilized at a lower dose, but addition of 100 g of good rhizosphere soil to the growth medium can increase the vegetative growth of maize compared with the plants fertilized at a higher dose without addition of rhizosphere soil. This indicates that there are the rhizosphere soils that can clearly enhance the growth of maize. In order to obtain the rhizosphere soils that can enhance the growth of maize, fertilizer and water holding capacity of growth medium were designed differ as in the second planting. Assay in the third planting showed a significant increase in growth by TR D treatment in drought condition. In addition to obtain the compatible bacterial community that directly affect the growth, using *in planta* screening can also to estimate the other factors may affect the growth of plants associated with the bacterial community in given environment.

Plant growth is not only affected by physical and chemical but also microbial communities of soil. These factors interact each other to affect plant growth. In some cases, one of these factors can affect more strongly depend on the condition of limiting factors in the environment. Soil pH is one of the main factor affecting bacterial community of the soil (Fierer and Jackson, 2006; Lauber *et al*., 2009). Environmental stresses such as high salinity and drought can also affected the microbial life (Bouasria *et al*., 2012; De Oliveira *et al*., 2013).

Beneficial effect of the rhizobacteria for plant growth and health were recorded (Glick, 2012; Van Loon, 2007; Saharan and Nehra, 2011). In this study also suggested that variation in the composition of microbial communities may affect the growth performance of plants together with other environmental factors. More variation and higher diversity in rhizobacterial community of TR D may give better effect for maize growth. The profiles data as revealed by T-RFLP showed that there are variation in T-RF profiles among the rhizobacterial community inhabitant of TR D compared to TR A, TR K1 and K0. The T-RF is a unit that can be directly used to describe a community. Each different T-RF size represents a different group of bacteria. Closely related bacterial species usually have the same T-RF size, but one or more different taxonomic groups can also have the same T-RF (Ding *et al*., 2013). It means that the variety size of T-RFs communities found in the rhizosphere soils showed the variety of bacterial groups belong to the communities. Base on the number of T-RF (Fig. 3), bacterial community of TR D may have higher number of bacterial group than TR A, TR K1 and K0. The T-RFs size were obtained from the community indicated that

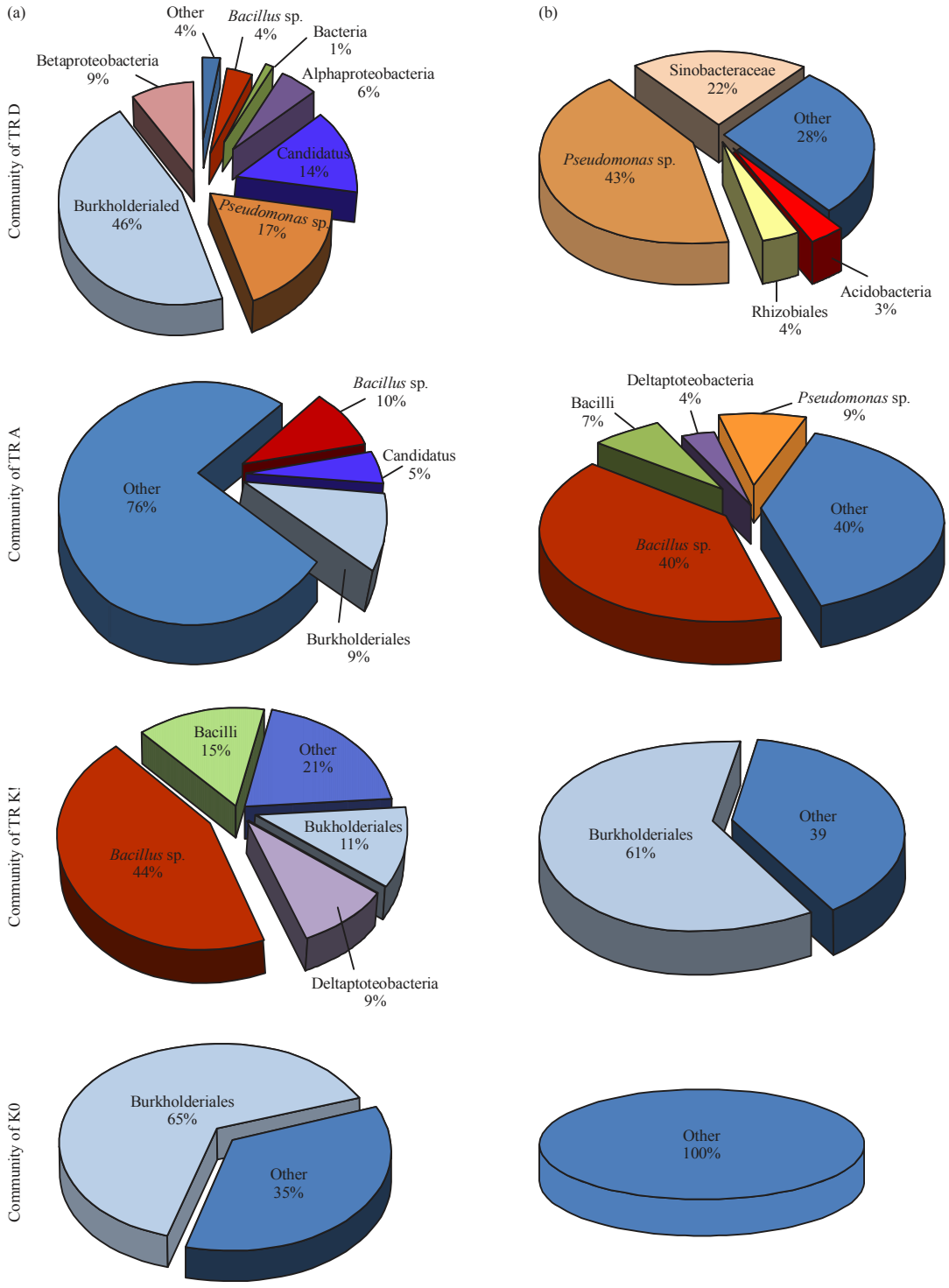


Fig. 3(a-b): Composition of bacterial community inhabitant of each (a) Soil sample in metagenomic and (b) Cultivation-dependent approaches

the T-RFs size may be owened by the dominant bacterial groups in the community. The actual number of bacterial groups in the community may be higher than the number of T-RFs assessed. This data also suggested that better growth performance of maize related to the higher number of bacterial group that composed the community. At least two groups of rhizobacteria, Burkholderiales and Pseudomonas, were detected more abundant in metagenomic approach

from TR D community (Fig. 3). These bacteria well known reputation as plant growth-promoting and have been developed commercially (Glick, 2012).

Bacterial community of maize and other commodity crops have studied in many aspects but need to assess to develope the bacterial inoculum from the communities. By using *in planta* screening, good bacterial community for maize plant can be obtain and develope all of microbe or only bacterial community in the rhizosphere soil sample selected using *in planta* screening. Culturable community can also develop to inoculum. Culturable abundant group can also develope for specific purpose, such as for inoculum fortivication, but the point to keep in mind that culturable bacteria only about 1% of the total community. The number of culturable depend on variation of cultivation media.

The results of this study showed that cultivation-dependent approach has lower level of diversity compared with metagenomic. It may because only the culturable bacteria that can be analysis as a source of diversity. While the higher level of diversity in the metagenomic approach may because the culturable bacteria and unculturable bacteria can be analysis as a source of diversity. Especially for community of TR A, higher level of diversity by cultivation-dependent approach compared with metagenomic can be occured if the TR A has more number of culturable bacteria than unculturable bacteria. Improvement in different types of media need for cultivating different types of bacterial group. As many researcher expalined that microbial community also referred as the second genome of plants because their significant role for growth and health of plants (Marri, 2015; Berendsen *et al*., 2012). Therefore, involvment of unculturable community important to be considered in the development of inoculum.

CONCLUSION

The TR D is a maize rhizosphere soil sample resulted from *in planta* screening. Bacterial community of TR D originated from maize rhizosphere planted in dryland farm. In the 50% water holding capacity of growth media, TR D treatment can increase the vegetative growth of maize. The TR D treatment generally showed increased fresh weight of upper biomass (47.1%), dry weight of upper biomass (45.2%) and highest increase of root dry weight (33.4%). The bacterial community structure of TR D included Burkholderiales, Pseudomonas, Bacillus, Candidatus, Alphaproteobacteria, Betaproteobacteria, Rhizobiales, Sinobacteraceae and Acidobacteria. Burkholderiales is the dominant group in metagenomic approach and *Pseudomonas* sp. is the dominant group in cultivation-dependent approach. The community also inhabited by bacterial groups commonly used for biofertilizer. This study indicated that *in planta* screening can use to obtain potential rhizosphere bacterial community for growth promoting of maize in dryland.

REFERENCES

Abdurachman, A., A. Dariah and A. Mulyani, 2008. [Strategy and technology of dryland management to support national food production]. Jurnal Litbang Pertanian, 27: 43-49, (In Indonesian).

Bashan, Y. and J.G. Dubrovsky, 1996. *Azospirillum* spp. participation in dry matter partitioning in grasses at the whole plant level. Biol. Fertil. Soils, 23: 435-440.

Bashan, Y., L.E. de-Bashan, S.R. Prabhu and J.P. Hernandez, 2014. Advances in plant growth-promoting bacterial inoculant technology: Formulations and practical perspectives (1998-2013). Plant Soil, 378: 1-33.

Berendsen, R.L., C.M.J. Pieterse and P.A.H.M. Bakker, 2012. The rhizosphere microbiome and plant health. Trends Plant Sci., 17: 478-486.

Bouasria, A., T. Mustafa, F. De Bello, L. Zinger, G. Lemperiere, R.A. Geremia and P. Choler, 2012. Changes in root-associated microbial communities are determined by species-specific plant growth responses to stress and disturbance. Eur. J. Soil Biol., 52: 59-66.

D'Odorico, P. and A. Bhattachan, 2012. Hydrologic variability in dryland regions: impacts on ecosystem dynamics and food security. Philos. Trans. R. Soc. B: Biol. Sci., 367: 3145-3157.

De Oliveira, A.B., N.L.M. Alencar and E. Gomes-Filho, 2013. Comparison between the Water and Salt Stress Effects on Plant Growth and Development. In: Responses of Organisms to Water Stress, Akinci, S. (Ed.). Chapter 4, InTech Publ., Rijeka, Croatia, ISBN: 978-953-51-0933-4, pp: 67-94.

Ding, T., M.W. Palmer and U. Melcher, 2013. Community terminal restriction fragment length polymorphisms reveal insights into the diversity and dynamics of leaf endophytic bacteria. BMC Microbiol., Vol. 13. 10.1186/1471-2180-13-1

Djaenudin, D., 2008. [The development of land resources research and its contribution to exceed requisite of agricultural land in Indonesia]. Jurnal Litbang Pertanian, 27: 137-145, (In Indonesian).

Dunbar, J., L.O. Ticknor and C.R. Kuske, 2001. Phylogenetic specificity and reproducibility and new method for analysis of terminal restriction fragment profiles of 16S rRNA genes from bacterial communities. Applied Environ. Microbiol., 67: 190-197.

Fierer, N. and R.B. Jakson, 2006. The diversity and biogeography of soil bacterial communities. Proc. Natl. Acad. Sci. USA., 103: 626-631.

Geetha, S.J. and S.J. Joshi, 2013. Engineering rhizobial bioinoculants: A strategy to improve iron nutrition. Scient. World J. 10.1155/2013/315890

Glick, B.R., 2012. Plant growth-promoting bacteria: Mechanisms and applications. Scientifica. 10.6064/2012/963401

Husen, E., A.T. Wahyudi, A. Suwanto and Giyanto, 2011. Soybean response to 1-aminocyclopropane-1-carboxylate deaminase-producing *Pseudomonas* under field soil conditions. Am. J. Agric. Biol. Sci., 6: 273-278.

Khalid, A., M. Arshad and Z.A. Zahir, 2004. Screening plant growth-promoting rhizobacteria for improving growth and yield of wheat. J. Applied Microbiol., 96: 473-480.

Lauber, C.L., M. Hamady, R. Knight and N. Fierer, 2009. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. Applied Environ. Microbiol., 75: 5111-5120.

MEA., 2005. Ecosystems and Human Well-Being: Synthesis. Island Press, Washington, DC., USA., ISBN-13: 9781597260404, Pages: 160.

Malusa, E., L. Sas-Paszt and J. Ciesielska, 2012. Technologies for beneficial microorganisms inocula used as biofertilizers. Scient. World J. 10.1100/2012/491206

Marri, P.R., 2015. The promise of the plant's second genome. J. Invest. Genomics, Vol. 2, No. 4.

Mazid, M. and T.A Khan, 2014. Future of bio-fertilizers in Indian agriculture: An overview. Int. J. Agric. Food Res., 3: 10-23.

Nannipieri, P., J. Ascher, M.T. Ceccherini, L. Landi, G. Pietramellara and G. Renella, 2003. Microbial diversity and soil functions. Eur. J. Soil Sci., 54: 655-670.

O'Neill, B., J. Grossman, M.T. Tsai, J.E. Gomes and J. Lehmann *et al*., 2009. Bacterial community composition in Brazilian anthrosols and adjacent soils characterized using culturing and molecular identification. Microb. Ecol., 58: 23-35.

Saharan, B.S. and V. Nehra, 2011. Plant Growth promoting rhizobacteria: A critical review. Life Sci. Med. Res.

Sengupta, A. and W.A. Dick, 2015. Bacterial community diversity in soil under two tillage practices as determined by pyrosequencing. Microb. Ecol., 70: 853-859.

Smith, C.J., B.S. Danilowicz, A.K. Clear, F.J. Costello, B. Wilson and W.G. Meijer, 2005. T-Align, a web-based tool for comparison of multiple terminal restriction fragment length polymorphism profiles. FEMS Microbiol. Ecol., 54: 375-380.

Subba-Rao, N.S., G.S. Venkateraman and S. Kannaiyan, 1993. Biological Nitrogen Fixation. Indian Council Agric. Res. (ICAR), New Delhi, India, pp: 112.

Thijs, S., N. Weyens, W. Sillen, P. Gkorezis, R. Carleer and J. Vangronsveld, 2014. Potential for plant growth promotion by a consortium of stress-tolerant 2,4-dinitrotoluene-degrading bacteria: Isolation and characterization of a military soil. Microb. Biotechnol., 7: 294-306.

Van Loon, L.C., 2007. Plant responses to plant growth-promoting rhizobacteria. Eur. J. Plant Pathol., 119: 243-254.

Zhang, R., V. Thiyagarajan and P.Y. Qian, 2008. Evaluation of terminal-restriction fragment length polymorphism analysis in contrasting marine environments. FEMS Microbiol. Ecol., 65: 169-178.

Zhao, Q., D.H. Zeng and Z.P. Fan, 2010. Nitrogen and phosphorus transformations in the rhizospheres of three tree species in a nutrient-poor sandy soil. Applied Soil Ecol., 46: 341-346.