The Pharma Innovation Journal 2021; 10(10): 2046-2053

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; 10(10): 2046-2053

www.thepharmajournal.com
Received: 14-08-2021
Accepted: 29-09-2021

N Olivia Devi
School of Crop Protection, College of Post Graduate Studies in Agricultural Sciences, Central Agricultural University, Umiam, Meghalaya, India

RK Tombisana Devi
School of Crop Protection, College of Post Graduate Studies in Agricultural Sciences, Central Agricultural University, Umiam, Meghalaya, India

D Thakuria
School of Natural Resource Management, CPGSAS, CAU, Umiam, Meghalaya, India

T Rajesh
School of Crop Protection, College of Post Graduate Studies in Agricultural Sciences, Central Agricultural University, Umiam, Meghalaya, India

K Ningthoujam
School of Crop Protection, College of Post Graduate Studies in Agricultural Sciences, Central Agricultural University, Umiam, Meghalaya, India

Corresponding Author:
N Olivia Devi
School of Crop Protection, College of Post Graduate Studies in Agricultural Sciences, Central Agricultural University, Umiam, Meghalaya, India

Isolation and characterization of Endophytic *Bacillus* isolated from tomato roots

N Olivia Devi, RK Tombisana Devi, D Thakuria, T Rajesh and K Ningthoujam

Abstract
Endophytic bacteria invade the host plant's tissues and develop a mutually beneficial relationship demonstrating a complex interaction inside the host plant. They are a vital part of plant micro ecosystem because they defend the plant against microorganisms and environmental stresses. The heat resistant spores and its ability to survive in diverse environmental situations make *Bacillus* a popular microbe for commercial use as biocontrol agent and plant growth promoting bacteria. In the study, total of 130 *Bacillus* endophytes were isolated from tomato roots from 20 locations under six districts of Meghalaya *viz.* South West Garo hills, West Garo hills, West Jaintia hills, East Jaintia hills, East Khasi hills and Ri Bhoi districts. Based on biochemical characters and bacterial identification software ABIS online all the 130 isolates were tentatively identified up to species level. The isolates belonged to the genera *Bacillus*, *Paenibacillus* and *Viridibacillus* with maximum of 17 isolates each identified as *Bacillus amyloliquefaciens* and *B. thuringiensis*. The study gives a good insight into the diversity of *Bacillus* endophytes found in tomato roots of Meghalaya.

Keywords: Endophytes, *Bacillus*, Meghalaya, isolates

Introduction
Tomato (*Solanum lycopersicum* L.) is a short duration vegetable which has been extensively cultivated mainly as a cash crop around the world (Adepoju, 2014) [2]. Tomato belongs to the Solanaceae family and have two assumptions about the origin of tomato one supporting Mexican and another Peruvian origin (Peralta and Spooner, 2007) [28]. Tomato is the third largest vegetable crop after potato and sweet potato but it ranks first as a processed or canned vegetable (Yeshiwas *et al*., 2016) [37]. During 2017-2018 the total area under tomato crop in Meghalaya was 2200 ha with production of 35510 metric tonne (Anon, 2018) [6]. Endophytes are known to colonize tomato plants and other vegetables. Plant-associated microorganisms called endophytes invade and occupy plant tissue without harming the host plant (Kandel *et al*., 2017) [18].

They are found in healthy plant parts such as the root, stem, and fruits (Kloepper, 1995) [21]. Endophytes have antimicrobial, anti-insect and anticancer properties since they contain secondary metabolite like phenolic acids, alkaloids, steroids, saponins, tannins, quinone and terpenoids (Gouda *et al*., 2016) [14] and they have plant growth promotion and disease suppression activities (Malfanova *et al*., 2011) [23]. *Bacillus* is one of the most commonly occurring genera of endophytic bacteria exploited as bio control agent (Nandhini *et al*., 2012) [26].

Bacillus are aerobic or facultative anaerobic, gram positive, rod shaped, flagellated motile bacteria, catalase positive belonging to the division Firmicutes (Vargas *et al*., 2004) [33] containing 60 species having different phenotype (Wulff *et al*., 2002) [35]. *Bacillus* spp. can form endospore which can withstand and resist adverse environmental conditions making them easily adapt to diverse habitats (Priest, 1993) [29]. It requires many biochemical test for their identification (Sneath *et al*., 1986) [31]. *Bacillus* species are identified on the basis of morphological characters and biochemical features described by Holt *et al*. (2000) [16]. Most *Bacillus* spp. are oxidase and catalase positive and can ferment carbohydrate (Aruwa and Ogunlade, 2016) [7]. *Bacillus* have been identified as plant growth promoting and biocontrol agents against diverse plant pathogens (Jayapala *et al*., 2019) [17]. *B. Subtilis* group (*B. subtilis*, *B. pumilus*, *B. atrophaeus*, *B. licheniformis* and *B. amyloliquefaciens*) and *B. cereus* group (*B. anthracis*, *B. cereus*, *B. thuringiensis*, *B. mycoides*, *B. pseudomycoides*, and *B. weihenstephanesis*) have both antagonistic and plant growth promoting potentials

(Ash *et al*., 1991) [9]. *Bacillus subtilis* is the main species of *Bacillus* that has been widely studied for its antagonistic activity along with other species like *B. megaterium* (Kanjanamaneesathian *et al.*, 2007) [19], *B. vallismortis* (Kaur *et al*., 2015) [20], *B. velezensis* (Palazzini *et al*., 2016) [27], *B. amyloliquefaciens* (Maung *et al*., 2017) [25], *B. pumilus* (Agarwal *et al*., 2017) [3], and *B. cereus* (Wang *et al*., 2019) [34]. The study of root associated endophytic *Bacillus* is necessary to understand their interaction with the plant and their role in rhizosphere. Examining various non-pathogenic endophytic *Bacillus* associated with tomato plants may help to recognise superior endophytic bacterial strains which have the tendency to be exploited as a bio control agent with plant growth promoting capabilities. Hence, the present study focuses on the isolation and characterization of endophytic *Bacillus* isolated from tomato roots grown in Meghalaya.

Materials and Methods

Isolation and purification of *Bacillus* endophytes

Bacillus endophytes were isolated from healthy tomato roots collected from 20 different locations of Meghalaya by the method described by Zinniel *et al.* (2002) [38] with slight modifications. The roots after washing in running tap water were surface sterilized with 70% ethanol for 1 min followed by immersing in 2% sodium hypochlorite solution for 3 min and again in 70% ethanol for 30 sec. The roots were cut and macerated in sterile mortar and pestle by adding phosphate buffer having 7.2 pH. From the total extract only 1.5 ml of the aliquot was taken and added to micro centrifuge tubes. They were given heat shock treatment by keeping the tubes in water bath at 80 ^{o}C for 5 min to eliminate other unwanted microorganisms. Tissue extract were diluted up to 10^5 in sterile saline and 0.1ml of the aliquot plated by spread plate method on Nutrient Agar plates. These plates were then incubated at 28 ± 1°C for 24 h. Bacterial colonies of *Bacillus* developed in dilution plates of NA were selected and single colonies were then picked up and streaked in a fresh NA medium with the help of a sterile loop to obtain pure culture. They were incubated at 28 ± 1 °C for 24 h.

Maintenance of isolates

The pure cultures were maintained by regular sub culturing at an interval of two months on NA slants and kept at 4 ±1°C in refrigerator. Long term preservation of the cultures were done by inoculating a loopful of 48 h old pure culture to 5 ml of sterile distilled water in screw caped storage vial of 10 ml and stored in refrigerator at 4 ± 1°C. Stock cultures were also made in Luria Bertani (LB) broth containing 20% (w/v) glycerol and stored at -20 °C.

Cultural and biochemical characterization of *Bacillus* endophytes

Cultural and biochemical tests of all the endophytic *Bacillus* spp. were achieved by the methods described by Cappucino and Sherman (2002) [10]. Cultural studies like gram staining using the Gram staining kit (K00-1Kt, Himedia) and KOH solubility Test (3%) were done for all the isolates. The *Bacillus* endophytes were biochemically characterized for the identification and classification of the isolates by referring the guidelines described by Holt *et al*. (2000) [16] in Bergey's Manual of Determinative Bacteriology. A total of twelve (12) biochemical test were performed on all isolates, which included oxidase production, catalase test, gelatin hydrolysis, arginine dehydrolase, urease test, nitrate reduction test, citrate utilization test, indole test, Methyl Red (MR) test, Voges - Proskauer (VP) test, Hydrogen sulphide (H_2 S) production test, and glucose fermentation test.

Tentative identification of the *Bacillus* isolates

Bacillus isolates were tentatively identified upto species level based on biochemical test and the bacterial identification software 'Advance bacteriological identification software' (ABIS) was also used for accurate identification.

Results and Discussion

Isolation of *Bacillus* endophytes

A total of 130 putative endophytic *Bacillus* isolates were isolated from tomato roots collected from 20 different locations of Meghalaya (Table 1). Among them maximum 10 isolates were obtained from tomato roots collected from Chibinang followed by Umshing-Mawkynroh (9 isolates) and Lokaichar (9 isolates) while least number of isolates were obtained from Umdihar (3 isolates). Bacterial endophytes tend to harbour with maximum densities in the roots of the host plant compared to other parts like stem, leaf (Gao *et al*., 2012) [13]. Since the environment around the root is complex and under the influence of both biotic an abiotic factors, the roots tend to have highest endophytic bacteria. Similarly, six gram positive endophytic Bacillus were isolated from tomato seeds (Amaresan *et al*., 2012) [5]. The present finding is also similar with that of Yanti *et al*. (2018) [36] who isolated 15 endophytic *Bacillus* from healthy tomato plants grown in Indonesia. Similarly, Abdallah *et al*. (2018) [1] isolated endophytic *Bacillus* from tomato roots, stems, leaves, flowers, fruits and seed collected from different places of Tunisia.

Table 1: Endophytic *Bacillus* isolates associated with tomato roots obtained from different parts of Meghalaya

Sl. No.	Districts	Locations name	Total number of *Bacillus* endophytes isolated
1	Ri Bhoi District	CPGS-AS	8
		Umiet	5
		Bhoirymbong	4
		Umsning	6
		Umdihar	3
2	East Khasi Hills	Sohryngkham	8
		Umshing-Mawkynroh	9
		Mylliem	8
3	East Jaintia Hills	Moolasngi	6
		Daistong	7
4	West Jaintia Hills	Moodymmai	8
		Larnai	6
		Ummulong	5
5	West Garo Hills	Chibinang	10
		Damalgre	8
		Chandabhoi	4
6	South West Garo Hills	Lokaichar	9
		Kalegaon	5
		Kodaldhuwa	5
		Bhoirakupi	6
		TOTAL	130

Characterization of *Bacillus* endophytes

Cultural studies

All 130 isolates were found to be gram-positive and rod-shaped. KOH test was additionally found to be negative for all the isolates which indirectly prove the isolates as gram-

positive (Table 2). Similar results were also found by Agarwal and Agarwal (2013) [4] and Chukeatirote *et al.* (2015) who reported *Bacillus* sp. to be gram positive and rod shaped. Gram staining may give variable result due to hasty decolourization of gram positive bacteria like *Bacillus* so alternative method like KOH test were used to confirm the gram positive or negative result (Dash and Payyappilli, 2016) [12]. So, all the 130 isolates were confirmed as *Bacillus* supported by the investigations of Holt *et al.* (2000) [16] and Sarode *et al.* (2019) [30] who also found *Bacillus* to be KOH negative and gram positive.

Biochemical characterization

Twelve biochemical tests were performed for tentative identification of the *Bacillus* isolates up to species level (Table 2 and Fig. 1). Among 130 *Bacillus* isolates tested, 60 isolates were oxidase positive indicated by the change in colour of the oxidase disc to purple colour. All 130 *Bacillus* isolates can produce bubble when a loopful of bacterial colony was mixed into hydrogen peroxide solution (3%) indicating a positive catalase test result.126 isolates were able to hydrolyse gelatin. Arginine Dehydrolase test was found positive for 75 isolates by giving a distinct red or dark pink colour of the arginine medium. 44 isolates could change the colour of urease broth from pink to red giving a positive reaction, rest 86 isolates were negative as the broth remained yellow colour. Out of 130 isolates, 99 isolates could reduce nitrate to nitrite by giving a pink or red colour of the nitrate broth that indicates positive reaction to nitrate reduction test. Only 17 isolates gave positive result by changing the green colour of simon citrate agar to blue colour indicating positive result of citrate utilization test. Only 4 isolates *viz.* ERBS40, ERBS71, ERBS82 and ERBS32 could convert tryptophan into indole by forming a red ring at the top of the tubes revealing that they were positive to indole test. Negative results were shown by all the isolates for methyl red test as the MR-VP broth did not change its colour to red and remained yellow. All 130 isolates gave positive reaction to produce acetylmethyl carbinol from glucose fermentation by changing the MR-VP broth to red colour indicating Voges - Proskauer (VP) positive. Only 34 isolates were able to reduce sulphur into sulphide by changing the Sulphide-indole-motility (SIM) agar medium into black colour which revealed its positive result to Hydrogen sulphide (H_2 S) production. Glucose fermentation test was found positive for majority of the isolates with 106 isolates changing the phenol red broth to yellow colour. Lu *et al*. (2018) [22] also found that *Bacillus* was methyl red and indole negative; however it was able to ferment glucose and was VP positive. H_2S production, oxidase, and simmons citrate negative *Bacillus* spp. were also reported by Hadi *et al.* (2019) [15] and they exhibited a catalase activity that was positive. *Bacillus* spp. were also identified as Gram-positive, rod-shaped, starch hydrolyzing, and catalse-positive in a previous study (Toppo and Naik, 2015) [32].

Tentative identification of the *Bacillus* isolates

The most frequent approach employed for identification of Gram-positive spore bearing bacilli in the laboratory is conventional methods based on biochemical studies. Based on biochemical tests, and Advance bacteriological identification software (ABIS), the 130 isolates were sorted into three genera (*Bacillus*, *Paenibacillus* and *Viridibacillus*) and 26 different species (Table 2 and Fig. 2).

17 isolates were tentatively identified as *Bacillus amyloliquefaciens* (ERBS1, ERBS8, ERBS20, ERBS23, ERBS38, ERBS43, ERBS47, ERBS48, ERBS51, ERBS52, ERBS56, ERBS57, ERBS62, ERBS67, ERBS81, ERBS89 and ERBS97) with a similarity per centage ranging from 85-95.20%, whereas 17 isolates were grouped as *Bacillus thuringiensis* (ERBS9, ERBS13, ERBS15, ERBS19, ERBS22, ERBS24, ERBS26, ERBS27, ERBS28, ERBS30, ERBS33, ERBS35, ERBS36, ERBS37, ERBS39, ERBS70 and ERBS88) with 83.90-99% similarity per centage. 15 isolates were designated as *Paenibacillus polymyxa* (ERBS5, ERBS18, ERBS29, ERBS31, ERBS32, ERBS34, ERBS59, ERBS75, ERBS90, ERBS102, ERBS103, ERBS105, ERBS110, ERBS115 and ERBS127) with 90.80-98.30% similarity per centage, while ERBS7, ERBS14, ERBS21, ERBS25, ERBS42, ERBS50, ERBS55, ERBS58, ERBS63 and ERBS76 and ERBS126 i.e. 11 isolates with a similarity per centage ranging from 83-90.8% were sorted as *B. licheniformis*. 10 isolates (ERBS2, ERBS41, ERBS44, ERBS49, ERBS64, ERBS74, ERBS77, ERBS107, ERBS109 and ERBS113) were identified as *B. siamensis* (83.80-92.4% similarity range), 6 isolates (ERBS3, ERBS17, ERBS96, ERBS108, ERBS114 and ERBS120) as *B. Pumilus* (87-97.4% range similarity), whereas 5 isolates from each of *Paenibacillus pectinilyticus* (ERBS79, ERBS92, ERBS94, ERBS104 and ERBS106) and *B. subtilis* (ERBS72, ERBS78, ERBS80, ERBS111 and ERBS122) with 83.9-99% and 87.7-95.5% similarity range respectively. Others were tentatively identified as *B. acidiceler* (ERBS121, ERBS123, ERBS125, and ERBS128), *Paenibacillus macerans* (ERBS10, ERBS61, ERBS112, and ERBS119), *B. psychrophilus* (ERBS54, ERBS100, ERBS117, and ERBS130), and *Bacillus. tequilensis* (ERBS60, ERBS65, ERBS81 and ERBS83) with a similarity range of 83.2-90.8%, 90.8%, 83.2-90.8% and 91.5-99% respectively.

Only few isolates were identified as *Paenibacillus castaneae* (ERBS84 and ERBS85), *B. fumarioli* (ERBS86 and ERBS87), *B. coagulans* (ERBS116 and ERBS129), *Paenibacillus peoriae* (ERBS4 and ERBS93), *Viridibacillus neidei* (ERBS6 and ERBS16), *B. cereus* (ERBS69 and ERBS73), *Paenibacillus validus* (ERBS118 and ERBS126), *B. fumarioli* (ERBS66), *B. nealsonii* (ERBS12), *B. niacini* (ERBS40), *B.vietnamensis* (ERBS99), *Paenibacillus alvei* (ERBS82), *Paenibacillus assamensis* (ERBS11), *Paenibacillus koreensis* (ERBS68). Seven isolates were classified into unknown group as species could not be identified. Majority of the isolates were identified as *B. amyloliquefaciens*, *B. thuringiensis* and *P. polymyxa.* Similarly, Aruwa and Olatope (2015) [8] categorized 21 *Bacillus* species as *B. cereus*, *B. subtilis*, *B. megaterium*, *B. licheniformis*, *B. sphaericus* and *B. polymyxa* based on biochemical tests. The result is in agreement with that of Manoj *et al.* (2018) [24] who identified 38 strains of Bacteria as different *Bacillus*, *Paenibacillus*, *Psedomonas* and *Vibro* species based on biochemical test and ABIS biochemical identification database.

Table 2: Biochemical characterisation and tentative identification of the endophytic *Bacillus* isolates

Isolate Names	Biochemical tests														Tentative identification	
	GR	KOH	Ur	Glu	Oxi	Nit	Cit	Gel	In	MR	VP	Cat	H_2S	Arg	Organism name	Similarity per cent
ERBS1	+	-	+	+	+	+	-	+	-	-	+	+	-	+	*Bacillus amyloliquefaciens*	87.70%
ERBS2	+	-	++	+	-	+	-	+	-	-	+	+	-	+	*Bacillus siamensis*	84.90%
ERBS3	+	-	-	-	-	-	+	+	-	-	+	+	-	-	*Bacillus pumilus*	94.50%
ERBS4	+	-	-	+	-	-	-	+	-	-	+	+	-	+	*Paenibacillus peoriae*	87.70%
ERBS5	+	-	-	-	-	+	-	+	-	-	+	+	-	-	*Paenibacillus polymyxa*	98.30%
ERBS6	+	-	-	+	-	-	-	+	-	-	+	+	-	-	*Viridibacillus neidei*	83.80%
ERBS7	+	-	++	+	+	+	+	+	-	-	+	+	-	+	*Bacillus licheniformis*	90.80%
ERBS8	+	-	-	-	+	+	-	+	-	-	+	+	-	-	*Bacillus amyloliquefaciens*	95.20%
ERBS9	+	-	-	+	+	+	+	+	-	-	+	+	-	+	*Bacillus thuringiensis*	91.50%
ERBS10	+	-	-	-	+	+	-	+	-	-	+	+	-	-	*Paenibacillus macerans*	90.80%
ERBS11	+	-	+	+	+	-	-	+	-	-	+	+	-	+	*Paenibacillus assamensis*	80.20%
ERBS12	+	-	-	+	-	-	-	-	-	-	+	+	-	-	*Bacillus nealsonii*	83.80%
ERBS13	+	-	+	+	-	+	-	+	-	-	+	+	-	+	*Bacillus thuringiensis*	91.50%
ERBS14	+	-	++	+	+	-	++	+	-	-	+	+	-	+	*Bacillus licheniformis*	90.80%
ERBS15	+	-	-	+	+	+	+	+	-	-	+	+	-	+	*Bacillus thuringiensis*	99%
ERBS16	+	-	-	+	-	-	-	+	-	-	+	+	-	+	*Viridibacillus neidei*	83.80%
ERBS17	+	-	-	+	-	-	++	-	-	-	+	+	-	-	*Bacillus pumilus*	94.50%
ERBS18	+	-	-	+	-	+	-	+	-	-	+	+	-	-	*Paenibacillus polymyxa*	90.80%
ERBS19	+	-	-	+	-	+	-	+	-	-	+	+	-	+	*Bacillus thuringiensis*	99%
ERBS20	+	-	-	-	+	+	-	+	-	-	+	+	-	+	*Bacillus amyloliquefaciens*	95.20%
ERBS21	+	-	++	+	+	+	-	+	-	-	+	+	-	+	*Bacillus licheniformis*	90.80%
ERBS22	+	-	-	+	+	+	+	+	-	-	+	+	-	+	*Bacillus thuringiensis*	99%
ERBS23	+	-	-	+	+	+	-	+	-	-	+	+	+	+	*Bacillus amyloliquefaciens*	95.20%
ERBS24	+	-	++	+	-	+	-	+	-	-	+	+	-	+	*Bacillus thuringiensis*	91.50%
ERBS25	+	-	++	+	+	+	-	+	-	-	+	+	-	+	*Bacillus licheniformis*	83.20%
ERBS26	+	-	-	+	-	+	-	+	-	-	+	+	-	+	*Bacillus thuringiensis*	91.50%
ERBS27	+	-	-	+	-	+	-	+	-	-	+	+	-	+	*Bacillus thuringiensis*	91.50%
ERBS28	+	-	-	-	-	+	-	+	-	-	+	+	-	+	*Bacillus thuringiensis*	91.50%
ERBS29	+	-	-	-	-	+	-	+	-	-	+	+	-	-	*Paenibacillus polymyxa*	90.80%
ERBS30	+	-	-	+	-	+	-	+	-	-	+	+	-	+	*Bacillus thuringiensis*	91.50%
ERBS31	+	-	-	+	-	+	-	+	-	-	+	+	-	-	*Paenibacillus polymyxa*	98.30%
ERBS32	+	-	-	+	-	+	-	-	-	-	+	+	+	-	*Paenibacillus polymyxa*	98.30%
ERBBS3	+	-	++	+	-	+	-	+	-	-	+	+	-	+	*Bacillus thuringiensis*	91.50%
ERBS34	+	-	-	+	-	+	-	+	-	-	+	+	-	-	*Paenibacillus polymyxa*	90.80%
ERBS35	+	-	-	+	-	+	-	+	-	-	+	+	+	+	*Bacillus thuringiensis*	91.50%
ERBS36	+	-	-	+	-	+	-	+	-	-	+	+	-	+	*Bacillus thuringiensis*	91.50%
ERBS37	+	-	++	+	-	+	-	+	-	-	+	+	-	+	*Bacillus thuringiensis*	83.90%
ERBS38	+	-	-	+	+	+	-	+	-	-	+	+	-	-	*Bacillus amyloliquefaciens*	95.20%
ERBS39	+	-	++	+	-	+	-	+	-	-	+	+	-	+	*Bacillus thuringiensis*	83.90%
ERBS40	+	-	+	+	-	-	-	+	+	-	+	+	-	-	*Bacillus niacini*	94.80%
ERBS41	+	-	-	+	-	+	-	+	-	-	+	+	+	+	*Bacillus siamensis*	91.40%
ERBS42	+	-	++	+	+	+	+	+	-	-	+	+	+	+	*Bacillus licheniformis*	90.80%
ERBS43	+	-	-	+	+	+	-	+	-	-	+	+	+	+	*Bacillus amyloliquefaciens*	95.20%
ERBS44	+	-	-	+	-	+	-	+	-	-	+	+	+	+	*Bacillus siamensis*	91.40%
ERBS45	+	-	+	+	+	-	-	+	-	-	+	+	+	+	*unknown*	-
ERBS46	+	-	+	+	+	-	-	+	-	-	+	+	+	+	*unknown*	-
ERBS47	+	-	-	-	+	+	-	+	-	-	+	+	+	-	*Bacillus amyloliquefaciens*	95.20%
ERBS48	+	-	-	+	+	+	-	+	-	-	+	+	+	+	*Bacillus amyloliquefaciens*	87.70%
ERBS49	+	-	-	+	-	+	-	+	-	-	+	+	+	+	*Bacillus siamensis*	91.40%
ERBS50	+	-	++	+	+	+	-	+	-	-	+	+	+	+	*Bacillus licheniformis*	83.20%
ERBS51	+	-	-	-	+	+	-	+	-	-	+	+	+	-	*Bacillus amyloliquefaciens*	95.20%
ERBS52	+	-	-	+	+	+	-	+	-	-	+	+	+	+	*Bacillus amyloliquefaciens*	85%
ERBS53	+	-	+++	+	-	-	-	+	-	-	+	+	+	-	*unknown*	-
ERBS54	+	-	+++	+	+	-	-	+	-	-	+	+	+	+	*Bacillus psychrophilus*	90.80%
ERBS55	+	-	++	+	+	+	-	+	-	-	+	+	+	+	*Bacillus licheniformis*	90.80%
ERBS56	+	-	-	+	+	+	-	+	-	-	+	+	+	+	*Bacillus amyloliquefaciens*	87.70%
ERBS57	+	-	-	+	+	+	-	+	-	-	+	+	+	+	*Bacillus amyloliquefaciens*	87.70%
ERBS58	+	-	++	+	+	+	-	+	-	-	+	+	+	+	*Bacillus licheniformis*	83.20%
ERBS59	+	-	+++	+	-	+	-	+	-	-	+	+	-	-	*Paenibacillus polymyxa*	90.80%
ERBS60	+	-	-	+	+	+	-	+	-	-	+	+	+	+	*Bacillus tequilensis*	91.50%
ERSB61	+	-	-	+	+	+	-	+	-	-	+	+	-	+	*Paenibacillus macerans*	90.80%
ERBS62	+	-	-	+	+	+	-	+	-	-	+	+	-	+	*Bacillus amyloliquefaciens*	87.70%
ERBS63	+	-	++	+	+	+	-	+	-	-	-	+	-	+	*Bacillus licheniformis*	85.30%
ERBS64	+	-	-	+	-	+	-	+	-	-	+	+	-	+	*Bacillus siamensis*	92.40%
ERBS65	+	-	-	-	+	+	++	+	-	-	+	+	-	+	*Bacillus tequilensis*	99%

ERBS66	+	-	-	+	-	-	-	+	-	-	+	+	-	-	*Bacillus fumarioli*	83.30%
ERBS67	+	-	-	+	+	+	-	+	-	-	+	+	-	+	*Bacillus amyloliquefaciens*	87.70%
ERBS68	+	-	-	+	+	+	-	+	-	-	-	+	-	+	*Paenibacillus koreensis*	87.60%
ERBS69	+	-	-	+	+	+	-	+	-	-	+	+	-	+	*Bacillus cereus*	95.60%
ERBS70	+	-	-	+	+	+	-	+	-	-	+	+	-	+	*Bacillus thuringiensis*	85%
ERBS71	+	-	-	+	+	+	-	+	+	-	+	+	-	+	*Bacillus tequilensis*	91.90%
ERBS72	+	-	-	+	+	+	++	+	-	-	+	+	-	+	*Bacillus subtilis*	95.20%
ERBS73	+	-	-	+	-	-	-	+	-	-	+	+	+	+	*Bacillus cereus*	87.90%
ERBS74	+	-	-	+	+	+	-	+	-	-	+	+	-	+	*Bacillus siamensis*	91.40%
ERBS75	+	-	-	-	-	+	-	+	-	-	+	+	+	-	*Paenibacillus polymyxa*	90.80%
ERBS76	+	-	++	+	+	+	-	+	-	-	+	+	+	+	*Bacillus licheniformis*	83.20%
ERBS77	+	-	++	+	-	+	-	+	-	-	+	+	+	+	*Bacillus siamensis*	83.80%
ERBS78	+	-	-	+	-	+	+	+	-	-	+	+	-	-	*Bacillus subtilis*	87.70%
ERBS79	+	-	-	+	-	+	-	+	-	-	+	+	+	-	*Paenibacillus pectinilyticus*	99%
ERBS80	+	-	-	+	-	+	-	+	-	-	+	+	-	-	*Bacillus subtilis*	89.20%
ERBS81	+	-	-	+	+	+	-	+	-	-	+	+	-	+	*Bacillus amyloliquefaciens*	95.20%
ERBS82	+	-	++	+	+	-	-	+	+	-	+	+	-	+	*Paenibacillus alvei*	94.50%
ERBS83	+	-	-	+	+	+	-	+	+	-	+	+	-	+	*Bacillus tequilensis*	91.50%
ERBS84	+	-	-	+	+	-	-	+	-	-	+	+	-	-	*Paenibacillus castaneae*	95.20%
ERBS85	+	-	-	+	+	-	-	+	-	-	+	+	-	-	*Paenibacillus castaneae*	95.20%
ERBS86	+	-	-	+	-	-	-	+	-	-	+	+	-	-	*Bacillus fumarioli*	83.80%
ERBS87	+	-	-	+	-	-	-	+	-	-	+	+	-	-	*Bacillus fumarioli*	83.80%
ERBS88	+	-	+	-	-	+	-	+	-	-	+	+	-	+	*Bacillus thuringiensis*	83.90%
ERBS89	+	-	-	+	+	+	-	+	-	-	+	+	-	+	*Bacillus amyloliquefaciens*	87.70%
ERBS90	+	-	-	+	-	+	-	+	-	-	+	+	-	-	*Paenibacillus polymyxa*	90.80%
ERBS91	+	-	++	+	+	-	-	+	-	-	+	+	+	+	*unknown*	-
ERBS92	+	-	-	-	-	+	-	+	-	-	+	+	+	-	*Paenibacillus pectinilyticus*	91.50%
ERBS93	+	-	-	+	-	-	-	+	-	-	+	+	-	+	*Paenibacillus peoriae*	95.20%
ERBS94	+	-	-	+	-	+	-	+	-	-	+	+	-	+	*Paenibacillus pectinilyticus*	83.90%
ERBS95	+	-	+++	+	-	+	+	+	-	-	+	+	-	+	*unknown*	-
ERBS96	+	-	-	+	-	-	+	+	-	-	+	+	-	-	*Bacillus pumilus*	87%
ERBS97	+	-	-	+	+	+	-	+	-	-	+	+	-	-	*Bacillus amyloliquefaciens*	95.20%
ERBS98	+	-	+++	-	+	+	-	+	-	-	+	+	-	+	*unknown*	-
ERBS99	+	-	+	+	+	-	-	+	-	-	+	+	-	+	*Bacillus vietnamensis*	91.40%
ERBS100	+	-	+	+	+	+	-	+	-	-	+	+	-	+	*Bacillus psychrophilus*	83.20%
ERBS101	+	-	+	+	+	-	-	+	-	-	+	+	-	+	*unknown*	-
ERBS102	+	-	-	+	-	+	-	+	-	-	+	+	-	-	*Paenibacillus polymyxa*	90.80%
ERBS103	+	-	-	-	-	+	-	+	-	-	+	+	-	-	*Paenibacillus polymyxa*	98.30%
ERBS104	+	-	-	+	-	+	-	+	-	-	+	+	-	-	*Paenibacillus pectinilyticus*	91.50%
ERBS105	+	-	-	+	-	+	-	+	-	-	+	+	-	-	*Paenibacillus polymyxa*	98.30%
ERBS106	+	-	-	+	-	+	-	+	-	-	+	+	-	-	*Paenibacillus pectinilyticus*	83.90%
ERBS107	+	-	++	+		+	-	+	-	-	+	+	-	+	*Bacillus siamensis*	83.80%
ERBS108	+	-	-	+	-	-	+	+	-	-	+	+	-	-	*Bacillus pumilus*	87%
ERBS109	+	-	++	+	-	+	-	+	-	-	+	+	+	+	*Bacillus siamensis*	83.90%
ERBS110	+	-	-	-	-	+	-	+	-	-	+	+	-	-	*Paenibacillus polymyxa*	98.30%
ERBS111	+	-	-	+	-	+	+	+	-	-	+	+	-	-	*Bacillus subtilis*	87.70%
ERBS112	+	-	-	-	-	+	-	+	-	-	+	+	-	-	*Paenibacillus macerans*	90.80%
ERBS113	+	-	+	+	-	+	-	+	-	-	+	+	-	+	*Bacillus siamensis*	91.40%
ERBS114	+	-	-	+	-	-	++	+	-	-	+	+	-	-	*Bacillus pumilus*	87.90%
ERBS115	+	-	-	+	-	+	-	+	-	-	+	+	+	-	*Paenibacillus polymyxa*	98.30%
ERBS116	+	-	-	-	-	+	-	+	-	-	+	+	-	-	*Bacillus coagulans*	90.00%
ERBS117	+	-	+	+	+	+	-	+	-	-	+	+	+	-	*Bacillus psychrophilus*	90.80%
ERBS118	+	-	+	-	-	+	-	+	-	-	+	+	-	-	*Paenibacillus validus*	96.90%
ERBS119	+	-	-	-	+	+	-	+	-	-	+	+	-	-	*Paenibacillus macerans*	90.80%
ERBS120	+	-	-	-	-	-	-	+	-	-	+	+	+	-	*Bacillus pumilus*	87%
ERBS121	+	-	-	-	-	+	-	+	-	-	+	+	+	-	*Bacillus acidiceler*	90.80%
ERBS122	+	-	-	+	-	+	+	+	-	-	+	+	-	-	*Bacillus subtilis*	87.70%
ERBS123	+	-	+	+	+	+	-	+	-	-	+	+	-	+	*Bacillus acidiceler*	83.20%
ERBS124	+	-	+	+	+	+	-	+	-	-	+	+	-	+	*Bacillus licheniformis*	83.20%
ERBS125	+	-	++	-	-	+	-	+	-	-	+	+	-	-	*Bacillus acidiceler*	83.20%
ERBS126	+	-	+	+	+	-	-	+	-	-	+	+	-	-	*Paenibacillus validus*	89.30%
ERBS127	+	-	++	+	-	+	-	+	-	-	+	+	-	-	*Paenibacillus polymyxa*	90.80%
ERBS128	+	-	-	+	+	-	-	+	-	-	+	+	-	-	*Bacillus acidiceler*	90.80%
ERBS129	+	-	-	+	-	-	-	-	-	-	+	+	-	-	*Bacillus coagulans*	89.30%
ERSB130	+	-	+	+	+	+	-	+	-	-	+	+	-	-	*Bacillus psychrophilus*	84.60%

Note: GR-Gram reaction, KOH-KOH 3%, Ur-Urease Test, Glu-Glucose fermentation, Oxi-Oxidase Test, Nit-Nitrate Reduction, Cit-Citrate Utilization, Gel-Gelatin Hydrolysis, In-Indole Test, MR-Methyl Red, VP-VP Test, Cat-Catalase test, H_2S- H_2S Test, Arg-Argenine Dehydrolase

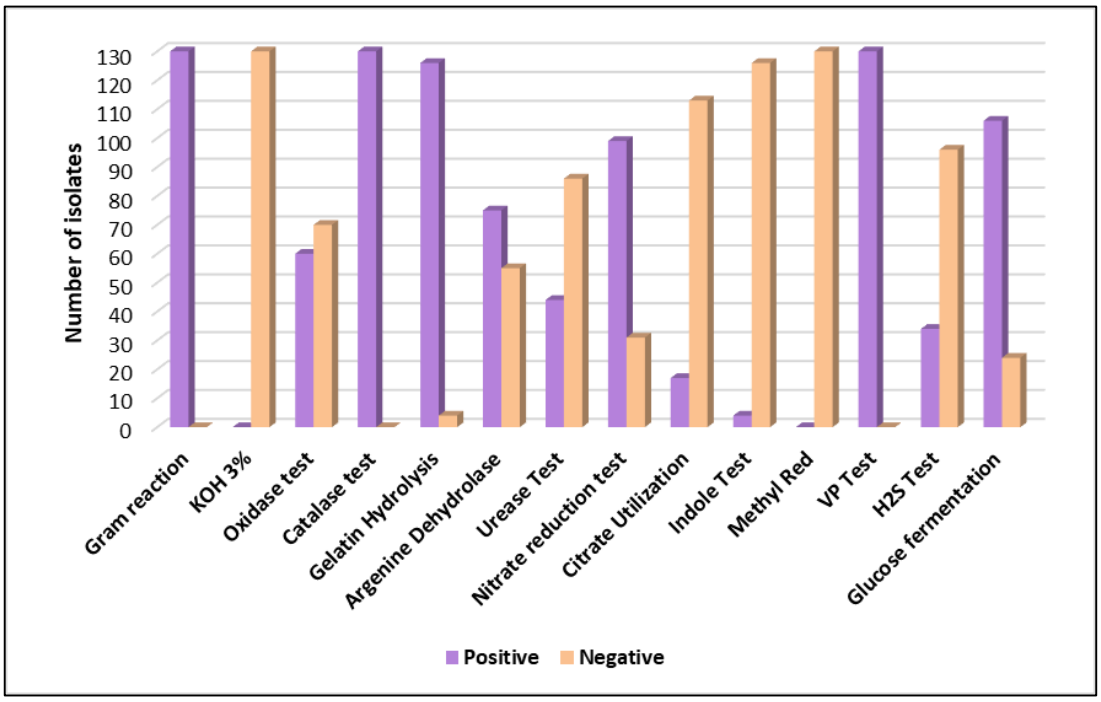

Fig 1: *Bacillus* isolates response to different biochemical tests

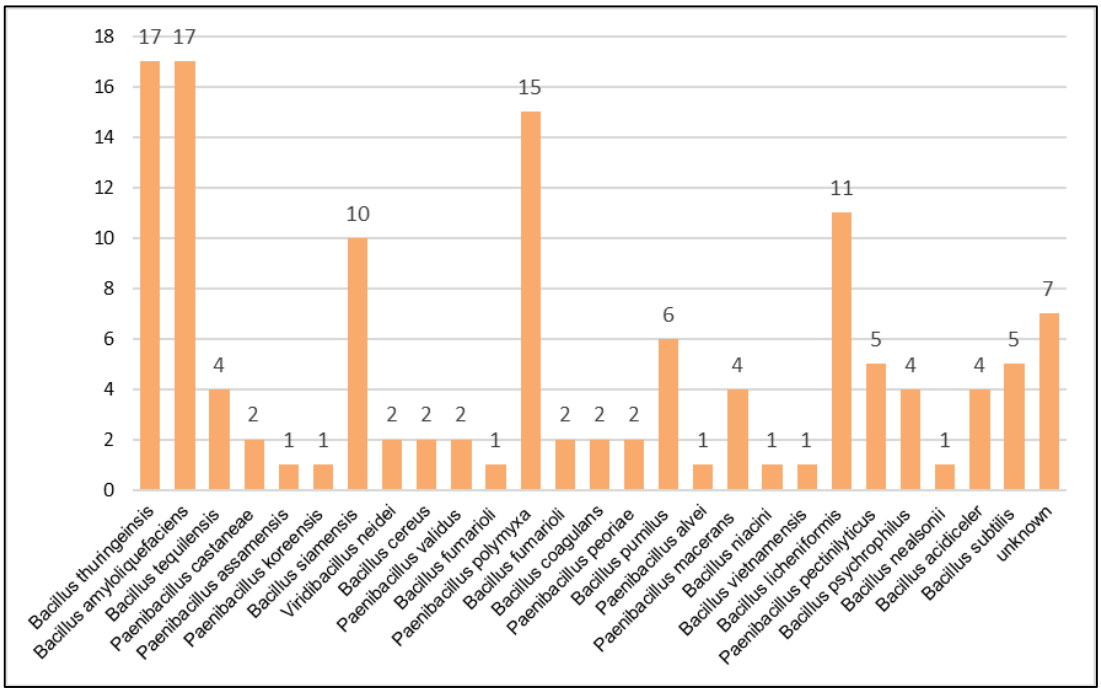

Fig 2: Bar graph showing the number of tentatively identified *Bacillus*, *Paenibacillus* and *Viribacillus* in each species

Conclusion

Tomato roots collected from different locations of Meghalaya harbor wide range of endophytic *Bacillus*. All the 130 isolates belonged to *Bacillus*, *Paenibacillus* and *Viridibacillus* genus. Maximum numbers of the isolates were identified as *Bacillus amyloliquefaciens* and *Bacillus thuringiensis*. The current study adds to our understanding of the diversity of endophytic *Bacillus* from tomato roots of Meghalaya. Further studies can be focused on exploitation of these *Bacillus* isolates as a bio control agent to control phytopathogens.

Acknowledgement

The essential materials needed to carry out the experiments was provided by the Dean, CPGS-AS, CAU(I), Umiam and College of Post graduate studies in Agricultural Sciences, Central Agricultural University, Umiam, Meghalaya

References

1. Abdallah RAB, Khiareddine JH, Nefzi A, Remadi MD. Evaluation of the growth-promoting potential of endophytic bacteria recovered from healthy tomato plants. Journal of Horticulture and Forestry 2018;5(2):234-244.
2. Adepoju AO. Post-harvest losses and welfare of tomato farmers in Ogbomosho, Osun State, Nigeria. Journal of Stored Products and Postharvest Research 2014;5(2):8-13.
3. Agarwal M, Dheeman S, Dubey RC, Kumar P, Maheshwari DK, Bajpai VK. Differential antagonistic responses of *Bacillus pumilus* MSUA3 against

Rhizoctonia solani and *Fusarium oxysporum* causing fungal diseases in *Fagopyrum esculentum* Moench. Microbiology Research 2017;205:40-47.
4. Agrawal DPK, Agrawal S. Characterization of *Bacillus* sp. strains isolated from rhizosphere of tomato plants (*Lycopersicon esculentum*) for their use as potential plant growth promoting rhizobacteria. International Journal of Current Microbiology and Applied Science 2013;2(10):406-417.
5. Amaresan N, Jayakumar V, Kumar K, Thajuddin N. Isolation and characterization of plant growth promoting endophytic bacteria and their effect on tomato (*Lycopersicon esculentum*) and chilli (*Capsicum annuum*) seedling growth. Annals of microbiology 2012;62(2):805-810.
6. Anonymous Horticultural Statistics at a Glance. Horticulture Statistics Division, Department of Agriculture, Cooperation and Farmers' Welfare, Ministry of Agriculture & Farmers' Welfare, Government of India 2018.
7. Aruwa CE, Ogunlade ST. Classical Identification, 16S rDNA sequencing and molecular characterization of Bacillus species from convenience foods. British Journal of Applied Science and Technology 2016;15(5):1-11.
8. Aruwa CE, Olatope SOA. Characterization of Bacillus species from convenience foods with conventional and API kit method: A comparative analysis. Journal of Applied Life Sciences International 2015;3(1):42-48.
9. Ash C, Farrow JA, Dorsch M, Stackebrandt E, Collins MD. Comparative analysis of Bacillus anthracis, Bacillus cereus, and related species on the basis of reverse transcriptase sequencing of 16S rRNA. International Journal of Systematic and Evolutionary Microbiology 1991;41(3):343-346.
10. Cappuccino JG, Sherman N. Microbiology: a Laboratory Manual, 6th edn. Menlo Park, CA: Benjamin Cumming, 2002.
11. Chukeatirote E, Arfarita N, Niamsup P, Kanghae A. Phenotypic and genetic characterization of *Bacillus* species exhibiting strong proteolytic activity isolated from Terasi, an Indonesian fermented seafood product. Journal of Northeast Agricultural University 2015;22(4):15-22.
12. Dash C, Payyappilli RJ. KOH string and vancomycin susceptibility test as an alternative method to Gram staining. Journal of International Medicine and Dentistry 2016;3(2):88-90.
13. Gao L, Xiaolong C, Tao J. Isolation and identification of endophytic nitrogen-fixing bacteria in rice with antipathogenic functions. Journal of Huazhong Agricultural University 2012;31:553-557.
14. Gouda S, Das G, Sen SK, Shin HS, Patra JK. Endophytes: a treasure house of bioactive compounds of medicinal importance. Frontier in Microbiology 2016;7:1-8.
15. Hadi SN, Dewi PS. Identification of the ultisol land indigenus bacteria from Banyumas Regency based on the characteristics of morphology, physiology and biochemistry. IOP Conference Series: Earth and Environmental Science 2019;250(1):012095.
16. Holt JG, Krieg NR, Sneath PHA, Stanley JT, Williams ST. In: Williams, L. and Wilkins (ed) Bergey's Manual of determinative bacteriology (9th ed), Philadelphia, USA 2000, 559-562.
17. Jayapala N, Mallikarjunaiah NH, Puttaswamy H, Gavirangappa H, Ramachandrappa NS. Rhizobacteria *Bacillus* spp. induces resistance against anthracnose disease in chili (*Capsicum annuum* L.) through activating host defense response. Egyptian Journal of Biological Pest Control 2019;29(1):45-54.
18. Kandel S, Joubert P, Doty S. Bacterial endophyte colonization and distribution within plants. Microorganisms 2017;5(4):77-103.
19. Kanjanamaneesathian M, Wiwattanapatapee R, Pengnoo A, Oungbho K, Chumthong A. Efficacy of novel formulations of *Bacillus megaterium* in suppressing sheath blight of rice caused by *Rhizoctonia solani*. Plant Pathology Journal 2007;6(2):195-201.
20. Kaur PK, Kaur J, Saini HS. Antifungal potential of *Bacillus vallismortis* R2 against different phytopathogenic fungi. Spanish Journal of Agricultural Research 2015;13(2):1-11.
21. Kloepper JW. Plant growth-promoting rhizobacteria (other systems). In: Y. Okon, (ed) Azospirillum/ Plant Associations, CRC Press, Boca Raton 1995, 137-166.
22. Lu Z, Guo W, Liu C. Isolation, identification, and characterization of novel *Bacillus subtilis*. Journal of Veterinary Medical Science 2018, 16-0572.
23. Malfanova N, Kamilova F, Validov S, Shcherbakov A, Chebotar V, Tikhonovich I, Lugtenberg B. Characterization of *Bacillus subtilis* HC8, a novel plant-beneficial endophytic strain from giant hogweed. Microbial Biotechnology 2011;4(4):523-532.
24. Manoj B, Ramesh CH, Mohanraju R. Isolation of plastic degrading and L-asparaginase enzyme producing bacteria from the mangrove environment of Guptapara, South Andaman. International Journal for Research in Applied Science and Engineering Technology 2018;6(10):29-36.
25. Maung CEH, Choi TG, Nam HH, Kim KY. Role of *Bacillus amyloliquefaciens* Y1 in the control of Fusarium wilt disease and growth promotion of tomato. Biocontrol Science and Technology 2017;27(12):1400-1415.
26. Nandhini S, Sendhilvel V, Babu S. Endophytic bacteria from tomato and their efficacy against *Fusarium oxysporum* f. sp. *lycopersici*, the wilt pathogen. Journal of Biopesticides 2012;5(2):178-185.
27. Palazzini JM, Dunlap CA, Bowman MJ, Chulze SN. *Bacillus velezensis* RC 218 as a biocontrol agent to reduce Fusarium head blight and deoxynivalenol accumulation: genome sequencing and secondary metabolite cluster profiles. Microbiology Research 2016;192:30-36.
28. Peralta IE, Spooner DM. History, origin and early cultivation of tomato (Solanaceae). In: Razdan, M.K. and Mattoo, A.K. (eds) Genetic improvement of solanaceous crops, Science Publishers, Inc., Enfield, New Hampshire, USA, 2007;2:1-27.
29. Priest FG. Systematics and ecology of Bacillus. In: Sonenshein, A., Hoch, J. and Losick, R. (eds), *Bacillus subtilis* and Other Gram-Positive Bacteria. ASM Press, Washington, 1993, 3-16.
30. Sarode CA, Bramhankar SB, Kakad SA, Labhasetwar AA, Bhure SS, Isokar SS *et al*. Biochemical and physiological characterizations of *Bacillus subtilis*. International journal of chemical studies 2019;7(1):1957-1960.
31. Sneath PHA, Mair NS, Sharpe ME, Holt JG. Bergey's manual of systematic bacteriology, The Williams and

Wilkins Co., Baltimore 1986;2:1104-1139.
32. Toppo SR, Naik UC. Isolation and characterization of bacterial antagonist to plant pathogenic fungi (*Fusarium* spp.) from agro based area of Bilaspur. International Journal of Research Studies in Biosciences 2015;8:6-14.
33. Vargas VA, Delgado OD, Hatti-Kaul R, Mattiasson B. Lipase-producing microorganisms from a Kenyan alkaline soda lake. Biotechnology Letters 2004;26(2):81-86.
34. Wang N, Wang L, Zhu K, Hou S, Chen L, Mi D, Guo JH. Plant root exudates are involved in *Bacillus cereus* AR156 mediated biocontrol against *Ralstonia solanacearum*. Frontiers in Microbiology 2019;10:98-112.
35. Wulff EG, Mguni CM, Mansfeld- Giese K, Fels J, Lübeck M, Hockenhull J. Biochemical and molecular characterization of *Bacillus amyloliquefaciens*, *B. subtilis* and *B. pumilus* isolates with distinct antagonistic potential against *Xanthomonas campestris* pv. *campestris.* Plant Pathology 2002;51(5): 574-584.
36. Yanti Y, Warnita W, Reflin R, Nasution CR. Characterizations of endophytic *Bacillus* strains from tomato roots as growth promoter and biocontrol of *Ralstonia solanacearum*. Biodiversitas Journal of Biological Diversity 2018;19(3):906-911.
37. Yeshiwas Y, Belew D, Tolessa K. Tomato (*Solanum lycopersicum* L.) yield and fruit quality attributes as affected by varieties and growth conditions. World Journal of Agricultural Science 2016;12:404-408.
38. Zinniel DK, Lambrecht P, Harris NB, Feng Z, Kuczmarski D, Higley P *et al*. Isolation and characterization of endophytic colonizing bacteria from agronomic crops and prairie plants. Applied and Environmental Microbiology 2002;68(5):2198-2208.