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Effect of Bacterial Endophytes *Lysinibacillus* sp. on Plant Growth and Fruit Yield of Tomato (*Solanum lycopersicum*)

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A B S T R A C T

Endophytes are known for its novel metabolites and benefits for host. Diversified role of endophytes are being studied in order to enhance their use in agriculture production system. Endophytes are being use for plant growth promotion, alleviation of biotic and abiotic stresses, enhancing agronomic traits, *etc*. In this study, bacterial endophytes isolated from earlier studies were tested for its plant growth promoting traits in tomato. Bacterial endophytes were tested for plant growth promoting attributes like phosphate solubilization and IAA production. These endophytes were inoculated to tomato plants in pot trial and their effect on dry matter accumulation and fruit yield were recorded. Results indicated that the endophyte 1TH16a had performed well in pot trial and significantly enhanced fruit yield as compare to control. Significantly higher root and shoot biomass were recorded from strain 1TH16a and 1016. Molecular identification using 16s rRNA shows its identity to *Lysinibacillus* sp. This strain is having potential plant growth promoting traits and detailed study is needed for its commercial use in tomato and other crops.

Introduction

The realm of ecofriendly agriculture has been flourishing with potential alternatives of harmful agrochemicals in last few decades. Microbes with tremendous capacities of PGPR, biocontrol and abiotic stress alleviation are being explored in rhizosphere, endosphere, phyllosphere and unique ecological niches (Abbamondi *et al.*, 2016). Endophytes have proven potential to be used in agriculture. Bacterial endophytes are present in different plant parts and impart beneficial effects on its host plant (Lodewyckx *et al.*, 2002).

Endophytes are the microorganisms which live inside the host and do not result in any significant loss of performance to it. Bacterial endophytes confers large array of advantages to its host plant like nitrogen fixation, mineral solubilization, siderophore, phytohormone, 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, secondary metabolites, enzymes, antibiotics, induce systemic resistance and tolerance, *etc.* (Lodewyckx *et al.,* 2002; Sahu *et al.,* 2016). Endophytes are reported to enhance performance of other beneficial microbes also like *Rhizobium* (Bai *et al.*, 2003). Endophytic inoculants are reported to solubilize mineral nutrients in the rhizosphere (Otieno *et al.,* 2015). This enhances the performance of crop as well as reduces necessity of adding higher amount of chemical fertilizers. Phytohormones control the physiology and performance of plants and endophytes enhances plant growth by controlling these phytohormone levels in the plant (Khan *et al.,* 2013; Szilagyi-Zecchin *et al.,* 2014). Therefore, it is a good strategy to screen for phytohormone producing bacterial endophyte for enhanced growth of plants.

Beneficial impact of bacterial endophytes has been reported from several crops like rice (Rho *et al.,* 2018), wheat (Herrera *et al.,* 2016), legumes (Bai *et al.,* 2003), cotton (Zhou, 2015), maize (Gond *et al.,* 2015), flowers (Engel *et al.,* 2016), sugarcane (Aguiar *et al.,* 2016), vegetables (Xie *et al.,* 2016), fruits (Kannan *et al.,* 2015), *etc.* Tomato is the commercial crop grown across the globe and India is the second largest producer of it (Saxena *et al.,* 2016). There are several microbial inoculants tested for its growth and development (Abbamondi *et al.,* 2016).

Enhancement of fruit yield and quality is important for the tomato farmers. Exploring endophytic treasure for plant growth promotion and fruit yield is required to sustainably boost agriculture production system. Endophytes can also be screened for its capacity to enhance fruit yield of tomato. This study was conducted to screen and evaluate bacterial endophytes for their plant growth promotion attribute. Screening was targeted for enhanced dry matter partitioning after vegetative growth.

Materials and Methods

Bacterial strain and growth media

The endophytic bacterial cultures from earlier studies and rhizospheric strains were taken from NAIMCC, ICAR-NBAIM, Mau. Six bacterial cultures were taken for this study (four endophytes 1TH16a, 4PH5a, 8PR3 and 8PH9) and two rhizospheric bacteria (1016 and 342). The mother cultures were stored in glycerol stock. Nutrient agar slants pre-monitored for purity was used for short term preservation till the study completed. Cultures of each microorganism were inoculated and grown for a period of 24 hours in nutrient broth. This active culture was used for inoculation to tomato plants.

Mineral phosphate solubilization

Phosphate solubilization by bacterial endophytes was tested in Pikovskaya's agar medium (Pikovskaya, 1948). Actively grown culture was spotted on Pikovskaya's agar medium and incubated at 28°C for 3-4 days. Development of a clear halo around the spotted colony indicates phosphate solubilization.

Production of IAA

Indole acetic acid (IAA) production was assessed in Luria bertani (LB) broth with and without 5µg/ml tryptophan. Inoculation was done in 15 ml LB broth and incubated at 28°C for 4-5 days. After this period, cultures were

centrifuged for 10 min at 5000 RPM. In 10 ml supernatant, two drops orthophosphoric acid and 5 ml Salkowski's reagent were mixed. (Salkowski's reagent =50 ml, 35% perchloric acid+ 1 ml 0.5 $FeCl_3$). Intensity of pink color developed indicated production of IAA (Patten and Glick, 1996).

Pot trial

Forty five days old tomato seedlings (Variety S-22) were used for transplanting. Plastic pots were filled with four kgs of autoclaved soil. Seedling roots were dipped in bacterial suspension containing 0.5% carboxy methyl cellulose (CMC) as sticking agent. Roots were dipped for a period of 10 minutes and planted in pots. Plants were watered based on moisture depletion in the pots. Observations were taken after 4 months of transplanting. Root biomass, shoot biomass and fruit weight were taken as key features of biomass accumulation. Samples are dried in oven at 60°C and it got stable weight in 5 days of drying.

Identification of prominent endophyte

Endophyte 1TH16a was found to have prominent effects on biomass accumulation and fruit yield. 1TH16a culture is grown for 24 hours in nutrient agar plate pre-monitored for its purity from any contamination. A loopful of this culture is taken for DNA isolation. DNA was extracted by ZR fungal/bacterial DNA Mini Prep™ kit for isolating genomic DNA (ZYMO Research Corporation, USA). DNA was isolated as per manufacturer's instruction and stored at -20 °C. Molecular identification was done by 16s rRNA partial sequencing. DNA from 1TH16a was amplified by universal 16S rRNA primers 27 forward (5'-AGAGTTTGATCCTGG CTCAG-3') and 1492 reverse (5'-TACGGYTACCTTGTTACGACTT-3'). PCR conditions were same as Thomas *et al.*, (2008). Purified PCR products were sent to M/s Eurofins for 16s rRNA partial sequencing. The obtained sequence was trimmed for poor sequence reads and identity was matched in EZbiocloud database (https://www.ezbiocloud.net) and NCBI. Sequence was submitted to NCBI- BankIt database. Phylogenetic relatedness of 1TH16a with other close relatives was assessed using neighbor joining method and tree was developed using MEGA7 (version=7.0.25).

Statistical analysis

This experiment was conducted in randomized complete block design (RCBD) with seven treatments and three replications each. Comparison has been done based on standard deviation values and means were separated by DMRT.

Results and Discussion

Exploration for better microbial isolates is a continuous process. Screening could bring potential strains for field application. In this study, most potent endophyte was *Lysinibacillus* sp. strain 1TH16a. *Lysinibacillus* sp. is reported as potent plant growth promoter by several workers in other crops (Sgroy *et al.,* 2009; Andrade *et al.,* 2014).

Phosphorus solubilization

Isolates had shown variable phosphate solubilizing potential in pikowskaya's medium (Table 1). Highest P-solubilization was recorded from 1TH16a and 8PH9. Isolate 8PR3 gave poor P solubilization. There is good number of reports indicating P solubilization by bacterial endophytes. The findings are corresponding to reports of Venden *et al.,* (2010) and Naureen *et al.,* (2017) where *Lysinibacillus* sp. had been reported as potential P-solubilizer.

Production of IAA

IAA production was highest in 1016 and 1TH16a (Table 1). 4PH5a had given low IAA production. Endophytes are known for their phytohormone production. The current findings are in line with results of Kuklinsky-sobral *et al.,* (2004) which show IAA production as chief function trait for plant growth promotion by bacterial endophytes. They have also indicated that 69% of their endophytes showing IAA production and P-solubilization, had capability of fixing dinitrogen. The screening based on these two features is in line with approaches chosen by other workers for plant growth promotion (Kuklinsky-sobral *et al.,* 2004; Andrade *et al.,* 2014).

Pot trial

Shoot dry weight was recorded significantly higher in the plants treated with 1TH16a, 342, 8PR3 and 8PH3 whereas other treatments were recorded lower shoot dry weight (Fig. 1, Table 2). Shoot dry weight also included dry weight of its fruits (Fig. 2 and Table 2). Root dry weight was highest in 1016, 1TH16a and 8PH9 inoculated plants whereas other treatments were recorded lower root dry weight (Fig. 3 and Table 2).

Fresh weight of fruits was highest in 1TH16a inoculated plants (Fig. 4). Plants inoculated with isolate 342 and 8PR3 were also having significantly higher fruit weight than control. Lowest fresh fruit weight was recorded from control, 1016, 4PH5a and 8PH9 plants. Root to shoot ratio was lowest in 1TH16a, 342 and 8PR3 inoculated plants.

Identification based on 16s rRNA had shown highest similarity with *Lysinibacillus* sp. with 100% query cover. The sequence was submitted to NCBI with accession number MH194246 (Fig. 5).

Higher biomass accumulation by inoculation of *Lysinibacilus* sp. in this experiment is supported by the findings of Sgroy *et al.,* (2009) which reports *Lysinibacillus* as producer of phytohormones and nitrogen fixer. Enhanced shoot and root biomass may also be due to the phosphate solubilizing ability of the endophytes. It might be secreting some of the compounds which helped in mineral nutrient solubilization and then uptake. Application of P-solubilizing microorganism was reported to enhance plant growth, biomass and dry matter partitioning in plants (Bagyaraj and Krishnaraj, 2000; Sahu *et al.,* 2016). Enhanced dry matter accumulation might be due to the high nutrient availability to the plants. Phosphorus nutrition is also important for initial plant growth and strength. Initial strength of the plant translates further to robust development of plant. This is in line with the results of Andrade *et al.,* (2014). They had reported presence of *nifH* gene in *Lysinibacillus* sp. which could enhance the nitrogen nutrition and biomass accumulation in the plant. They have also reported high solubilization index for tricalcium phosphate in *Lysinibacillus* sp.

Plants inoculated with isolate 342, 8PR3 and 8PH9 had relatively better shoot biomass content but less was translated into fruit yield as compared to 1TH16a. This might be due to better phytohormone induction by 1TH16a for fruit bearing as compared to others (Ali *et al.,* 2017). Enhanced nutrient uptake also supports higher fruit bearing. Higher fruit yield in 1TH16a might be due to its ability to enhance nutrient uptake. Phytohormone production by bacterial endophytes is also a key reason for enhanced fruit yield. The results are supported by the findings of Ali and co-workers (2017).

Some of the indirect effects by microbial inoculants were also reported to influence growth by influencing phytohormone level in plants (Singh 2013).

Table.1 Assessment of biocontrol and plant growth promoting traits in bacterial endophytes

Sn.	Isolates	Phosphate solubilization	IAA production
1.	1016	+	++
2.	1TH16a	+++	++
3.	342	+	+
4.	4PH5a	+	-
5.s	8PR3	-	+
6.	8PH9	++	+

+++ Prominent activity; ++ Moderate activity; + low activity; - no activity

Table.2 Plant biomass by inoculation of endophytic bacteria

Sn.	Isolates	Shoot dry weight	Root dry weight	Fresh fruit weight	R:S ratio
1.	Control	13.60^{d}	4.70^{c}	56.97^{de}	0.35^{bc}
2.	1016	11.52^{d}	6.48^{a}	47.40^{e}	0.56^{a}
3.	1TH16a	23.60^{a}	6.33^{ab}	152.81^{a}	0.27^{c}
4.	342	20.39^{b}	5.44^{bc}	75.00^{c}	0.27^{c}
5.	4PH5a	13.58^{d}	5.30^{bc}	57.44^{de}	0.39^{b}
6.	8PR3	19.83^{b}	5.30^{bc}	89.89^{b}	0.27^{c}
7.	8PH9	17.40^{c}	5.94^{ab}	61.33^{d}	0.34^{bc}
	SEm±	0.68	0.31	3.28	0.03

Fig.1 Tomato plants indicating effects of 1TH16a inoculation on (A) plant growth and (B) fruit yield

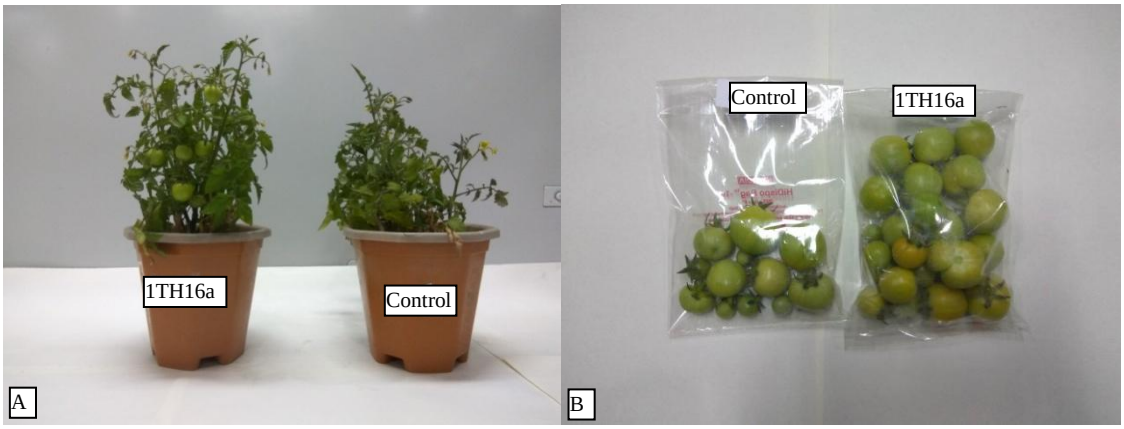


Fig.2 Effect of endophyte inoculation on shoot dry weight

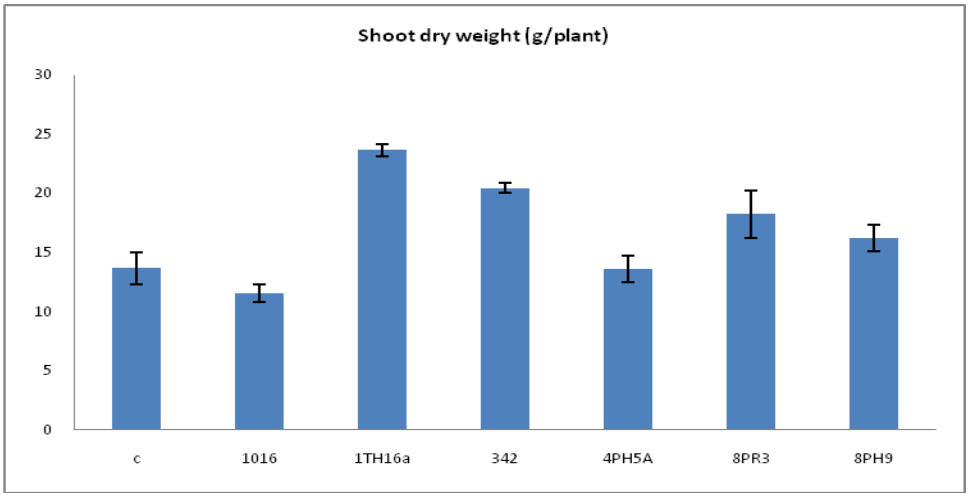


Fig.3 Effect of endophyte inoculation on root dry weight

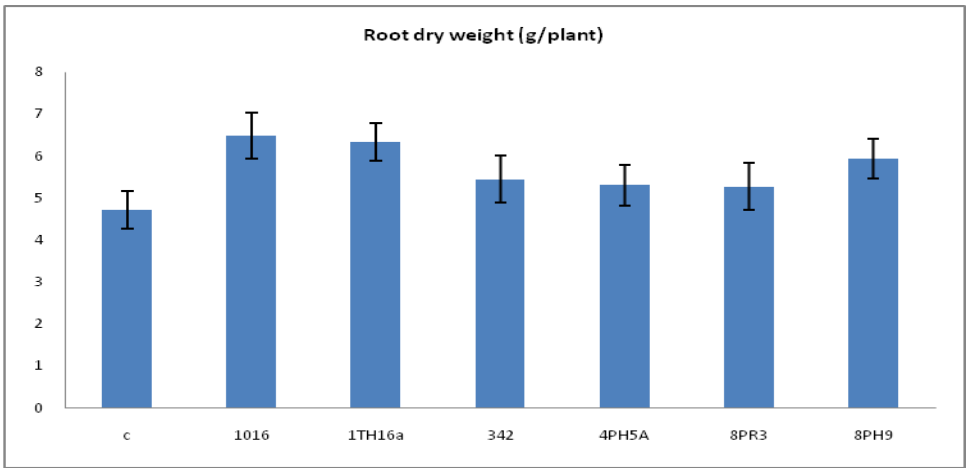


Fig.4 Effect of endophyte inoculation on fresh fruit weight

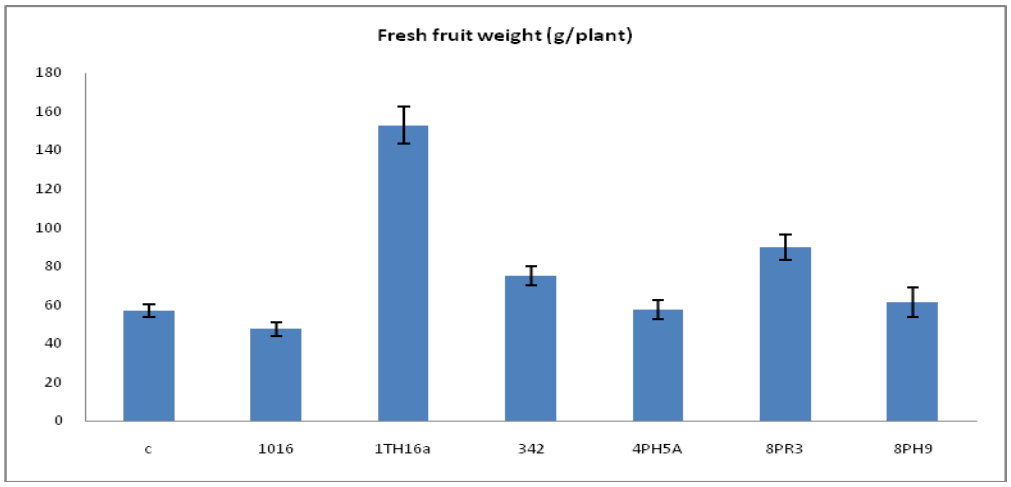
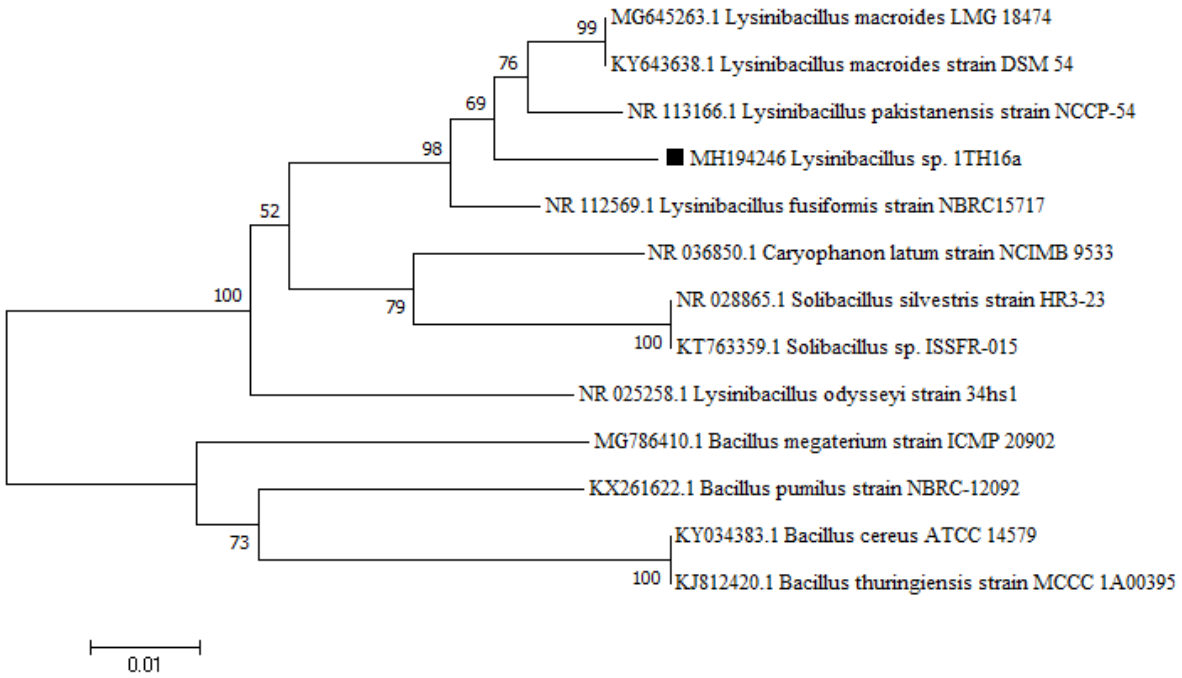


Fig.5 Phylogenetic tree indicating the relatedness of MH194246 *Lysinibacillus* sp. 1TH16a with other close relatives



This might be one of the reasons for higher biomass accumulation in plants. Venden and co-workers (2010) has reported higher solubilization of mineral phosphate and production of higher amount of IAA by *Lysinibacillus* sp. which contributed to higher plant growth in ginseng.

Zhang *et al.*, (2010) reported that *Lysinibacillus* sp. also has the ability to protect plants from pathogens. This could be an additional mechanism which might be contributing towards higher crop yield in tomato by *Lysinibacillus* sp. 1TH16a inoculation. Verma and co-workers (2014) reported that *Lysinibacillus* sp. also solubilize zinc and produce ACC-deaminase which might be reducing the ethylene stress in the plants and helping it in realizing higher fruit yield and biomass accumulation. Yadav and co-workers (2016) assessed the IAA production capabilities of different isolates and found that *Lysinibacilus fusiformis* strain IARI-AR8 produces higher amount of IAA even at adverse conditions (higher pH 8-10).

Naureen *et al.*, (2017) had studied *Lysinibacillus sphaericus* ZA9 in greater detail and shown its potential for plant growth promotion and biotic stress management. They indicated production of higher quantity of IAA (697µg/ml), siderophore, hydrolytic enzymes and HCN. This bacterium had shown potential solubilization of phosphorus, potassium and silicon. They had shown enhanced shoot growth in tomato and cucumber due to application of this bacterium. It is also reported to produce 2-pentyl-4-quinolinecarboxylic acid and 1-methyl cyclohexene which are strong antagonist to most of the fungi. There are several such reports indicating potential of *Lysinibacillus* sp. for plant growth promotion like phosphate solubilization and phytohormone production (Hardoim *et al.*, 2008). These could be the reasons for

enhanced the fruit yield by inoculation of *Lysinibacillus* sp. strain 1TH16a in tomato.

This strain of *Lysinibacillus* has potential to be used as inoculant for plant growth promotion in tomato. Other mechanisms of *Lysinibacillus* sp. and its interaction with plant need to be unravel so as to harness more for enhancing agriculture production. Experiments are also needed to standardize delivery technique and formulation for its field application. Further, extensive trials are needed to utilize the potential of *Lysinibacillus* sp. strain 1TH16a to be used as inoculant for tomato and other crops.

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