

ORIGINAL RESEARCH ARTICLE

Isolation and Characterization of Bacterial Endophytes From *Lycopersicon esculentum* Plant and Their Plant Growth Promoting Characteristics

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Abstract

The study was designed to isolate and characterize bacterial endophytes from root and stem of *Lycopersicon esculentum* plant which was collected form different region of Gujarat. Total 18 isolates of endophytic bacteria were selected in which, all the endophytic bacteria produced one or the other different characteristics involved in plant growth promotion. They either produced phytohormones like indole acetic acid, siderophore, protease, pectinase, organic acid showed antifungal activity, chromium tolerance and solubilized phosphate. Four of the strains among the 18 showed maximum positive results of plant growth promoting regulators (PGPR) test and among them best probable isolate was selected and thus its 16SrDNA was amplified and sequenced. Only HR7 endophyte of tomato turned out to be *Pseudomonas aeruginosa*. It's a gram negative coccobacili, sporeforming motile bacilli and show maximum PGPR activity. The results of our present studies indicated that above strains might be endophytic and therefore, were associated with the plant growth.

Keywords: *Lycopersicon esculentum*, endophytic bacteria, PGPR, IAA, 16SrDNA

Introduction

There are many endophytic and epiphytic bacteria are directly or indirectly involved in plant growth and development. Endophytic bacteria live in plant tissues without causing substantive harm to the host or gaining any benefit other than a noncompetitive environment inside the host. It has recently been demonstrated that bacterial endophytes may also have beneficial effects on host plants, such as growth promotion and biological control of pathogens [10, 25, 28].

Some studies have indicated that the plant growth-promoting potential of endophytes is higher than that of rhizosphere microbes [23,31], but the role of bacterial endophytes in plant growth are not yet fully understood. Most of these microorganisms are not pathogenic to the host plant. Moreover, the association between the plant and its endophytes is very often mutualistic. In 1926, endophytic growth was recognized as a particular stage in the life of bacteria, described as an advanced stage if infection and as having a close relationship with mutualistic symbiosis [22]. Since then, endophytes have been defined as microorganisms that could be isolated form surface-sterilized plant organ [15]. Although the presence of bacterial endophytes in plants is variable and, occasionally transient [32], they are also often capable of eliciting drastic physiological changes that modulate the growth and development in the plant [8].

The utilization of endophytic and epiphytic bacteria in agriculture production depends on our knowledge of the bacteria-plant interaction and our ability to maintain, manipulate and modify beneficial bacteria population under field condition [14]. Many PGPRs are known to promote plant growth by a variety of mechanisms: fixation of atmospheric nitrogen that is transferred to the plant, production of siderophores that chelate iron and make it available to the plant root, solubilization of minerals such as phosphorus, and synthesis of phytohormones [12]. PGPR have been reported to directly enhance plant growth by the production of plant growth regulators, and improvements in plant nutrient uptake [12,16] or indirectly by the production of metabolites like antibiotics, siderophores etc that decrease the growth of phytopathogens [12]. PGPRs can be of two different types when associated with host tissue that is endophytes or epiphytes; otherwise they can be even rhizospheric bacteria that are present in the root adhering soil.

The aim of the present study was to isolate and characterize the endophytic bacteria associated to root and stem part of *Lycopersicon esculentum*, to evaluate different characteristics involved in plant growth promotion. Result revealed that four of the strains showed maximum positive results of PGPR test and its 16SrDNA was amplified and sequenced.

Materials and methods

Isolation of endophytic bacteria form *Lycopersicon esculentum*

Endophytes strains were isolated from root and stem of *Lycopersicon esculentum* plant (Table 1). Roots and stem part of plant were thoroughly

washed with sterile n-saline (0.85%) and cut down in 1 cm long pieces through sterile forceps with the help of alcohol. The pieces were transferred to sterile N-agar plate and incubated for 24 hrs at 30^0C.

Morphological and physiological characterization of endophytic isolates

For the present study, total 18 endophytic bacteria were isolated whose systemic morphological characters done which includes: Size, Shape, Margin, Elevation, Consistency, Opacity, Pigmentation, was done by Systematic Microbiology [3].

Gram's staining bacterial suspension was prepared in sterile distilled water and from this suspension a smear was prepared on clean & dry glass slide, air dried and then heat fixed. The smear was treated with 1% crystal violet for 1-2 min. Gram's iodine was applied for 30 sec. to 1 min. Smears were then decolorized with 10% alcohol. The counter stain, saffranin was then applied for 45-60 seconds. The stained slide were washed with tap water, air dried & observed under oil immulsion.

For motility test, the culture was inoculated into the Edward's and Ewing motility agar stab medium by stabbing the medium right into the center of agar. The entire depth of the medium was punctured. The medium was incubated at 28 ± 2ºC for 24 hrs. After incubation, it was observed for the turbid growth across the line of inoculation, which indicates motile organisms.

For Antibiotic assay, top agar (1.5%) was prepared and autoclaved. It was cooled to 45º C, 100µl of culture was added to this and overlaid preset N-agar plates. Using sterile forceps, disc containing the antibiotic of interest was placed on the agar and incubated at 28±2ºC for 48 hrs.

Estimation of plant growth promoting properties

(i) Detection of siderophore production: This was performed by a method described by Schwyn and Neilands [27], which involved the use of chrome azurol S containing indicator plates. Siderophore detection was performed by mixing equal volumes of chrome azurol S (CAS) assay solution and the culture supernatant. Colour change from blue to yellowish orange was indicative of presence of Siderophore. Two percentage of overnight grown culture was inoculated in magnetotactic bacterium *Magnetospirillum magneticum* AMB-1 (AMB) and grown for 48 hrs. Then the culture was centrifuge at 8000 rpm for 20 min and the supernatant was examined for the presence of siderophore by CAS solution.

(ii) Evaluation of endophytes for chromium tolerance: Isolated strains were tested for resistance to Cr (VI) by plate dilution method using yeast extract mannitol agar (YEMA) medium. In a plate dilution method, agar plates amended with $K_2Cr_2O_7$ at 50-500 µg/ml were inoculated with 48 hrs grown cultures and incubated at 28 ± 2ºC for 72hrs. The lowest concentration of Cr (VI) inhibiting on YEMA plates was defined as minimum inhibitory concentration [35].

(iii) Phosphate solubilization ability: The phosphate solubilizing ability of the cultures were examined by growing the cultures on Pikovskaya's agar plate and looking for the zone of clearance after incubating at 28 ± 2°C for 48-72h.

(iv) Antifungal activity: The spores of fungal cultures (*Fusarium oxysporium*, *Alternaria,*, *Trichoderma* and *Rhizoctonia solani*) grown on Potato dextrose agar (PDA) blocks were placed in the centre of PDA plates and the bacterial cultures were streaked at four ends of the plate. This was incubated at 28 ± 2°C for 48-96 hrs and examined for zone of growth inhibition.

(v) Protease production: It is indicated by casein degradation, which was determined by observing clearing zones in Nutrient casein agar plate. All isolated culture was streak on Nutrient casein agar plate and incubated at 28 ± 2°C for 24-48 hrs. Next day flood the plate with Frazier's reagent to detect clear zone around the colony.

(vi) Indole 3-acetic acid (IAA) production test: IAA in presence of $FeCl_3$ develops pink color. This fact is utilized in determination of IAA. Different mineral acids like hydrochloric acid, perchloric acid, phosphoric acid, nitric acid and sulphuric acid can be used to develop the color. $FeCl_3$ –$HClO_4$ reagent is the most sensitive and shows least interference by other iodole compounds like, tryptophan, skatole, acetyletryptamine etc.

Loopful of each culture was inoculated in luria broth (LB) 2ml containing 50µg/ml tryptophan and incubated at 28^{o}C for 24 hrs on shaking condition, centrifuged at 9000 rpm for 15min, 2ml of supernatant was taken in fresh tube and 2-3 drops of orthophosphoric acid was added. A quantity of 4ml of reagent (1ml of 0.5 M $FeCl_3$ in 50 ml of 35% $HClO_4$) was added to this aliquot and incubated for 25 min at RT. Absorbance was measured at 530 nm. Auxin quantification values were recorded by preparing standard calibration curve made by using IAA standard in the range of 10-100 µg/ml. IAA stock solution was prepared as 100 µg/ml in 50% ethanol. Standard graph of IAA concentration was plotted against O.D 530 and

the concentration of IAA in samples used was calculated.

Organic acid production: It was studied by growing the cultures in Calcium carbonate agar plate and observing for a clear zone around the colony.

Chitinase production: It was observed by spotting the culture on chitin agar plate and observing zone of clearance after incubating at 28 ± 2°C for 48- 72h. Chitinase activity (degradation of β- 1,4- N- acetylglucosamine polumer) were tested in a minimal medium. There were clear zones were detected after 5 days incubation period at 30°C.

Pectinase production: It was detected by spotting the culture on pectin agar plate and observing zone of clearance after incubating at 28 ± 2°C for 48- 72h.

16S-rDNA sequencing of PGPR isolates

Well isolated colonies (2-3 colonies) of the culture whose 16S-rDNA had to be amplified were suspended in 20µl-30µl of sterile distilled water. The suspension was heated at 95ºC for 20 min and centrifuged at 9000 rpm for 1min. The supernatant was used as template DNA in the PCR system [26]. The 16S-rDNA gene fragment was amplified using universal eubacterial full-length primers. The amount of DNA taken for amplification was 10ng.

Primer sequences

Forward Primer (PF) 5' AGA GTT TGA TCC TGG CTC AG 3'

Reverse Primer (PR 5' ACG GCT ACC TTG TTA CGA CTT 3'

The PCR components and conditions (to set a system of 30 µl) used for amplification. Amplifications were performed in Eppendroff gradient thermal cyclers programmed for 30cycles. The PCR thermal cycle consist of an initial denaturation step of 3 min at 94°C, Then 30 sec at 94°C for denaturation, 30 sec at 57°C for primer annealing and in last step primer extension done by 2 min at 72°C. Steps 2, 3, 4 repeated for 30 cycles followed by a final extension of 10 min at 72°C. The amplified products were then examined by an aliquot of the DNA (2µl) was analyzed on a 1.0 % agarose gel along with 500bp ladder and stained with ethidium bromide (0.5µg/ml). The gels were visualized under UV light in a transilluminator and photographed subsequently.

Sequence Analysis: The product was sequenced and matched with the already available sequences in the Gene Bank by uploading the obtained sequence in its FASTA format in nucleotide sequence match available at the online tool of RDP Database Project II.

Results and discussion

Isolation of endophytic bacteria

We have isolated endophytic bacteria from the *Lycopersicon esculentum* (tomato) plants from different field areas on the Nutrient agar (NA) medium. Colonies showing different morphological characteristics on the Nutrient agar plates were selected for further characterization. About 18 strains were isolated. The number of isolates, the source of their plant and field from where the samples were procured are mentioned in the Table 1.

Table1: Bacterial endophytes isolates form *Lycopersicon esculentum*

Sample	Location	No. of isolates	Name of the isolates
1	AAU (Anand)	5	HR 1 HR 2 HR 3 HR 4 HR 5
2	Mansa (Gandhinagar)	7	HR 6 HR 7 HR 8 HR 9 HR 10 HR 11 HR 12
3	Gana (Anand)	6	HR 13 HR 14 HR 15 HR 16 HR 17 HR 18

Morphological and physiological Characterization

In this work all the 18 isolates strains were picked on the basis of different morphological characteristics. The morphological characteristics of the final four short-listed isolates are shown in Table 2.

Gram's staining and Motility

Result showed that out of 18 isolates tested 9 were gram negative coccobacilli, 5 were gram positive bacilli and only 4 were gram negative cocci. This indicated that majority (50%) of the bacteria in our studies belonged to gram negative coccobacilli strains followed by 22.22% gram positive bacilli and gram negative cocci seemed to be the most uncommon one constituting only 27.77% of the total isolates. While in case of motility 66.66% were motile and remaining 43.44 % were non-motile (Table 3).

Antibiotic assay

The endophytic isolates were also checked for their sensitivity (S) and resistance (R) against antibiotics like Ampicillin, Gentamycin, Spectinomycin, Tetracycline. The result of the antibiotic assay of the rhizospheric isolates is tabulated (Table 4).

Table 2: Morphologyical and Physiological characteristics of 18 isolates

Colony character	Size	Shape	Margin	Elevation	Texture	Opacity	Pigmentation
HR1	Medium	Round	Entire	Raised	Smooth	Transparent	No pigmentation
HR2	Medium	Round	Entire	Flat	Smooth	Transparent	No pigmentation
HR3	Small	Round	Entire	Slightly Raised	Smooth	Transparent	Yellow pigmentation
HR4	Small	Round	Entire	Slightly Raised	Smooth	Opaque	Yellow pigmentation
HR5	Small	Round	Entire	Flat	Rough	Transparent	No pigmentation
HR6	Small	Round	Entire	Slightly Raised	Smooth	Transparent	Yellow pigmentation
HR7	Medium	Round	Entire	Flat	Smooth	Opaque	Pitch pigmentation
HR8	Medium	Round	Entire	Raised	Smooth	Opaque	Yellow pigmentation
HR9	Medium	Irregular	Irregular	Flat	Rough	Opaque	White pigmentation
HR10	Medium	Round	Entire	Flat	Smooth	Transparent	Yellow pigmentation
HR11	Small	Round	Entire	Raised	Smooth	Transparent	Yellow pigmentation
HR12	Medium	Irregular	Irregular	Flat	Rough	Opaque	White pigmentation
HR13	Small	Round	Entire	Flat	Rough	Transparent	No pigmentation
HR14	Medium	Round	Entire	Flat	Smooth	Transparent	Yellow pigmentation
HR15	Medium	Irregular	Irregular	Flat	Rough	Opaque	White pigmentation
HR16	Medium	Round	Entire	Flat	Smooth	Transparent	Yellow pigmentation
HR17	Medium	Irregular	Irregular	Raised	Rough	Transparent	Golden yellow pigmentation
HR18	Small	Irregular	Irregular	Flat	Rough	Opaque	White pigmentation

Siderophore production

Assay of siderophore production performed by CAS agar plate method in which following isolates HR1, HR3, HR4, HR7, HR18 showed production of siderophore. So further estimation of siderophore was performed to determine which types of siderophores are produced, either cathecolate or hydroxymates type of sideophore. Unfortunately we could not obtain the result.

IAA production test

All the isolates were tested for their IAA production. After 24 hrs of incubation with tryptophan all the strains exhibited a significant amount of IAA production. The production of IAA by isolates indicated that the tested strains

Table 3: The Gram nature and Motility of the 18 isolated strains.

Isolates from Tomato plant	Gram's Nature	Motility
HR 1	Gram –ve cocco bacilli	+
HR 2	Gram –ve cocco bacilli	+
HR 3	Gram –ve cocco bacilli	+
HR 4	Gram –ve cocco bacilli	+
HR 5	Gram –ve cocco bacilli	+
HR 6	Gram –ve cocco bacilli	+
HR 7	Gram –ve cocco bacilli	+
HR 8	Gram –ve cocco bacilli	+
HR 9	Gram -ve cocci	-
HR 10	Gram -ve cocci	-
HR 11	Gram +ve bacilli	+
HR 12	Gram +ve bacilli	-
HR 13	Gram +ve bacilli	+
HR 14	Gram +ve bacilli	-
HR 15	Gram +ve bacilli	-
HR 16	Gram -ve cocci	+
HR 17	Gram –ve cocco bacilli	+
HR 18	Gram –ve cocco bacilli	+

+ : Indicates motile organism, - : Indicates non-motile organism.

utilized tryptophan as a precursor for growth and produced IAA, the primary auxins in the majority of plant species as a plant growth promoter. Data indicated that all the bacterial endophytes from plant were able to produce IAA in the presence of tryptophan (Table 5). Production of IAA is widespread among bacteria-plant associated. Several bacteria having the ability to anabolise indole-3-acetic acid (IAA) with supplemented L- tryptophan have been isolated from the plant surfaces. Bacterial IAA producers (BIPs) have the potential to interfere with any of these processes by input of IAA into the plant's auxin pool [1]. Patten and Glick [20,21] have shown that bacterial IAA stimulates the development of the root system of the host plant and Brandi and Lindow (1998) have studied the contribution of IAA for bacterial epiphytic fitness, observation supported by the investigation of other workers [12,20,2,9,33].

Chromium tolerance of the endophytic strains

Almost 17 out of 18 isolates from *Lycopersicon esculentum* tolerated a chromium concentration upto 500µg/ml. One of the isolate HR11 tolerated upto 300µg/ml, wheres all the isolates showing tolerance above 450µg/ml. There are reports of certain *Bacillus* spp. tolerating upto

Table 4: Antibiotic assay of isolated strains

Isolates	Ampicillin	Streptomycin	Tetracycline	Chloramphenicol
HR1	10	16	18	23
HR2	R	19	13	22
HR3	14	21	19	24
HR4	R	9	15	R
HR5	R	17	R	14
HR6	R	9	R	13
HR7	R	18	R	17
HR8	R	11	R	8
HR9	28	29	27	38
HR10	R	R	7	19
HR11	7	16	18	21
HR12	R	13	11	19
HR13	R	R	9	16
HR14	13	21	15	14
HR15	8	R	11	14
HR16	R	R	8	18
HR17	R	12	R	R
HR18	21	20	19	24

Resistance microorganism- **R**, Number mentioned is zone of inhibition in mm

550 μg/ml [35] and *Bacilli* spp. is a well known PGPR strain. All the standard strains except *R. leguminosarum* and *S. meliloti* showed very less tolerance to chromium. Both the strains *R. leguminosarum* and *S. meliloti* are well known for their PGPR activity in leguminous plants. A *Rhodococcus erythropolis* MTCC 7905 strain has been shown to be resistant to 300 mg l^{-1} of Cr^{6+} isolated from metal contaminated soil samples from a site near Indian Himalayan region has been reported to reduce substantial amounts of Cr^{6+} to Cr^{3+} as well as showed to have plant growth promotion of pea (*Pisum sativum*) in the presence of toxic Cr^{6+} concentration [30].

Table 5: Indole Acetic Acid production by endophytic bacterial isolates

Isolates	OD at 530nm
HR1	0.061
HR2	0.020
HR3	0.050
HR4	0.241
HR5	0.199
HR6	0.067
HR7	0.057
HR8	0.114
HR9	0.056
HR10	0.097
HR11	0.181
HR12	0.007
HR13	0.029
HR14	0.270
HR15	0.112
HR16	0.094
HR17	0.166
HR18	0.079

Phosphate solubilisation

Phosphorous is one of the most important plant nutrient and a large portion of inorganic phosphate applied to soil as fertilizer is rapidly immobilized [19,24]. Endophytic bacteria possess the capacity to solubilize immobilized mineral phosphates. In this study all the 18 isolates were tested for their phosphate solubilizing activity on Pikovasky agar medium. It was interesting to note that out of 18 endophytic isolates, 8 showed phosphate solubilisation activity (Table 6). Result revealed that majority of the PGPR strains do have phosphate solubilizing activity and such organisms play a major role in plant growth promotion [24].

Table 6: Phosphate Solubilization by endophytic bacterial isolates

Isolate No.	Growth on PV	Zone (mm)
HR 1	Full growth	20
HR 2	Full growth	-
HR 3	Full growth	21
HR 4	No growth	-
HR 5	No growth	-
HR 6	No growth	-
HR 7	Full growth	17
HR 8	No growth	-
HR 9	No growth	21
HR10	No growth	-
HR 11	Less growth	8
HR 12	Less growth	11
HR 13	No growth	-
HR 14	No growth	-
HR 15	No growth	-
HR 16	No growth	-
HR17	Full growth	31
HR 18	Less growth	9

mm zone of clearance (Pink colour Zone)

Organic acid production

Out of the 8 endophytic isolates showing phosphate solubilization, all 8 showed organic acid production. The isolates number HR7, HR8, HR9, HR10, HR14, HR15 showed slight organic acid production by forming a very thin zone of clearance on the plates of Pikovasky with methyl red as pH indicator dye. This gave the pink coloured zone that indicated shift in pH change from alkaline to acidic. Some isolates like HR4, HR7, HR13, HR16 were unable to solubilize phosphate and also did not produce organic acid. This could be because the amount

of organic acid produced might be very less to do so (Table 7).

Chitinase production

In the present study none of the strain revealed a clear zone, but 5 isolates out of 18 showed growth on the chitin agar plate, remaining 13 strains did not show any growth (Table 8). Biological control of plant pests and diseases is much more attractive than chemical treatment methods due to its greater specificity and less harmful impact on the environment [34,35]. Major component of fungal cell is chitin. Thus organism having the ability to produce chitinase might have antifungal property.

Pectinase production

For pectinase production, 17 out of 18 isolates of *Lycopersicon esculentum* revealed the production of pectinase. The strains showing

Table 7: Isolates showing organic acid production

Sample No.	Isolate No.	Production of Organic acid	Zone (mm)	Pink colour of Zone
Sample 1.	HR 1	Medium	13	+++
	HR 2	Medium	10	++
	HR 3	Very less	8	++
	HR 4	No	-	-
	HR 5	No	-	-
Sample 2.	HR 6	Very less	7	-
	HR 7	Medium	11	++
	HR 8	Medium	13	-
	HR 9	Medium	10	-
	HR 10	Very less	9	-
	HR 11	Medium	12	+
	HR 12	Very less	8	++
	HR 13	No	-	-
Sample 3.	HR 14	Medium	11	-
	HR 15	Very less	8	-
	HR 16	No	-	-
	HR 17	Medium	11	+++
	HR 18	Medium	13	+

+ : 1.0 mm ZOC (zone of clearance), ++: 1.2 mm ZOC, +++: 1.4 mm ZOC, -: No zone

production of pectinase on pectin agar plate are listed in Table 9. Maximum research indicated that pectin methyl esterase (PME) (EC 3.1.1.11) catalyzes the hydrolysis of methyl-ester groups of cell wall pectins. It has been found in all plant tissues and in some of plant cell wall-degrading microorganisms or insects [5,6] and has been implicated in a number of processes including cell growth [18], fruit ripening [11, 29], abscission and senescence [17], pathogenesis [7] and cambial cell differentiation [13].

Table 8: Isolates showing Chitinase production

Isolates	Chitinase Production
HR1	-
HR2	+
HR3	-
HR4	+
HR5	-
HR6	-
HR7	+
HR8	-
HR9	-
HR10	-
HR11	-
HR12	-
HR13	-
HR14	+
HR15	-
HR16	-
HR17	-
HR18	+

+ : positive - : negative

Table 9: Isolates showing Pectinase production

Isolates	*Pectinase production*
HR1	+
HR2	+
HR3	+
HR4	+
HR5	+
HR6	+
HR7	+
HR8	+
HR9	+
HR10	+
HR11	+
HR12	+
HR13	+
HR14	+
HR15	+
HR16	-
HR17	+
HR18	+

+ : positive - : negative

16S-rDNA sequencing of PGPR isolates

Colony PCR

All the plant growth promoting results when compiled together showed one strain (HR7) showed maximum positive features and thus the 16SrDNA of the strain was amplified using universal full length primers. An amplicon of 1.5kb was obtained and sent for sequencing to Bangalore Genei, Pvt, Ltd India. The sequence obtained was matched with the online available sequences in RDP (Ribosomal Database Project II) bioinformatics tool.

Multiple sequence alignment phylogenetic analysis

BLAST (Basic local alignment search tool) search was done for partial 16s rDNA of the isolates HR7 by submitting queries to NCBI-BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi.) and homologous sequences obtained by standard nucleotide-nucleotide BLAST (blastn) were aligned with the different 16s rDNA isolates after sequencing and various related sequence were retrieve after blasting the partial sequence of the isolates obtained after sequencing. Accession No. of the related species was retieved and Multiple sequence alignment (Fig 1) was performed using CLC free protein workbench 5.0. Evolutionary tree for the same

data was obtained by neighbor joining method with Bootstrap values (expressed as percentages of 100 replications) as shown in (Fig 2). Except HR7 other do not give the sequencing results. Accession no. of some isolates used for multiple alignment with HARDIKSEQ-1(HR7) isolate were, JF423918, JF281099, HQ995502, HQ268732, HQ202541, HQ202540, HQ259948, FM995816, FM995815, FM995811, FM995802, FM995800, FM995798, FM995797, FM995796.

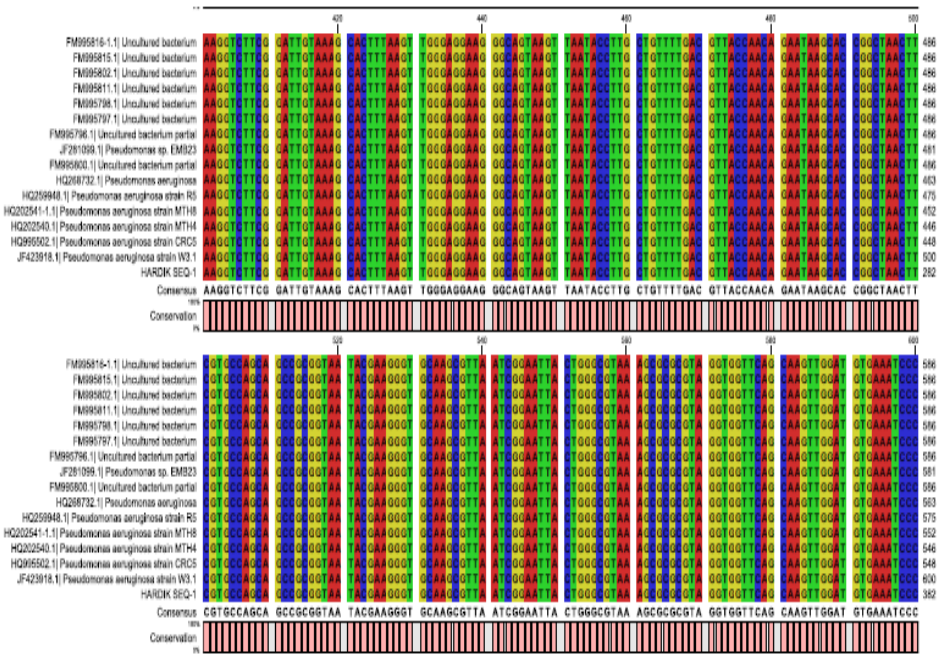

Fig. 1: Multiple sequence alignment for the partial 16s rDNA sequence of hardik seq-1 (HR7) isolate with other related species retrieve after BLAST, resulted in versatile coloring scheme that highlighted the conserved sequence in Aligned sequences.

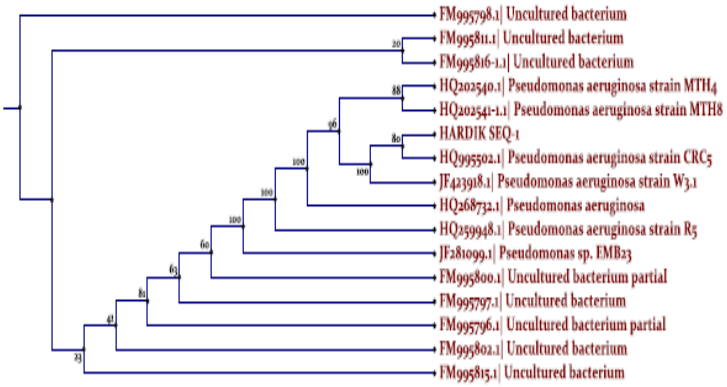

Fig. 2: Phylogenetic tree of partial 16S rRNA genes of Hardik seq-1(HR7) islolates from closely related of resistant bacteria obtained after BLAST. The tree was constructed based on partial 16S rRNA sequences of the isolates and the reference strains. Bootstrap values (expressed as percentages of 100 replications) are shown at branch points. Bootstrap values over 50% are shown. The scale bar 0.500 indicates 50% nucleotide sequence substitution

Precisely, the research concluded that endophytic bacteria isolated form *Lycopersicon esculentum* produced one or the other different characteristics involved in plant growth promotion. They either produced phytohormones like indole acetic acid, siderophore, protease, pectinase, organic acid showed antifungal activity, chromium tolerance and solubilized phosphate. Only HR7 endophyte of tomato turned out to be *Pseudomonas aeruginosa*, It is a gram negative coccobacili, sporeforming motile bacilli,which showed maximum PGPR activity. It may be concluded that the above strains may be endophytic and was associated with the plant probably because they may benefit the plant by stimulating its growth.

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