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Isolation and Characterization of Potential Phyllosphere Bioinoculants from Coriandrum sativum

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Abstract

Coriandrum sativum, also known as Coriander, is an annual herb in the apiaceae family. It is a common leguminous plant that grows abundantly in India. It has also been used for intercropping with short irrigation intervals. This plant is well-known for its ability to thrive in low-phosphate and drought-prone soils. This study aims to identify isolates from the phyllosphere region that have potential to be used as bioinoculants. Phyllosphere sample was collected from the native farmland and neighbourhood and screened for bioinoculants. A total of 98 bacterial strains were isolated and tested for production of ammonia. The nitrogen fixing strains were chosen based on their ammonia production. The selected 28 isolates were tested for various PGP factors such as HCN production, IAA production and Phosphate solubilization and also tested for extracellular enzymatic activities such as Amylase production, Protease degradation, Gelatinase activity, Catalytic activity and Lipase production, based on the results, few exhibited potent antagonistic activity against *Colletotrichum capscici* and *Rhizoctonia solani.* Based on the results of extracellular activities and antagonistic assay, five potential strains were subjected to germination study. One best PGPP was identified through amplification and sequencing of the 16S rRNA gene. The research uncovered a previously unknown diversity of plant growth promoting bacteria with enormous potential for use as a bioinoculant in agriculture. This study emphasises the importance of Phyllosphere microbiota for plant growth, in addition to identifying promising candidates for bioinoculation.

1. Introduction

Agriculture is a major field that has attained a very advanced level when compared to the last few decades. Scientific developments have a vital role in the improvement of agriculture in many ways such as mechanical inventions, irrigation techniques, chemical fertilizers, pesticides, insecticides, etc. In 2050, the world would need a 65% higher production of food products when compared to the current market production rate. However, that may be achieved by excessive input of chemical fertilizer, but the biological and physicochemical characterization of soil may degrade eventually. Dumping more unbalanced amounts of chemical fertilizer may increase the plant yield and productivity for a single crop cultivation cycle, the non-absorbed chemical or synthetic fertilizer may remain in the soil and can acidify soil nature (Sharma and Singhvi, 2017).

Current research studies have shown that the degradation of soil can be prevented and also restored by the application of bio-fertilizer, which can be considered a greater and safer replacement for chemical fertilizers. On the formation of biofertilizer, the plant symbiotic rhizosphere- and phyllosphere- bacteria had shown plant growth-promoting activity. Based on traits, isolates were screened for antagonistic activity among themselves and also with the plant to prevent any future accidents (Ghosh., 2004). Microbes that were used in the formation of biofertilizer can be bacteria, fungi, algae, etc. Those were generally described as bio-inoculants. Bio-inoculants were substances derived from microbes or live microbes that could be applied to plants as an additive source of nutrients and could also increase the plant growth parameters.

Despite the importance of phyllosphere bacteria with different PGP characteristics in sustainable agriculture, little is known about their impact on shaping plant performance traits. For better survival in the phyllosphere, bacteria have developed mechanisms such as ultraviolet variation (UV) resistance, biofilm formation, and aggregation.

Coriander (*Coriandrum sativum* L.), an annual herb, is commonly used for seasoning. Its plant seeds, leaves, and roots are edible, with distinct flavors and applications. The flavor of the herb is light and refreshing. Coriander can be used as a flavoring agent in various food preparations due to the perishable nature of the leaves and seeds are used to increase the palatability of ripe fruits (seeds). Coriander is used in cooking in its entirety, primarily for fresh leaves and ripe fruits. Coriander leaves have a different flavor than coriander seeds, with citrus undertones (Kumar, J., et al, 2021). The Coriander plant is a rich reservoir of micronutrients and nutritional elements and is luxurious in growth, so we decided to isolate potential phyllobacteria from it. Coriander is a good source of tocopherol and vitamin K, low in saturated fat but high in linoleic acid. Plant leaves are high in vitamins, while seeds are high in polyphenols and essential oils.

In general, phyllosphere bacteria originate primarily in soil, seed, and air and adapt to life on leaf tissue, where several factors such as environmental parameters shape their community composition. The phyllosphere hosts a variety of bacterial isolates at the genus and species levels, depending on plant genotype and climate. The capabilities of plant-microbe interactions in the phyllosphere can contribute to our comprehension of how plants benefit from their microbial partners. As a result, having more information on phyllosphere bacteria from various geographical locations in terms of their PGP traits may make it possible to find qualified applicants for organic amendments (Stone et al., 2018).

The primary objectives of this study were to isolate and characterize the leaf-colonizing bacteria with the potential for plant growth–promoting activities from *Coriandrum sativum* in a village region of Coimbatore district, Tamil Nadu State, India. The bacteria were screened in vitro for PGP traits and the most promising isolate was identified by sequencing of 16S rRNA genes. We also examined the germination property of the selected isolate compared with the control under the greenhouse effect (Lau, S.-E., et al, 2022).

2. Materials and methods

2.1. Isolation of Phyllobacteria:

Coriandrum sativum leaves were collected from agricultural fields, which were not subjected to any chemical fertilizer or synthetic pesticides and herbicides. On employment to enrichment technique to increase the papulation of symbiotic bacteria leaves were inoculated in nutrient broth for overnight. Serial dilution plate method was adapted for isolating microorganisms from enriched broth culture. After incubation for 24 hours, unique colonies were selected based on colour, shape, elusion and growth (Schlegel and Jannasch, 1967).

2.2. Characterization of PGPR:

2.2.1. Production of ammonia:

It was done by growing the isolates in peptone water at 30°C for 4 days. To each culture tube, 1mL of Nessler's reagent was added. Development of faint yellow colour indicates small amounts of ammonia and deep yellow to brownish colour indicates maximum amount of ammonia production (Tsegave. Z et al.,2019).

2.2.2. Determination of IAA:

Luria Bertani (LB) broth medium (25 mL) was used to enrich isolates in which 50 µg/mL tryptophan, incubated for 24 h at 28°C on rotary shaker. Cultures were centrifuged at 10,000 rpm for 15 min with addition of two drops of ortho phosphoric acid and 4 ml of Salkowsky reagent were mixed with supernatant (2 mL). Development of pink colour was achieved by incubating for 25 min at room temperature confirms qualitative IAA production (Tsegave. Z et al., 2019).

2.2.3. HCN Production:

A 4.4 g/l glycine amended King's B medium was used to streak isolates. Whatman No.1 filter paper discs were dipped in 0.5% picric acid in 2% sodium carbonate solution. On lid of each petriplate the discs were placed and sealed. Plates were incubated for 4 days at 28 ± 2℃. Production of HCN was recorded from colour change of the filter paper from deep yellow to orange and orange to brown (Bharucha U.D., et al, 2013).

2.3. Extra cellular enzyme production:

2.3.1. Phosphate solubilization:

To check phosphate solubilisation, isolates were streaked on Pikovaskya's (Pikovaskya, 1948) agar medium and incubated for 4 days at 28°C. Phosphate solubilising activity was confirmed by clearing zones around the bacterial colony plates.

2.3.2. Catalase activity:

Catalase test was performed by growing bacterial culture for 48 hr on trypticase soy agar medium followed by adding three to four drops of H_2O_2 on bacterial culture which was grown. The prescence of effervescence indicates Catalase activity (Kumar, et al, 2012).

2.3.3. Protease Production:

Protease production qualitative assay was performed on sterile skim milk agar plates containing casein 5.0 g, Yeast extract 2.5 g, Glucose 1.0 g, Agar 15.0 g, Distilled water 1000 mL, Skim milk 7% was added as inducer. Isolates were inoculated and zone of clearance around the colony was observed by incubation at 30 C and indicating the enzymatic degradation of protease.

2.3.4. Production of lipase:

Nutrient agar amended with egg yolk was used to test the bacterial isolates. Saturated aqueous solution of copper sulphate ($CuSO_4$) was used to flood agar medium and kept for 10–15 min after 48 h of growth. The excess reagent was poured off. Formation of greenish blue colour zones around the colony indicated the production of lipase (Malleswari D and Bagyanarayana G., 2013).

2.3.5. Production of amylase (starch hydrolysis):

Amylase assay medium comprises Beef extract 3.0, Peptone 5.0, Soluble Starch 2.0, Agar 15.0, Distilled water 1lit and inoculated isolates incubated at 30°C for 48 h., The plates were flooded with iodine solution at the end of incubation period, kept for a minute and then poured off. Iodine reaction with starch forms a blue colour compound. This blue colour fades rapidly. The production of amylase is indicated by the colour less zone surrounding colonies (Malleswari D and Bagyanarayana G., 2013).

2.3.6. Gelatinase test:

The culture was inoculated into gelatine containing tubes followed by incubation for 5 to 7 days. Upon refrigeration of the tubes for an hour confirms if gelatinase present, failing of liquid medium to solidify upon refrigeration (Geetha K., et al, 2014).

2.4. Salt tolerance:

Isolates were tested for their salt tolerance ability by spot inoculating the isolates on nutrient agar plates containing different concentrations of NaCl (2%, 4%, 6%, 8% and 10%), incubated at 32°C for 5 days. Observe the growth (Geetha K., et al, 2014).

2.5. Antagonistic test:

Selected five potential strains were screened for antagonism against *Colletotrichum capscici* and *Rhizoctonia solani* using potato dextrose agar (PDA) medium by using potato dextrose agar medium, placing the pathogen and bioagent in opposite direction and allowing them to grow for 7 days (Geetha K., et al, 2014).

2.6. Germination test:

Green gram seeds were surface sterilized with 0.1% $HgCl_2$ for 3 min, followed by successive washing with sterile distilled water and then the water was decanted. The seeds were kept for 10 min in 48 h old cultures. The seeds were shown on paper cup filled with sterilized sand collect from and incubated at 30℃ for 2–7 days. Seeds were treated with sterilized medium was treated as control. The root and shoot lengths were recorded on 7th days (Geetha K., et al, 2014).

2.7. Morphological characterization of isolates:

The selected bacterial isolates were examined on Nutrient Agar plates for their morphological features. After 3 days of incubation, different colony characteristics such as Gram reaction, shape, motility,

elevation, surface, etc. were recorded (Mącik, M., et al, 2020).

2.8. Seed Germination and Seedling Growth:

Test Seed germination tests were carried out to determine the effect of inoculated rhizobacterial species or strains on the rates of seed germination and seedling growth. For this, green gram seeds were used as plant materials. Healthy seeds were surface sterilized with 70% alcohol for 3 min and followed with 1% sodium hypochlorite for 5 minutes and rinsed 5 times with sterile distilled water. Five PGPR species or strains were grown in nutrient broth on shaking incubator (180 rpm) at 30°C for 24 h. The surface-sterilized seeds of green gram were inoculated in broth culture of the PGPR species or strain cultures for 30 min including normal water (C) as control. 25 inoculated seeds of each treatment were placed in separate plastic pot filled with sterilized red soil and the pots were incubated at room temperature for 7 days. Percentages of germination and seedling growth were calculated and evaluated the effect of single seed bacterization on a shoot and root growth of green gram crops. Vigor index of seedling was measured at the end of the experiment according to the formula. Seed vigor index = (Mean shoot length + Mean root length) × germination %.

2.9. Identification of bacterial strains using 16SrRNA gene sequencing:

The DNA template was prepared by picking an individual colony of each bacterial strain, and amplification of the 16S rRNA gene was carried out by the PCR. PCR amplification of DNA was performed following Katsivela et al. (1999) using universal primers (27F: 5′- ' AGAGTTTGATCTGGCTCAG-3'; 1492R: 5′-TACGGTACCTTGTTACGACTT-3′) in a reaction mixture (25 µL). The amplification program for the full-length 16S rRNA gene consisted of an initial denaturing at 94°C for 2 min, followed by 30 cycles of denaturation at 94°C for 2 min, primer annealing at 55°C for 1 min and primer extension at 72°C for 2 min, followed by a final extension at 72°C for 10 min, in a thermocycler. Amplified PCR products of the 16S ribosomal gene were separated on 1% agarose gel in 0.5× TE (Tris-EDTA) buffer containing 2 µL ethidium bromide (20 mg/ml). The purification process for PCR product was done by using Montage PCR Clean up kit (Millipore). Sequencing reactions were performed using a ABI PRISM® BigDyeTM Terminator Cycle Sequencing Kits with AmpliTaq® DNA polymerase (FS enzyme) (Applied Biosystems). Single-pass sequencing was performed on each template using below 16s rRNA universal primers. The fluorescent-labeled fragments were purified from the unincorporated terminators with an ethanol precipitation protocol. The samples were resuspended in distilled water and subjected to electrophoresis in an ABI 3730xl sequencer (Applied Biosystems).

The 16s rRNA sequence was blast using NCBI blast similarity search tool. The phylogeny analysis of query sequence with the closely related sequence of blast results was performed followed by multiple sequence alignment. The program MUSCLE 3.7 was used for multiple alignments of sequences (Edgar 2004). The resulting aligned sequences were cured using the program Gblocks 0.91b. This Gblocks eliminates poorly aligned positions and divergent regions (removes alignment noise) (Talavera and Castresana 2007). Finally, the program PhyML 3.0 aLRT was used for phylogeny analysis and HKY85 as

Substitution model. PhyML was shown to be at least as accurate as other existing phylogeny programs using simulated data, while being one order of magnitude faster. PhyML was shown to be at least as accurate as other existing phylogeny programs using simulated data, while being one order of magnitude faster. The program Tree Dyn 198.3 was used for tree rendering. (Dereeper et al., 2008).

3. Experimental Results

Over all 98 bacterial strains were isolated from the phyllosphere region of coriander, from enrichment technique and followed by serial dilution method. The Phyllobactera have been screened for ammonia production to identify nitrogen fixative bacteria. Among the 98 isolates, only 28 isolates were positive for ammonia production test. Isolates CS02, CS04, CS07, CS10, CS13, CS16, CS19, CS22, CS24, CS28, CS32, CS38, CS39, CS41, CS43, CS46, CS48, CS49, CS50, CS56, CS61, CS63, CS66, CS70, CS76, CS79, CS82, and CS85 exhibited significant ammonia production. Isolate CS10, CS24, and CS61 has shown high level of ammonia production. All the 28 nitrogen fixative strains were tested for growth promotion traits. Plant growth promotion was assessed by qualitative determination of Indole Acetic Acid (IAA), ammonia production and Hydrocyanic acid production.

Among 28 nitrogen fixative, only 3 (CS10, CS24, CS61) were the best producers of ammonia, High production of IAA was associated with CS10, CS24, CS28, CS56, and CS82 and whereas isolates CS02, CS07, CS43, CS50, and CS66 were the minimum indole acetic acid producers. Overall, 14 isolates were positive for HCN production (Sharma, K., et al, 2007). Isolate CS31, CS32 and CS49 were the maximum producer of Hydrocyanic acid.

Table I: Production of NH_3, IAA and HCN by PGP isolates from phyllosphere of Coriander.

S. No	Isolate Name	NH_3 Production	IAA Production	HCN Production
01.	CS02	++	+	+
02.	CS04	++	-	-
03.	CS07	++	+	++
04.	CS10	+++	+++	++
05.	CS13	++	+	+
06.	CS16	++	++	+
07.	CS19	++	++	+
08.	CS22	+	-	-
09.	CS24	+++	+++	-
10.	CS28	++	+++	+
11.	CS32	++	-	+
12.	CS38	+	++	+
13.	CS39	+	++	+
14.	CS41	+++	++	-
15.	CS43	++	+	-
16.	CS46	+	++	-
17.	CS48	+	+	+
18.	CS49	++	+	-
19.	CS50	+	+	++
20.	CS54	+	-	-
21.	CS56	++	+++	+
22.	CS61	+++	++	-
23.	CS63	++	+	-
24.	CS66	+	+	-
25.	CS70	++	-	+
26.	CS76	+	++	-
27.	CS79	+	-	-

- = No production; + = Weak production; ++ = medium production; +++ = high production

S. No	Isolate Name	NH_3 Production	IAA Production	HCN Production
28.	CS82	+++	+++	-
- = No production; + = Weak production; ++ = medium production; +++ = high production				

Production of extracellular enzymes by microorganisms plays an important role in the management of plant pathogens as well as holds enormous economic potential. In view of the significance of extracellular enzymes all the 28 PGP isolates were tested for their extracellular enzyme production like phosphatases, lipase, catalase, lipase, amylase, and gelatinase activity. The results of extracellular activities were listed on below table (Table II).

Table II: Extracellular enzyme activity of selected phyllobacterial isolates from Coriandra phyllosphere.

S. No	Isolate Name	P.Solibiliz-ation	Catalase activity	Protease activity	Lipase activity	Amylase activity	Gelatinase activity
01.	CS02	0.5	++	-	0.6	0.2	++
02.	CS04	-	+	-	-	0.4	++
03.	CS07	0.3	+++	-	0.4	-	++
04.	CS10	-	+	-	0.4	0.2	++
05.	CS13	0.6	+	2.7	-	0.4	++
06.	CS16	0.6	-	2.5	0.4	0.6	++
07.	CS19	1.2	-	1.9	0.4	0.9	++
08.	CS22	0.5	++	1.1	0.7	0.5	++
09.	CS24	1.1	+	2.4	-	0.3	-
10.	CS28	0.8	+++	0.8	1.5	0.9	+++
11.	CS32	0.4	+	2.2	0.3	0.5	++
12.	CS38	1.1	-	0.5	-	0.5	++
13.	CS39	0.7	++	-	-	0.5	++
14.	CS41	0.2	++	0.5	-	-	+++
15.	CS43	0.3	+	1.2	-	0.4	-
16.	CS46	0.7	++	2.1	-	-	++
17.	CS48	0.6	++	-	0.3	-	++
18.	CS49	1.2	-	1.5	-	0.3	-
19.	CS50	-	+	-	0.4	0.5	+++
20.	CS54	0.9	-	1.5	0.5	-	-
21.	CS56	-	+	2.1	0.3	0.9	++
22.	CS61	-	-	-	-	0.5	++
23.	CS63	0.4	-	1.2	-	0.4	++
24.	CS66	0.7	-	1.6	0.4	0.2	-
25.	CS70	0.8	-	2.2	0.3	0.9	-
26.	CS76	-	+	1.9	-	0.5	++
27.	CS79	0.2	+	-	0.3	-	++

S. No	Isolate Name	P.Solibiliz-ation	Catalase activity	Protease activity	Lipase activity	Amylase activity	Gelatinase activity
28.	CS82	0.6	-	-	0.4	0.4	-

- = No production; + = Weak production; ++ = medium production; +++ = high production; numerical values represent the radius of zone formed in centimeter.

Based on PGP and enzyme activities of five isolates viz., CS02, CS13, CS19, CS28, CS38 were selected for further studies. The colony morphology, growth at different concentrations of NaCl was studied for all the five selected isolates. These isolates are showing motility. They are salt tolerant at 2% and 4% and mesophilic i.e., all the selected isolates were able to grow at 20–50°C and at 10°C, 60°C the growth was not observed (Table III).

Table III: Morphological characterization of isolates and growth on different NaCl concentration and different temperature.

Isolate code	Gram staining	Shape of bacteria	Motility	Growth at NaCl (%)					Growth at temperature (^{0}C)				
				2	4	6	8	10	10	20	30	40	50
CS02	(-)ve	Rod	+	++	+	-	-	-	-	++	+	+	+
CS13	(+)ve	Cocci	+	+	+	-	-	-	-	++	++	+	+
CS19	(-)ve	Rod	+	+	+	-	-	-	-	+	+	+	+
CS28	(-)ve	Rod	+	++	+	+	-	-	-	++	++	+	+
CS38	(-)ve	Rod	+	+	+	-	-	-	-	++	+	+	+
-=No Growth; + = Medium growth; ++ = moderate growth													

All 5 isolates were tested for antagonistic activity against fungal pathogens *Rhizoctonia solani* (Otten and Gilligan, 1998) and *Colletotrichum capscici* (Naziya et al., 2019). The isolates CS19 inhibited *R. solani* and zone of inhibition was found to be 0.35 cm. The isolate CS02 showed antagonistic activity against *Colletotrichum capscici* with an inhibition zone of 0.26 cm and no inhibition were found on other isolates (Whipps, J.M., 2001).

Potential chosen five strains were applied for germination test and growth rate percentage. Overall study indicates the higher growth of plant shoot and root region when compared to control or non-treated seeds. Seeds treated with CS19 and CS28 isolates shown 92% of seed germination (Table IV). Maximum root length and shoot length was observed from seeds treated with CS28 isolate (Biswas et al., 2000).

Table IV: Plant growth promoting potential of selected isolates by germination study.

S. No	Isolate Name	Germination percentage (%)	RL Average (cm)	SL Average (cm)	Moisture Weight (g)	Dry Weight (g)
1.	Control	68	6.3	12.2	3.97	0.94
2.	CS02	80	14.1	21.6	8.71	1.52
3.	CS13	88	10.5	20.5	6.89	1.55
4.	CS19	96	9.5	19.7	6.83	1.16
5.	CS28	96	20.9	20.9	8.06	1.89
6.	CS38	84	20.4	20.4	6.88	1.01

4. Discussion

A wide array of beneficial phyllosphere bacteria have been categorized as PGPR including mainly diazotrophs, bacilli, pseudomonads and rhizobia. PGPR may induce plant growth promotion through different direct or indirect modes of action. In the present study, Isolation of bacterial cultures from phyllosphere samples of *Coriandrum sativum* from rural regions of Coimbatore district. The phyllosphere region supported a total of 98 PGP isolates with varied characteristics. Among 98 isolates 28 Bacterial strains were selected based on nitrogen fixative ability. All the isolates were screened for their plant growth promoting activities viz., Indole acetic acid production (IAA, Phosphate solubilization, HCN production, other lytic enzymes like Catalase, Protease, Lipase, Amylase, Gelatinase, Antagonistic activities and Seed germination parameters. Characterization of selected phyllobacterial isolates by using conventional methods like morphological characters, cultural characteristics on agar plate, growth on broth media, growth on NaCl, and growth at different temperature was done as described in Bergey's Manual of Systematic Bacteriology. All isolates are gram positive, motile and rod shaped. Most of the PGP's are known to produce IAA. Another important trait of PGP isolates, that may indirectly influence the plant growth, is the production of ammonia. All the selected isolates were positive for ammonia production. A secondary metabolite produced commonly by rhizosphere pseudomonads is Hydrogen Cyanide (HCN), a gas known to negatively affect root metabolism and root growth and is a potential and environmentally compatible mechanism for biological control of weeds. Phosphorous is a major essential macronutrient for biological growth and development. In our study, from 28 bacterial isolates 22 were able to solubilize phosphate in the plate-based assay, by showing a clear halo zone around the colony. Among which CS19, CS24, CS38, CS49 showed more production then the other isolates. Fungal plant diseases are one of the major concerns to agricultural production all worldwide. Antagonistic microorganisms are a potential non-chemical mean of plant disease control. Many strains of Bacillus have been shown to be potential biocontrol agent against fungal pathogens. In the present study, 28 isolates were selected for Coriandrum plant growth promoting and bio protecting activity which was originally isolated from Coriandrum phyllosphere.

Most of the rhizobacteria showed multiple traits for plant growth promotion and disease suppression. The predominant trait observed was IAA production, HCN production, phosphate solubilization followed by Ammonium production. Bacterial inoculants are able to increase plant growth and germination rate, improve seedling emergence, responses to external stress factors and protect plants from disease. In the present study it was observed that the length of root and shoot were enhanced by 70% compared to the control due to seed treatment with PGPR. This might be due to the production of growth hormones like IAA, gibberellins, auxins by bacteria. More than 80% of isolates showed positive response towards germination. The highest response for seed germination was observed with isolate CS19 and CS28 showing 96%. Gelatinase and protease activity was detected in 20 and 17 isolates. Among 28 isolates 11 isolates not shown catalase activity. 16 isolates shown lipase activity. Salinity is an ever-increasing problem in many regions of the world, particularly in arid and semi-arid regions. Salinity stress decreases crop growth and productivity due to salt-induced reduction in the photosynthetic activity. In this study, the ability of the isolates to withstand high salt concentration was a unique feature which may facilitate competitiveness in soil ecosystem, where these isolates could be used. Selected five isolates were tested for salt tolerance capabilities and observed that all the 5 isolates were observed to grow at high salt concentration 2% and 4%. While only CS28 strain was capable of growing at 6% of NaCl, none of the isolates were able to show growth at 6% and at 8%, 10% of NaCl concentrations. All the selected isolates were able to grow at 20^0C – 50°C, 60°C and at 10°C the growth was not observed.

Based on the results of above mention test results, CS28 isolate was selected for molecular identification through 16S rRNA Sequencing. A BLAST search of nucleotide sequences was done using multiple sequencing alignment method and a common similarity were found between two genus *Stenotrophomonas maltophilia* as 93.58% and *geniculate* as 93.44%. The program Tree Dyn 198.3 was used for tree rendering.

Declarations

CONFLICTS OF INTEREST:

The authors declare that they have no conflict of interest.

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Figures

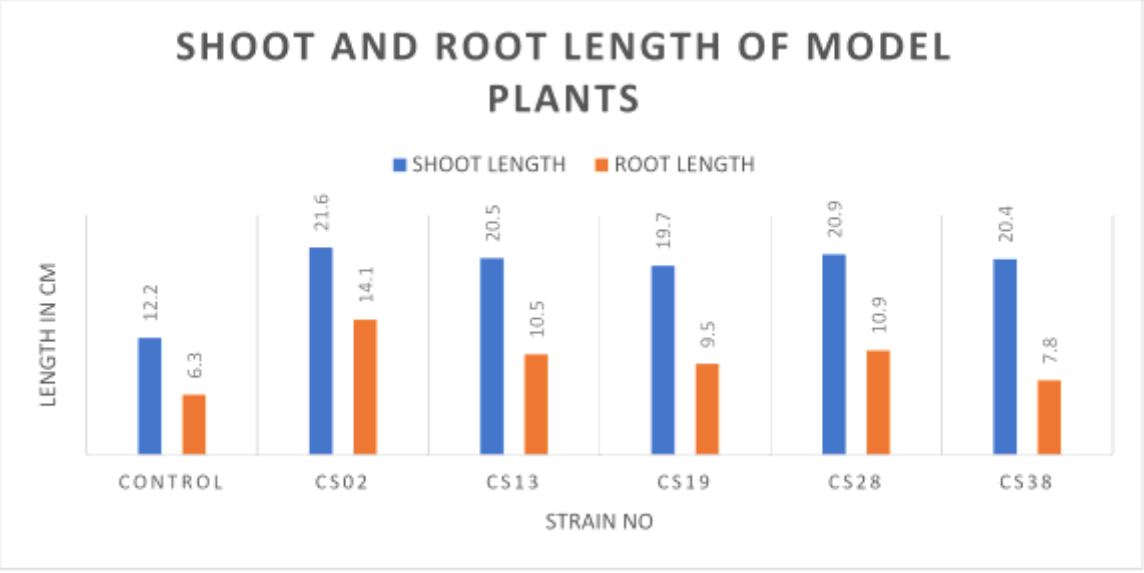


Figure 1

Comparsion of shoot and root length of model plant (Green gram) between control and treated plant.

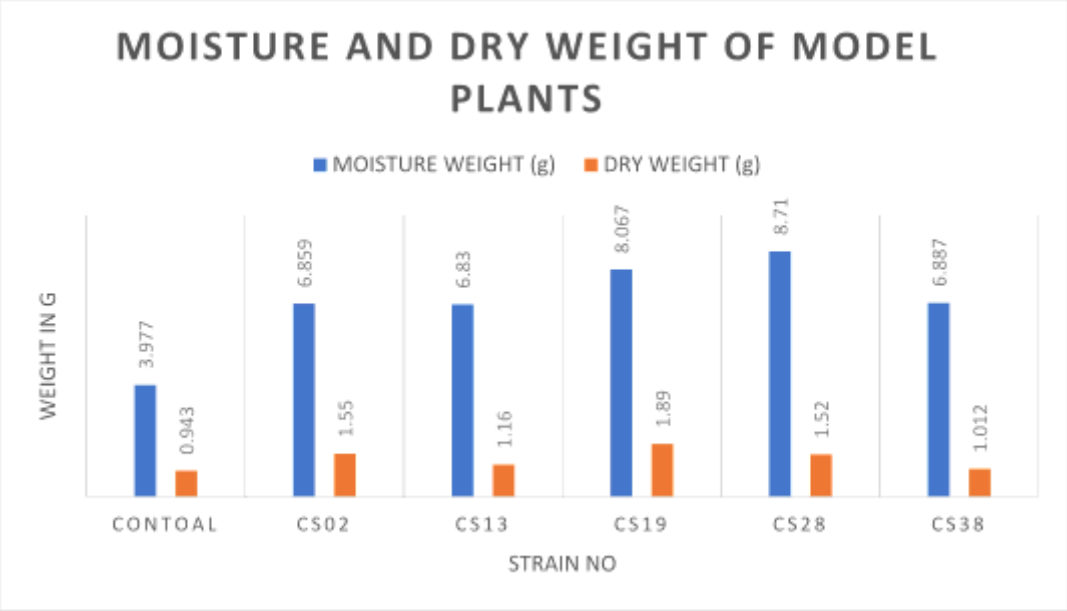


Figure 2

Comparison of moisture and dry weight of model plant (Green gram) between control and treated plant.

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Bacterium strain BS1784 16S ribosomal RNA gene, partial sequence	1605	1605	94%	0.0	93.68%	MK824972.1
Bacterium strain BS1627 16S ribosomal RNA gene, partial sequence	1596	1596	94%	0.0	93.58%	MK824815.1
Bacterium strain BS1625 16S ribosomal RNA gene, partial sequence	1596	1596	94%	0.0	93.58%	MK824813.1
Bacterium strain BS0232 16S ribosomal RNA gene, partial sequence	1596	1596	94%	0.0	93.44%	MK823420.1
Stenotrophomonas maltophilia strain D8 16S ribosomal RNA gene, partial sequence	1596	1596	94%	0.0	93.58%	OM235093.1
[Pseudomonas] geniculata ATCC 19374 = JCM 13324 16S ribosomal RNA gene, partial sequence	1596	1596	94%	0.0	93.44%	MT672503.1
Bacterium strain BS0233 16S ribosomal RNA gene, partial sequence	1594	1594	94%	0.0	93.43%	MK823421.1
Bacterium strain BS0228 16S ribosomal RNA gene, partial sequence	1594	1594	94%	0.0	93.50%	MK823416.1
Stenotrophomonas maltophilia strain R1076 16S ribosomal RNA gene, partial sequence	1594	1594	94%	0.0	93.57%	OP861522.1

Figure 3

BLAST interaction shown similarities with sequences from the database.

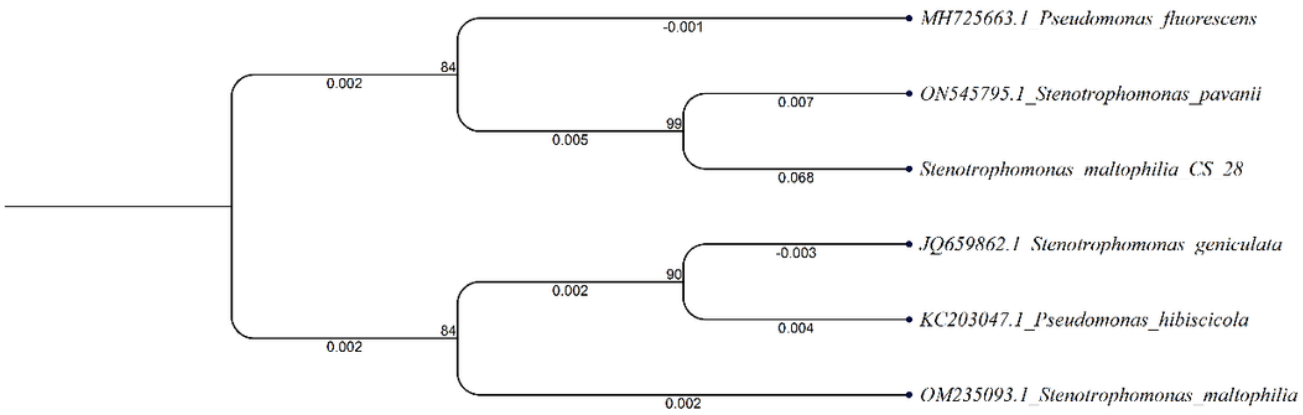


Figure 4

Phylogenetic tree constructed from the data obtained from 16S rRNA Sequencing.