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Research article

Isolation and Molecular Characterization of Endophytic Bacteria from Pigeon Pea along with Antimicrobial Evaluation against *Fusarium udum*

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ABSTRACT

Aims: The aim of this study was to isolate and identify endophytic bacteria from pigeon pea and evaluating their antimicrobial potential against *Fusarium udum*.

Methods and results: Isolation, biochemical and molecular characterization of endophytic bacteria from roots, stems and leaves of pigeon pea were investigated. Total 40 endophytic bacteria were isolated from roots, stems and leaves of pigeon pea. Molecular characterization and identification was done for 18 isolates selected from 40 isolates, by 16SrRNA gene. All identified isolates corresponded to genera *Chryseobacterium endophyticum*(SS1), *Paenibacillus castaneae*(SR1), *Streptomyces sp.* (SR2), *Lactobacillus plantarum* DR1, *Bacillus proteolyticus* DS1, *Pseudomonas sp.* (DS2), *Serratia rubidaea* (CL1), *Klebsiella aerogenes* (CS1), *Paraburkholderia sp.* (CS2), *Burkholderia sp.* (KR1), *Bacillus cereus* (KR2), *Bacillus subtilis* (KS1), *Enterobacter cloacae* (JL1). Antimicrobial potential of selected 18 endophytic isolates evaluated against *Fusarium udum*. 7 endophytic bacterial isolates *Streptomyces* sp. (SR2), *Bacillus proteolyticus* (DS1), *Bacillus subtilis* (KS1), *Bacillus cereus* (KR2), *Bacillus subtilis* (MR1), *Bacillus* sp. (MR2), *Bacillus thuringiensis* (ML1) were showing its antimicrobial potential against *Fusarium udum*.

Conclusion: Pigeon pea plant possesses bacterial endophytes *viz*, *Chryseobacterium endophyticum, Paenibacillus castaneae, Streptomyces sp., Lactobacillus plantarum, Bacillus proteolyticus, Pseudomonas sp., Serratia rubidaea Klebsiella aerogenes, Paraburkholderia sp., Burkholderia sp., Bacillus cereus, Bacillus subtilis, Enterobacter cloacae*. Among these *Streptomyces sp., Bacillus proteolyticus, Bacillus subtilis, Bacillus cereus, Bacillus subtilis, Bacillus sp., Bacillus thuringiensis* showing antimicrobial potential against *Fusarium udum*.

Significance and Impact of the Study: Bacterial endophytes can be useful in agriculture under integrated crop production technology and can be used as biocontrol agents.

Keywords: Endophytic Fungi; Medicinal Plants; AntimicrobialActivity; *Chryseobacterium* endophyticum; Phytostimulation

INTRODUCTION

Plants can be considered as complex micro-ecosystems that provide different habitats to a variety of microorganisms. These habitats are represented by the plant external surfaces as well as internal tissues. [1] Endophytes are bacterial or fungal microorganisms that colonize healthy plant tissue intercellularly and/or intracellularly without causing any apparent symptoms of disease [2]. These microbes enter inside the host plant to avoid environmental stresses, microbial competition and to get nutrients. Association of bacterial endophytes can be obligate or facultative and causes no harm to the host plants. Bacterial endophyte shows complex interactions with their hosts which includes mutualism and antagonism.In order to retain stable symbiosis, endophytes produce several compounds that promote growth of plants and help them adapt better to the environment. Endophytic population differs from plants to plants and from species to species. Within the same species it not only varies

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from region to region but also differs with change in climatic conditions of the same region.Different applications of bacterial endophytes include Phytostimulation in which Endophytes play an important role in the uptake of nutrients. Endophytic bacteria produce a wide range of phytohormones, such as auxins, cytokinins, and gibberellic acids which promotes the growth of the plant. Many commercially important enzymes are produced by several bacterial endophytes. Endophytic bacteria show their promising potential for deployment in biotechnological processes involving production of pectinases, cellulases, xylanases, and proteases. Endophytes are capable of synthesizing bioactive compounds that are used by plants for defence against pathogens and some of these compounds have proven to be useful for novel drug discovery.Endophytic microorganisms are also regarded as an effectual biocontrol agent, substitute to chemical control. These microbes helps the legume and non-legume plants to acquire nutrients by nitrogen fixation, phosphate solubilization [3] or iron chelation [4], by preventing pathogen infections via antifungal or antibacterial agents, by out competing pathogens for nutrients by siderophores production, or by establishing the plants systemic resistance [5]. All this has profound effects on agricultural traits of crop plants which hold promises for eco-friendly and economically sustainable agriculture [6]. Pigeon pea (Cajanus cajan) is an important legume crop belonging to family Fabaceae. India is the main producer of pigeon pea. Over 85% of the world's pigeon pea is produced in India. Pigeon pea has immense adaptability to diverse climates and soils. It is most preferred crop of small holder dry land farmers because it can grow well under subsistence level of agriculture and provides nutritive food, fodder and fuel wood. Pigeon pea improves soil characteristic by fixing atmospheric nitrogen along with fertility status ensuring better growth to succeeding crop that is considered to be an important asset. Nutritionally pigeon pea contains high levels of proteins and important amino acids lysine, methionine and tryptophan. Dry pigeon pea seeds contain protein (20-22%), carbohydrate (57.3%), fat (1.5%) and ash (8.1%). Its protein has two globulins, cajanin and concajanin accounting for 58% and 8% respectively [7].Among the various fungal diseases Fusarium wilt (Fusarium udum Butler) has been reported to be the most destructive all over the world including India. Hence, the present study has been planned to carry out work on pigeon pea.

MATERIALS AND METHODS

Collection of the sample

Plant sample was collected from different location of Madhya Pradesh and Maharashtra mentioned in Table 1.

Table 1: Location of plant sample and date of collection.

S.No.	Location	Plant material	Date of collection
1	Sehore	Stem, Root	12 June 2018
2	Damoh	Root, Stem	19 June 2018
3	Chhindwara	Leaf, Stem	22 June 2018
4	Kundam	Stem, Root	25 June 2018
5	Jabalpur	Leaf, Root, Stem	28 June 2018
6	Maharashtra (Amravati)	Root, Leaf	02 July 2018

Isolation of endophytic bacteria from pigeon pea roots, leave tissue and stems

Surface sterilization: The root, stem and leave sample of the pigeon pea plant were taken and processed separately in triplicate. Healthy and undamaged leave, stem and root were collected from the plant. These were then washed under running water and dried followed by surface sterilization. Subsequently these leaves, stems and pieces of roots were treated with sodium hypochlorite (2.5% Cl-) for 4 min, ethanol for 30 s, and finally 3 rinses in sterile distilled water.

Isolation of endophytic bacteria: The samples of leaves, stems and roots were cut into small pieces and macerated separately in phosphate buffer of pH 7.2 with a sterile pestle and mortar. Tissue extract were then prepared for tenfold serial dilution. For inoculations 0.1 ml of the aliquot was used on TSA medium (pH 7.3). The inoculations were done in triplicates separately for roots, stems and leaf tissue extract. These plates were then incubated at 37°C. Observations were taken after 48 to 72 hrs. Bacterial isolates were picked from plates and purified by streaking techniques on nutrient agar medium and incubated at 37°C (Figure 1).

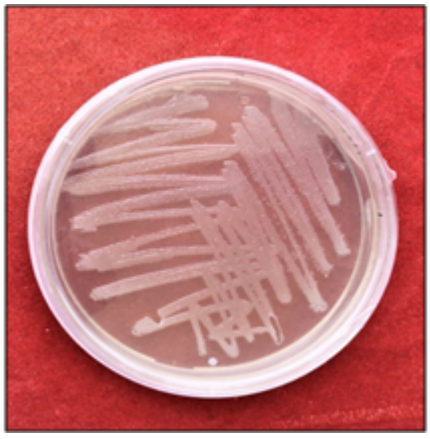

Figure 1: Streak plate purification result of endophytic bacterial isolates.

CHARACTERIZATION OF ENDOPHYTIC BACTERIA

Morphological characterization

KOH test: Gram nature of isolates was tested by KOH string test which was carried out by following standard procedures and techniques.

Physiological characterization

Effect of temperature on growth of isolates: The aim of this study is to identify the thermostable endophytic bacterial isolates. Effect of temperature on growth of isolates was determined by inoculating the isolates in nutrient broth and incubated at different temperature that is at 28°C, 37°C, for 48°C for 48 hours [8].Optical density at 520 nm was measured.

Effect of pH on growth of isolates: Optimization and standardization of growth of the isolates on range of pH was studied by inoculating the isolates on nutrient broth media having pH range 6, 8, and 9. These were then incubated at 37°C for 48 hours [9]. The growth was determined by taking optical density at 520 nm.

Biochemical characterization

Catalase test: Catalase activity of the isolates was detected by transferring the isolated colony on clean glass slide by sterile nicrome wire loop. Afterwards 3% hydrogen peroxide was added on a slide and evolution of oxygen bubbles showed positive catalase activity of the isolates and the absence of gas bubbles showed negative catalase activity.

Amylolytic activity: Amylolytic activity of the isolates was estimated by inoculating the isolates on nutrient agar with 1% starch, which were incubated at 37°C for 24-48 h, (Figure 2) individual plates were flooded with Gram's iodine to produce a deep blue colored starch-iodine complex. The isolates which were showing zone of clearance in starch agar plates were producing amylase.

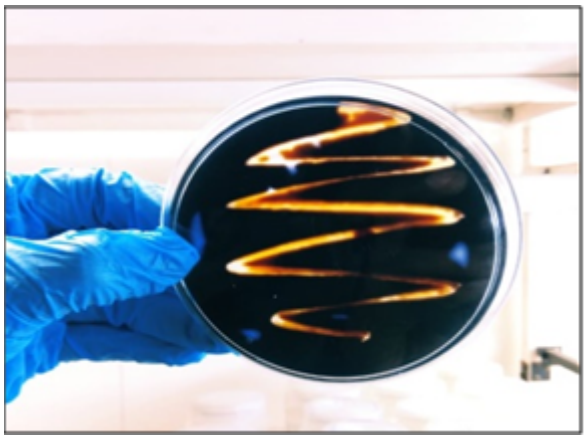

Figure 2: Positive Amylase activity of endophytic isolates.

Urease activity: The isolates were streaked on urea agar slants. The slants were then incubated at 37°C for 28 to 48 hours. Development of pink colouration in the slants was observed for detecting the urease activity of the isolates (Figure 3).

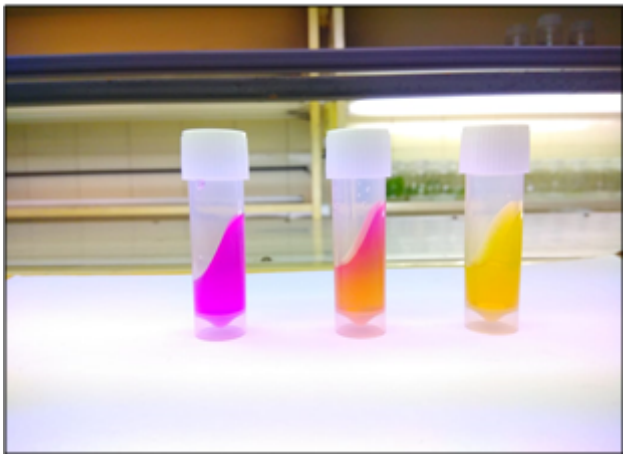

Figure 3: Positive urease activity of endophytic isolates.

Indole acetic acid production by selected isolates: Production of indole acetic acid was detected by a calorimetric method using the Salkowski reagent [10]. The pure bacterial isolates were aseptically inoculated into sterile nutrient broth, and incubated for 72 hours. The cultures were then centrifuged at 12,000 g, for 5 minutes, at 25°C to obtain cell free broth. Few drops (0.5 ml) of Salkowski's reagent were then added to the cell free broth and incubated for thirty minutes at room temperature after which observations on colour change were done (Figure 4).

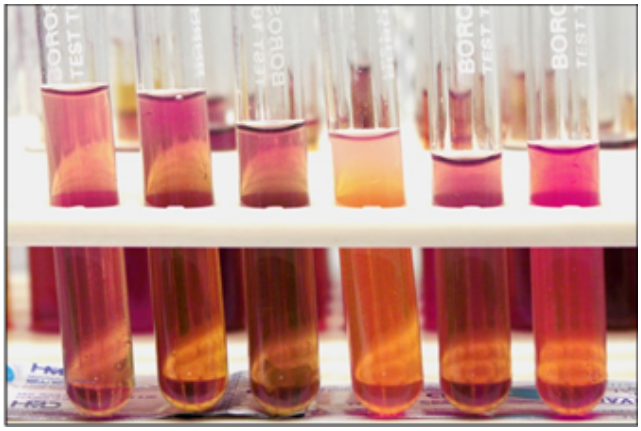

Figure 4: IAA production of by endophytic isolates.

Phosphate solubilization by selected isolates: Phosphate solubilisation was performed for screening phosphate solubilizing microorganisms. Isolates were inoculated at the centre in the form of spot on Pikovskaya's medium (Figure 5). These plates were then incubated at 37°C for 48 to 72 h. Phosphate solubilization was checked in the form of a clear halo zone formed around the colony representing the production of organic acids as a possible mechanism of the phosphate solubilization.

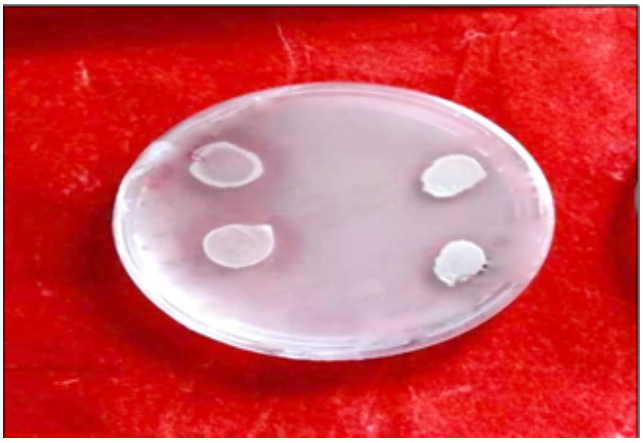

Figure 5: Phosphate solubilization activity of endophytic bacteria.

MOLECULAR CHARACTERIZATION OF ENDOPHYTIC BACTERIAL ISOLATES

Extraction of genomic DNA from bacterial root endophytic isolates

Genomic bacterial DNA isolation was performed by the method described by Wilson [11].

PCR amplification of the isolates

The identification of the bacterial endophyte isolates by the 16S rRNA gene partial sequencing were performed by using the universal primers 27F (5′ AGAGTTTGATYMTGGCTCAG-3′) and 1492R (5′ TACCTTGTTACGACTT-3′; Frank et al., 2008). The amplification were carried out in a 20 µL reaction volume containing 2 µl DNA, 2 µl Taq buffer, 1.4 µl Mgcl2, dNTPs 0.4 µl, Primers 2 µl, Taq DNA Polymerase 0.4 µl, Nuclease free water 11.8 µl. Reaction mixtures were then subjected to the optimized temperature conditions: Initial denaturation of the template at 94°C for 5 minutes, denaturation at 94°C for 30 seconds, annealing at 55°C for 30 sec, elongation at 72°C for 2 minutes and a final elongation at 72°C for 10 minutes. Denaturation, annealing and elongation cycles were repeated for 35 cycles. Amplification products were separated on a 1% (w/v) agarose gel in 1X TAE buffer and visualized by ethidium bromide staining (Figure 6).

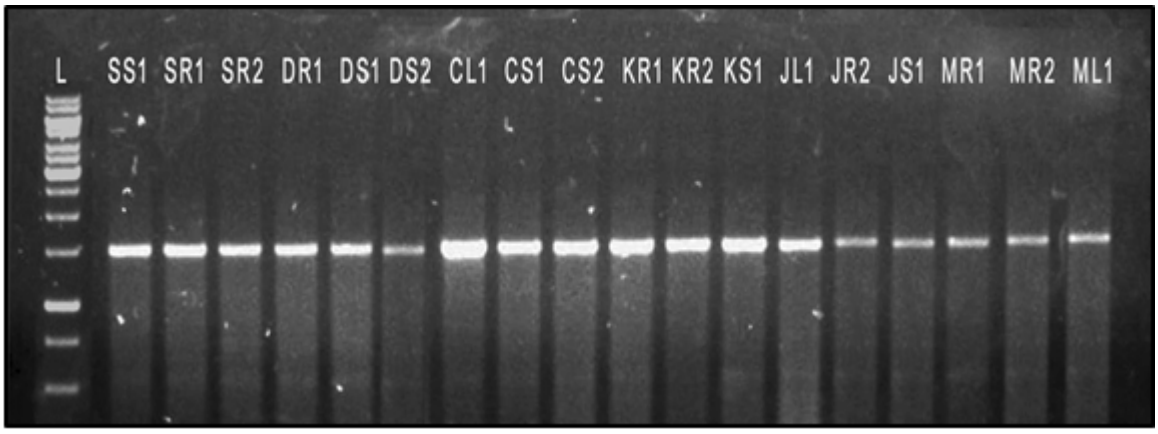

Figure 6: PCR Product was electrophoresis on 1.2% agarose gel. Lane 1: 1Kb ladder, Lane 2-19: indicate PCR amplification by 16S rRNA gene of the selective endophytic bacterial isolates.

DNA sequencing and phylogenetic analysis

Samples for sequencing were sent to AGRIGENOME Pvt. Ltd. 16S rRNA gene sequences obtained from bacterial isolates were analysed using BLASTn tool at the National Centre for Biotechnology Information database (NCBI) GenBank database using the Basic Local Alignment Search Tool (BLAST) analysis tools (http://blast. ncbi.nlm.nih.gov/Blast.cgi) [12]. To identify the most similar 16S rRNA sequences available in the GenBank. Phylogenetic relationship analysis was performed using neighbour joining method with 1000 bootstrap value using DARwin software.

ANTIMICROBIAL ACTIVITY OF ISOLATES AGAINST *Fusarium udum*

Antimicrobial activity of the isolated endophytes was examined by duel culture method. *Fusarium udum* culture was inoculated on PDA (potato dextrose agar) medium. The endophytic bacterial isolates were screened for the ability to inhibit by *Fusarium udum* employing dual culture method [13] on nutrient agar plates. The fungal disc and the endophytic bacteria were inoculated side by side on a petri plate containing solidified nutrient agar medium along with maintaining control plate containing inoculation of fungal disc only. They were incubated at 28°C. Observations were recorded when there was a full growth of bacteria and fungus in the plate (4-7 days) and in control plate (Figures 7 and 8).

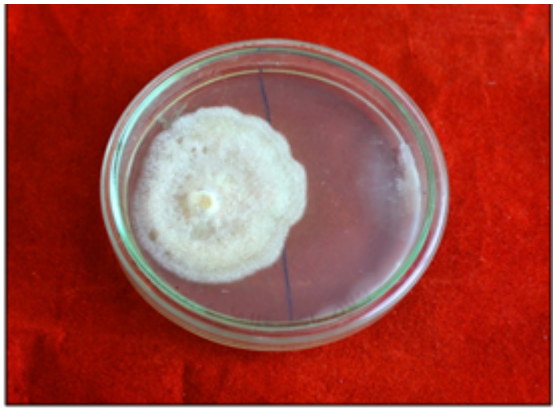

Figure 7: Control plate with fungus Fusarium udum.

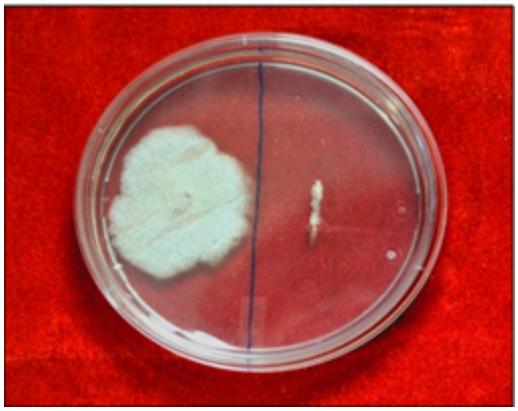

Figure 8: Antimicrobial activity of endophytic bacterial isolate (Bacillus subtilis) against Fusarium udum.

RESULTS

Collection and Isolation of endophytic bacteria from roots, stems, and leaves of pigeon pea

The healthy plant samples of pigeon pea were collected from Sehore, Damoh, Chhindwara, Kundam, and Jabalpur of Madhya Pradesh and from Amravati of Maharashtra. Total 40 strains of endophytic bacteria were isolated after proper surface sterilization from root, stem and leaves portion of pigeon pea plants which are mentioned in Table 2.

Table 2: Nomenclature of endophytic bacterial isolates from Pigeon pea plant.

Location	Plant parts	Nomenclature of endophytes
Sehore	Stem	SS1
		SS2
	Root	SR1
		SR2
		SR3
	Root	DR1
		DR2
Damoh		DR3
	Stem	DS1
		DS2
		DS3
		DS4
		DS5
	Leaf	CL1
Chhindwara	Stem	CS1
		CS2
	Stem	KS1
Kundam	Root	KR1
		KR2
		KR3
		KR4
		KR5
		KR6
		KR7
	Leaf	JL1
Jabalpur		JL2
	Root	JR1
		JR2
		JR3
		JR4
	Stem	JS1
		JS2
		JS3
		JS4
		JS5
	Root	MR1
Maharashtra(Amravati)		MR2

	Leaf	ML1
		ML2
		ML3

Biochemical and molecular characterization of isolates

The preliminary identification of the bacterial isolates was done based on morphological and biochemical characteristics.

Morphological characterization and physiological characterization

Gram nature of endophytic bacteria were detected in which out of 40 isolates 25 of the endophytic bacterial isolates were gram negative while 15 were gram positive.The results obtained in this regard have been recorded in Table 3.

Table 3: Morphological and biochemical characterization of selected bacterial endophytes.

Sample	KOH	Strach test	Catalase test	PSB	Urease test	IAA test
CODE	TEST			TEST		
SEHORE						
SS1	-	+	+	+	-	+
SS2	+	+	+	+	-	-
SR1	-	+	+	+	+	+
SR2	+	+	+	-	+	+
SR3	+	+	+	+	-	-
DAMOH						
DR1	+	+	-	+	-	-
DR2	+	+	+	+	-	-
DR3	-	+	-	-	+	-
DS1	+	+	+	+	-	-
DS2	-	+	+	+	-	-
DS3	-	+	+	+	-	-
DS4	-	-	+	+	-	-
DS5	+	+	-	-	+	-
CHHINDWARA						
CL1	-	-	+	+	-	-
CS1	-	+	+	-	-	-
CS2	-	-	+	+	-	+
KUNDAM						
KS1	+	+	+	+	-	+
KR1	-	+	+	+	+	+
KR2	+	+	+	+	+	+
KR3	-	+	+	+	-	-
KR4	-	+	+	+	-	-
KR5	+	+	+	+	-	-
KR6	+	+	+	+	-	-
KR7	+	+	+	+	-	-
JABALPUR						
JL1	-	+	+	+	-	+
JL2	+	+	+	-	-	-
JR1	-	-	+	+	+	+
JR2	-	-	+	+	-	-
JR3	-	-	+	+	-	-
JR4	-	-	+	+	-	-
JS1	-	-	+	+	-	+
JS2	+	+	+	-	-	-
JS3	-	+	+	-	-	-
JS4	+	-	+	+	-	-
JS5	-	-	+	-	-	-
MAHARASHTRA (Amravati)						
MR1	-	+	+	-	+	+
MR2	-	+	+	-	+	+

ML1	-	+	+	-	-	+
ML2	-	-	+	+	+	-
ML3	-	-	+	+	+	-

Effect of temperature on growth of isolates

The total 40 isolates showing moderate growth at 28°C, good growth at 37°C and less growth at 48°C. The temperature growth curves of the isolates were shown in the line graph (Figure 9A-Figure 9F).

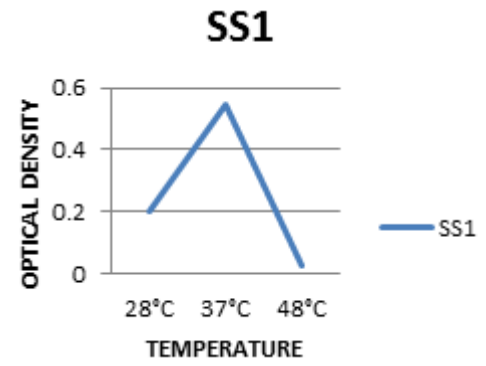

Figure 9A: Effect of temperature on isolate SS1.

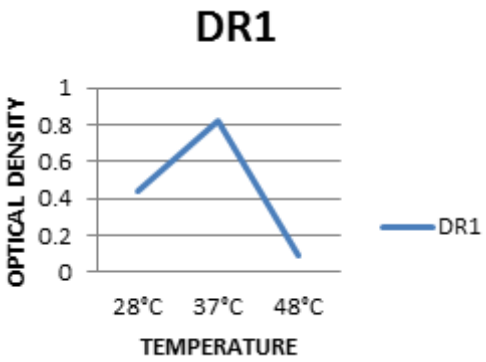

Figure 9B: Effect of temperature on isolate DR1.

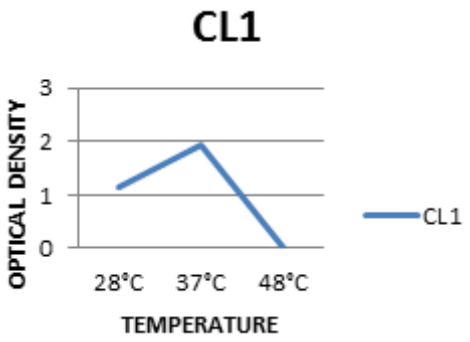

Figure 9C: Effect of temperature on isolate CL1.

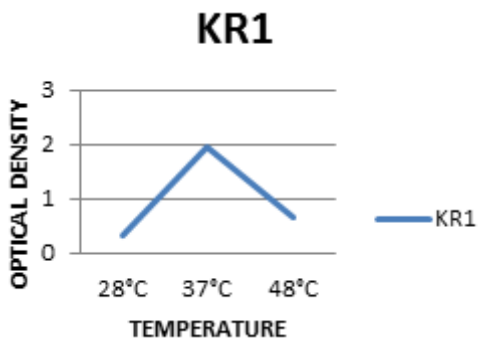

Figure9D: Effect of temperature on isolate KR1.

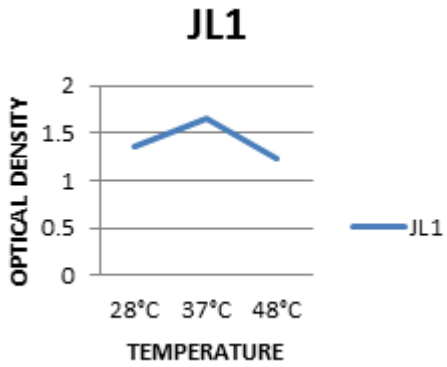

Figure 9E: Effect of temperature on isolate JL1.

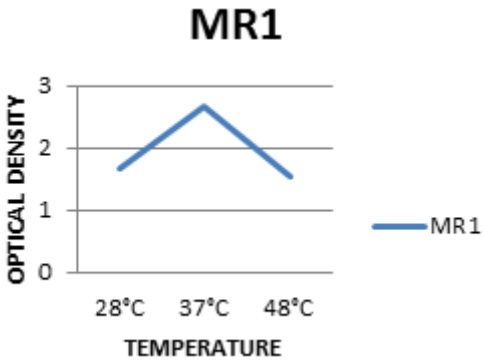

Figure 9F: Effect of temperature on isolate MR1.

Effect of pH on growth of isolates

The total 40 isolates were showing optimum growth between pH 6 to 8, and poor growth at pH 9. The growth of isolates in varying range of pH was shown in line graph (Figure 10A – Figure 10F).

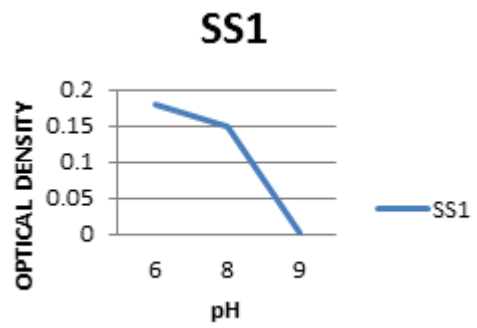

Figure 10A: Effect of pH on isolate SS1.

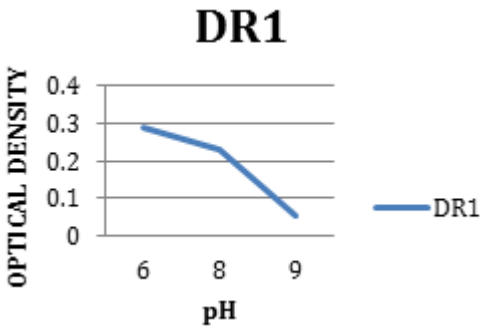

Figure 10B: Effect of pH on isolate DR1.

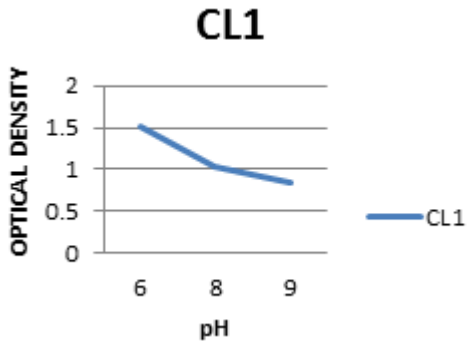

Figure 10C: Effect of pH on isolate CL1.

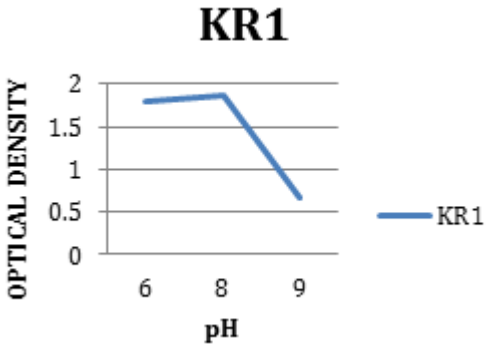

Figure 10D: Effect of pH on isolate KR11.

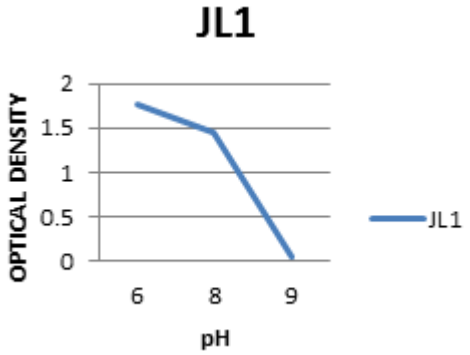

Figure 10E: Effect of pH on isolate JL1.

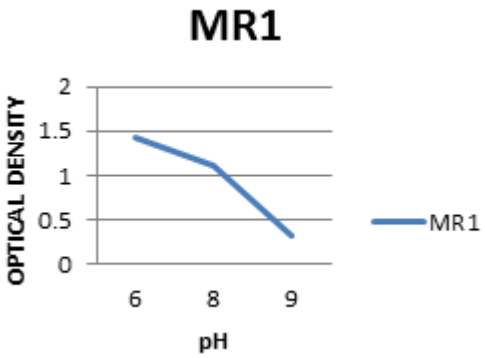

Figure 10F: Effect of pH on isolate MR1.

Biochemical characterization

The biochemical analysis results are summarized in Table 3. The result shows that out 40 isolates 37 endophytic bacterial isolates were showing the positive catalase activity and only 3 endophytic isolates DR1, DR3, DS5 were showing negative catalase activity. 28 isolates were producing amylase while 12 isolates were not producing amylase, 11 isolates showed positive urease activity while the 29 were showing negative urease test. Further results showed that 13 bacterial isolates were able to produce IAA while the other 27 isolates did not produced IAA. It was observed that 29 isolates were showing phosphate solubilization activity and 11 were not performing the phosphate solubilization activity.

Molecular characterization of endophytic isolates

Isolation of Genomic DNA was done for 18 isolates, selected 3 isolates from each location following the protocol described by Wilson [11].

PCR amplification of the isolates

PCR amplified products expectedly ranged at (1500 bp) amplicon using primer27F and 1492R. Total 18 sequences were subjected to online BLASTn in NCBI (www.ncbi.nlm.nih.gov/blast/) for analysis to identify the species of different bacterial isolates.

Phylogenetic analysis of PCR product

The analysis of 16SrRNA gene sequence showed the evolutionary relationship of bacterial strain with the respective

genera mentioned in Table 4. Isolate SS1 phylogenetically related to *Chryseobacterium endophyticum*, SR1 related to *Paenibacillus castaneae*, SR2 related to *Streptomyces sp.*, DR1 related to *Lactobacillus plantarum*, DS1 related to *Bacillus proteolyticus*, DS2 related to *Pseudomonas sp.*, CL1 related to *Serratia rubidaea*, CS1 related to *Klebsiella aerogenes*, CS2 related to *Paraburkholderia sp.*, KR1 related to *Burkholderia sp.*, KR2 related to Bacillus cereus, KS1 related to *Bacillus subtilis*, JL1 related to Enterobacter cloacae, JR1 related to *Enterobacter sp.*, JS1 related to *Arthrobacter sp.*, MR1 related to *Bacillus subtilis*, MR2 related to *Bacillus* sp., ML1 related to *Bacillus thuringiensis* (Figure 11).

Table 4: Identification of endophytic bacterial isolates by 16SrRNA.

Sr. No.	Sample code	Species identified	Length of sequence (bp)	Per. Identity
				100%
1	SS1	*Chryseobacterium endophyticum*	1458	
				100%
2	SR1	*Paenibacillus castaneae*	1483	
				100%
3	SR2	*Streptomyces sp.*	1410	
				100%
4	DR1	*Lactobacillus plantarum*	1500	
				100%
5	DS1	*Bacillus proteolyticus*	1326	
				100%
6	DS2	*Pseudomonas sp.*	1396	
				100%
7	CL1	*Serratia rubidaea*	1408	
				100%
8	CS1	*Klebsiella aerogenes*	1496	
				100%
9	CS2	*Paraburkholderia sp.*	1385	
				100%
10	KR1	*Burkholderia sp.*	1396	
				99.54%
11	KR2	*Bacillus cereus*	1432	
				100%
12	KS1	*Bacillus subtilis*	1499	
				100%

13	JL1	*Enterobacter cloacae*	1215	
				100%
14	JR1	*Enterobacter sp.*	1441	
				100%
15	JS1	*Arthrobacter sp.*	1440	
				100%
16	MR1	*Bacillus subtilis*	1457	
				100%
17	MR2	*Bacillus sp.*	921	
				100%
18	ML1	*Bacillus thuringiensis*	1389	

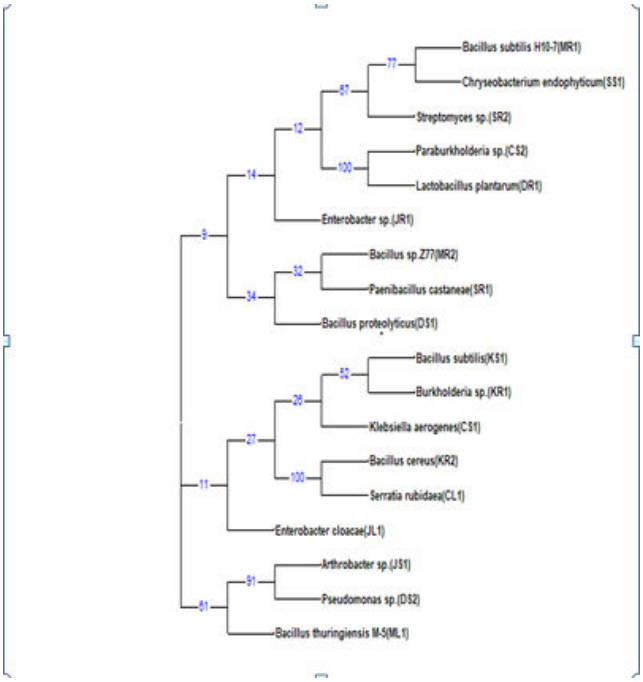

Figure 11: Phylogenetic analysis depicting the relation between the 16S rRNA sequence of isolates of endophytic bacteriaAntimicrobial activity of isolates against *Fusarium udum*.

Selected 18 bacterial endophytic strains were displayed for the antimicrobial activity against *Fusarium udum*. Among the 18 isolated bacteria 7 were (SR2, DS1, KS1, KR2, MR1, MR2, ML1) showing visible zone of inhibition on nutrient agar medium confirming its antimicrobial potential against *Fusarium udum*.

DISCUSSION

Successful isolation of endophytic bacteria from pigeon pea was done from different location of Madhya Pradesh (Sihore, Damoh, Chindwada, Kundam, and Jabalpur) and Maharashtra (Amravati). Total 40 endophytic bacterial strains were isolated from root, stem and leaf of pigeon pea. Narula et al. [14] isolated 60 endophytic bacteria from nodules of field pea following the same isolation protocol.

Characterization of endophytic bacteria

Gram positive and gram negative nature of endophytic bacteria were identified in which out of 40 isolates 25 of the endophytic bacterial isolates were gram negative while 15 were gram positive. Mbai et al. [15] also isolated 46 Gram negative endophytic bacteria and 26 Gram positive bacteria from Kenyan Basmati Rice.

Physiological characterization

Physiological characterization based on temperature was done, to evaluate whether the endophytic bacteria showing thermostable nature. All the 40 isolates showing moderate growth at 28°C, good growth 37°C, and less growth at 48°C. Optimum temperature for almost all the isolate was predicted 37°C and no thermostable endophytic bacteria were detected.

Another physiological characterization based on pH was done, isolates were showing optimum growth between pH range 6 to 8 and the growth of isolates abruptly declines at pH 9. Gupta et al., [16] obtained similar results during the study of isolation of endophytic bacteria from *Prosopis cineraria* plant from root and leaf tissue. In physiological characterization they found the optimum growth of the isolates at pH 7 and optimum temperature 37°C and thus confirm the findings.

Biochemical characterization

Catalase activity is an important aspect required by the bacteria to reproduce by avoiding cellular toxicity. 37 endophytic bacterial isolates were showing the positive catalase activity and

only 3 endophytic isolates DR1, DR3, DS5 were showing negative catalase activity

Amylases are among the most important enzymes. Starch degrading bacteria are most important for industries such as food, fermentation, textile and paper. 28 were producing amylase, whereas 12 isolates were not producing amylase. El-Deeb et al., [17] similarly isolated endophytic bacteria from *P. tenuiflorus* and were evaluated for the amylolytic activity, result obtained showed isolates *Bacillus sp., B. licheniformis, B. megaterium, B. pumilus, and Pseudomonas sp.* we're showing positive amylase activity.

The urease test was performed to find out the ability of the isolates to break down urea in simple forms of nitrogen which can be quickly absorbed by the plants. 11 isolates have potential to break down urea in simple form which can be readily available to the plant and 29 were showing negative results for urease test. Gupta et al., [16] also reported high urease activity of *Bacillus subtilis* strain isolated from roots of *Prosopis cineraria*.

Endophytes promote and stimulate plant growth by production of Phytohormones such as IAA. Isolates were screened for IAA production. Results showed that 13 of the isolates (SS1, SR1, SR2, CS2, KS1, KR1, KR2, JL1, JR1, JS1, MR1, MR2, and ML1) were able to produce IAA and therefore have the potential to promote plant growth. Similarly in a study on characterisation of bacterial root endophytes with potential to enhance plant growth from Kenyan basmati rice tested endophytic bacteria for IAA production, it was found that ten isolates (14%) were able to produce IAA while the other sixty three (86%) did not show IAA production[15].

Phosphate solubilizing microorganisms grow in media with tricalcium phosphate or similar insoluble materials as the only phosphate source. Microorganisms not only assimilate the element but also solubilize quantities in excess of their nutritional demands, thereby making it available for plants [18]. Phosphate solubilisation for the isolated endophytic bacteria during this characterization process was demonstrated. 29 endophytic bacteria were assayed positive for this test. Similar findings obtained by Gupta et al., [19] isolated endophytic bacteria (e.g. *Bacillus sp.and pseudomonas fluorescens*) from Carex kobomugi roots, which exhibited inorganic phosphate solubilization to increase P uptake.

Molecular characterization of bacterial endophytes

Molecular characterization of 18 endophytic isolates and PCR amplification by using 16S rRNA was done. The 16S rRNA gene nucleotide sequences provide bacterium species specific signature and hence, 16S rRNA gene sequence based bacterial identification is considered as a precise method of bacterial identification [20]. Thus, this method has been used for the rapid and accurate identification of endophytic bacterial isolates. Genetic diversity was observed among the 18 endophytic isolates upon PCR products. The PCR product was sequenced through outsourcing. The resulted sequences were further aligned with the available sequences in NCBI using BLAST programme.

BLAST search of the sequences revealed the isolates DS1, KR2, KS1, MR1, MR2, and ML1 shows 98 to 100% similarity with family bacillaceae. Whereas JL1, JR1, CL1, CS1 with family Enterobacteriaceae, KR1, CS2 with Burkholderiaceae, DS1 with family Pseudomonadaceae, SR1 with family Penibacillaceae, SS1 with Flavobacteriaceae, SR2 with family Actinomycetaceae, DR1 with family Lactobacillaceae, JS1 with family Micrococcaceae.

El-Deeb et al. [17] also isolated and characterized endophytic bacteria Found from *Plectranthus tenuiflorus*. Among 28 endophytic bacterial isolates, 8 isolates were identified by sequencing of their 16S rRNA gene. The isolated endophytic bacteria were identified as *Bacillus sp., Bacillus megaterium, Bacillus pumilus, Bacillus licheniformis, Micrococcus luteus, Paenibacillus sp., Pseudomonas sp., and Acinetobacter calcoaceticus.*

Antimicrobial activity of isolates against *Fusarium udum*

Out of total 40 isolated strains 18 bacterial endophytic strains were displayed for the antimicrobial activity against *Fusarium udum*. Among the 18 isolated bacteria 7 isolates were able to supress the growth of *Fusarium udum* which was checked by duel culture method. Thus *Streptomyces sp.* (SR2), *Bacillus proteolyticus* (DS1), *Bacillus subtilis* (KS1), *Bacillus cereus* (KR2), *Bacillus subtilis* (MR1), *Bacillus sp.* (MR2), *Bacillus thuringiensis* (ML1) were found to have antimicrobial potential against *Fusarium udum*. Cho et al. [21] also examined 13 isolates belonging to low G+C Gram-positive bacteria (LGCGPB) and Proteobacteria, were assessed for their antifungal activity against phytopathogenic fungi such as *Rhizoctonia solani*. Among them, they found similar result as *Paenibacillus polymyxa* GS01, *Bacillus sp.* GS07, and *Pseudomonas poae* JA01 show potential activity as biocontrol agents against phytopathogenic fungi.

CONCLUSIONS

The present study aimed to isolate 40 endophytic bacteria from root, stem and leaves of pigeon pea plant collected from different location of Madhya Pradesh and Maharashtra.

Morphological and biochemical characterization of 40 bacterial isolates was done. Under morphological characterization 25 endophytic bacteria were showing gram negative nature and 15 endophytic bacteria showing gram positive nature. Physiological characterization based on varying rang of temperature and pH was performed, all the 40 the isolates showed good growth at 38°C and pH rang 6 to 8. Biochemical characterization of all the 40 isolates performed in which, 37 isolates showed catalase activity, 28 showed amylase activity, 11 showed urease activity, 13 showed IAA activity, 29 showed phosphate solubilization activity.

Molecular characterization and identification was done for 18 isolates selected from 40 isolates, by 16SrRNA gene. BLAST analysis revealed sequence similarity of 99 to 100% with the sequences in database of NCBI GenBank. All identified isolates corresponded to genera *Chryseobacterium endophyticum*(SS1), *Paenibacillus castaneae*(SR1), *Streptomyces sp.* (SR2), *Lactobacillus plantarum* DR1, *Bacillus proteolyticus* DS1, *Pseudomonas sp.* (DS2), *Serratia rubidaea* (CL1), *Klebsiella aerogenes* (CS1), *Paraburkholderia sp.* (CS2), *Burkholderia sp.* (KR1), *Bacillus cereus* (KR2), *Bacillus*

subtilis (KS1), *Enterobacter cloacae* (JL1), *Enterobacter sp*. (JR1), *Arthrobacter sp*. (JS1), *Bacillus subtilis* (MR1), *Bacillus sp*. (MR2), *Bacillus thuringiensis* (ML1).

Antimicrobial potential of selected 18 endophytic isolates were evaluated against *Fusarium udum*. 7 endophytic bacterial isolates *viz Streptomyces sp*. (SR2), *Bacillus proteolyticus* (DS1), *Bacillus subtilis* (KS1), *Bacillus cereus* (KR2), *Bacillus subtilis* (MR1), *Bacillus sp*. (MR2), *Bacillus thuringiensis* (ML1) were showing its antimicrobial potential against *Fusarium udum*.

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