Screening of Enophytic Bacteria From Chilli (*Capsicum Annum L*) For Growth Promotion And Antagonistic Properties

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Abstract- *Endophytes are microorganisms that spend at least part of their life cycle inside plants without causing apparent disease. They have both plant growth promotion as well as antagonistic activities against phytopathogens. The present study mainly focuses on isolating and identifying best endophytic bacterial isolates from chilli plant (Capsicum annuum L) collected from five different locations of Thrissur and Palakkad districts of Kerala. A total of 67 bacterial isolates were isolated from root, stem and leaves of the plant on four different media namely, Nutrient agar medium, Pikovskaya's agar medium, Jensens's agar medium and Aleksandrow's agar medium. All the 67 isolates were screened for plant growth promotion and antagonistic properties under in vitro. The plant growth promoting activities included nitrogen fixation, phosphate solubilisation, potassium solubilisation and IAA production. Ammonia production, hydrogen cyanide production and siderophore production were carried out for antagonistic properties. Out of 67 endophytic bacterial isolates three bacterial isolates were selected as most promising endophytes, KPR, PJS and CPL. These isolates were characterized morphologically, culturally, biochemically and finally at molecular level. Based on the morphological and biochemical characters, PJS was tentatively identified as Acenetobacter sp. The isolate KPR was identified as Enterobacter hormaeche and CPL as Bacillus subtilis using 16S rRNA sequencing. In this study,Enterobacter hormaeche was found to be more efficient endophyte.*

Keywords- Endophytic bacteria, Enterobacter hormaeche, Acenetobacter sp, Bacillus subtilis, 16S rRNA sequencing

I. INTRODUCTION

Endophytes are microorganisms that spend at least part of their life cycle inside plants without causing apparent disease. Endophytes are ubiquitous and have been found in all plant species studied to date. However most of the endophyte/plant relationships are not well understood. Endophytes were first described by French botanist Heinrich Friedrich (1809). He used the term *endophytae* to describe a partly parasitic fungi living in plants. It was Victor Galippe who (1887) discovered bacteria normally occurring inside plant tissues.

Hallmann and co-workers (1997) stated that "endophytes are those that can be isolated from surface-disinfected plant tissue or extracted from within the plant and that do not visibly harm the plant. Currently endophytes are described as any microbe that can be isolated from asymptomatic plant tissue which includes beneficial, commensal, pathogens and neutral microorganisms (Hardoim *et al*., 2015). Within the hosts, endophytes inhabit all available tissue including petioles, stems, wigs, bark, root, fruit, flower and seeds. Endophytic bacteria colonize a large number of plants, which also include plant growth promoting bacteria. They have both plant growth promotion as well as antagonistic activities against phytopathogens. Plant growth promotions by these bacteria are facilitated *via* three interrelated mechanisms: phytostimulation, biofertilization and biocontrol. Phytostimulation includes ACC (1- aminocyclopropane – 1 – carboxylate) utilization and indole acetic acid production, biofertization includes nitrogen fixation, phosphate solubilisation, potassium solubilisation, and biocontrol includes siderophore production, hydrogen cyanide production and ammonia production. The most commonly found genera of bacterial endophytes are *Pseudomonas, Bacillus, Burkholderia, Strenotrophomonas, Micrococcus, Pantoea, Microbacterium etc.* (Chaturvedi, Singh and Gupta, 2016).

Indole acetic acid is the most abundant phytohormone belonging to auxin family which can promote plant growth by producing longer roots and also by increasing the density for more nutrient uptake. Endophytic bacteria promote plant growth significantly by the production of phytohormones. It also acts as biofertilizres by nitrogen fixation, phosphate solubilisation and potassium solubilisation. These are essential elements for plants and their deficiency depletes the plant growth. Endophytic bacteria can make these minerals into a soluble form foe easy accession to plants and

thus helping their growth. Examples of these endophytic bacteria include *Pseudomonas, Bacillus, Klebsiella, Serratia, Azoarcus, Rhizobium* etc. Endophytes can also protect the plants from phytopathogens by producing siderophore, hydrogen cyanide, ammonia etc. and thus indirectly helps plant growth. Through siderophore production it makes the unavailable form of iron to a soluble form to plants and also by removing iron from phytopathogens. (Priyanka Batra *et al.*, 2018). Hydrogen cyanide and ammonia is considered as a biocontrol based on its toxicity against phytopathogens.

Chilli plant is an essential and one of the most valuable spice crops that is grown all over the world. It is the second most important vegetable crop after tomato which estimated to be grown over 1.7 million hectares worldwide. It belongs to the family Solanaceae and genus *Capsicum*. It is economically important and valuable cash crop that is produced and consumed fresh and processed. India is the largest producer, consumer and exporter of chilli where, most of the cultivated varieties belong to the species *Capsicum annuum L*. Chilly grows best in loam or silt loam soil with good water holding capacity and requires a soil pH between 5.5 and 6.8. In Kerala chilly is grown in an area of 820 ha with production of 1192 tones. Reports shows that the current productivity levels of chilli are far below the satisfactory level to meet domestic demands. Optimum and balanced fertilization is the key for sustained higher production. (Priya, 2009). There are various diseases associated with chilly caused by bacteria, fungus, and viruses. Although there are several chemical treatments, biological control is ecologically safer. The tendency of bacterial endophytes for antagonism and growth promotion will be able to increase the productivity of crop.

The present study was undertaken with an objective to identify promising endophytic bacteria for growth promotion and disease management in chilli for the benefit of Kerala farmers.

II. MATERIALS AND METHODOLOGY

2.1 Isolation of endophytic bacteria

Endophytic bacteria were isolated from five chilli plants collected from different areas of Palakkad district (Kollengode, Malampuzha, Cherpulasseri) and Thrissur district (Puthur and Vellanikkara. Young plants (less than two weeks) and old plants (more than two weeks) were obtained from the above mentioned areas. The whole plant was uprooted manually and brought to the laboratory in a polythene bag. The samples were washed under running tap water to remove the excess soil. Root and stem were made into segments of 1-2 cm length using a sterilized scalpel. Leaf segment was made as $1cm^2$ area using sterilized scalpel.

The samples were surface sterilised by first immersing in 70% ethanol for 30 seconds followed by second immersion in sodium hypochlorite (1%) for three minutes (Anjum and Chandra, 2015). They were rinsed six times with sterile distilled water and dried on sterile blotting sheets under laminar air flow. The surface sterilized tissue segments were kept in Petri plates containing nutrient agar medium, Pikovskaya's agar medium, Jensen's agar medium and Aleksandrow's agar medium. Two segments were kept per plate. The effectiveness of surface sterilization was checked by spreading 0.1ml sterile water from final wash on agar plate and observing for any growth. It served as control and was kept for each media. All the plates were wrapped using parafilm and kept for incubation at 28°C for 1-2 days for nutrient agar media and 4-5 days for other media respectively. Isolated bacterial colonies were purified on the respective media. The colonies were maintained on agar slants for further use.

2.2 SCREENING OF ENDOPHYTIC BACTERIAL ISOLATES FOR GROWTH PROMOTION ACTIVITIES UNDER *IN VITRO*

2.2.1 IAA production

The bacterial isolates were inoculated in sterile Luria-Bertani broth supplemented with tryptophan at the rate of 1mg/ml. (Ahmed *et al.*, 2008). The tubes were inoculated in dark for seven days. After incubation, the cultures were centrifuged at 3000 rpm for 30 minutes and the supernatant was collected. To the supernatant 4 ml Salkowski reagent (2% 0.5 M $FeCl_3$ solution in 35% perchloric acid) was added. The development of pink colour indicated the production of IAA.

2.2.2 Estimation of IAA production

The tubes that showed pink colouration were used for quantification by measuring the optical density at 540 nm using a spectrophotometer (Ahmed *et al.*, 2008). The OD values were plotted on a standard graph to obtain the quantity of the IAA produced by the isolates per unit volume of broth.

2.2.3 Screening for nitrogen fixation

The bacterial colonies obtained in Jensen's agar medium (Appendix) was carefully isolated and streaked in the same media and incubated at 28°C for 4-5 days and observed for the bacterial growth.

2.2.4 Screening for phosphate solubilisation activity

All the bacterial isolates grown in four media were screened for their ability to solubilise phosphate in Pikovskaya's agar medium (Appendix). The cultures were spot inoculated on Pikovskaya's agar plates and incubated at room temperature for 5-7 days for the presence of clear halo zone around the colony (Devendra singh *et al.,* 2017). The diameter of the halo zone was measured using formula: Total diameter (colony + halo zone) – colony diameter.

2.2.5 Screening for potassium solubilisation activity

All the bacterial isolates grown in four media were screened for their ability to solubilise potassium in Aleksandrow's agar medium (Appendix). The cultures were spot inoculated on Aleksandrow's agar plates and incubated at room temperature for 5-7 days for the presence of clear halo zone around the colony (Anukriti *et al*., 2016).

2.3 SCREENING OF ENDOPHYTIC BACTERIAL ISOLATES FOR ANTAGONISTIC ACTIVITIES UNDER *IN VITRO.*

2.3.1 Screening for HCN production

The isolates were screened for their ability to produce hydrogen cyanide using the method described by Loeck (1948). Nutrient agar was supplemented with glycine at the rate of 4.4g/L. The bacterial isolates were streaked on the media and a Whatman No.1 filter paper dipped in picric acid solution (2% sodium carbonate in 0.5% picric acid) was kept in the upper portion of the Petri plates. The plates were sealed and incubated at 28°C for 5 days. The production of HCN was indicated by the formation of orange to red colour in the Whatman No.1 filter paper placed in the top of the Petri plates.

2.3.2 Screening for ammonia production

Screening of bacterial isolates for ammonia production was carried out by inoculating the isolates in 4 per cent peptone water and incubating at 28°C for 48 hours. After incubation, 0.5 ml of Nessler's reagent was added to the tubes. Formation of brown to yellow colour indicated the presence of ammonia. (Devendra singh, 2017).

2.3.3 Screening for siderophore production

The screening of bacterial isolates for siderophore production was done by Chrome Azurol Sulfonate (CAS) assay (Ajith Kumar *et al.,* 2015). The assay medium was prepared by dissolving 60.5 mg CAS in 50 ml distilled water, which was then mixed with Iron (III) solution (1Mm $FeCl_3.6H_2O$ in 10Mm HCl). The solution was then slowly added to 72.9 g hexadecyltrimethyl ammonium bromide (HDTMA) dissolved in 40 ml distilled water. The blue green solution obtained was mixed with 100 ml nutrient agar, which was then used for screening. The bacterial isolates were spot-inoculated on the media and incubated at 30°C for 5-7 days. The presence of yellow to orange halo around the colony indicates the siderophore production by isolates.

2.4MORPHOLOGICAL, CULTURAL AND BIOCHEMICAL CHARACTERIZATION OF THE SELECTED ENDOPHYTIC BACTERIAL ISOLATES

After the evaluation of endophytic bacteria for plant growth promotion and antagonistic activities, the three best isolates (KPR, PJS, and CPL) were selected and characterized.

2.4.1 Morphological studies

Morphological studies such as shape, elevation, texture, margin, colour, size, of colonies of endophytic bacteria were recorded on nutrient agar medium incubated at 28°C for 2-3 days.

2.4.1.1 Gram's reaction

Gram's staining was done by preparing a heat fixed smear of the endophytic bacterial culure in a clean slide followed by the addition of the stains and washing with tap water. (Cuppuccino and Sherman, 1992). The slide was dried and observed under microscope.

2.4.1.2 Endospore staining

For this the endophytic bacterial smear was prepared and heat fixed. It was flooded with primary stain – malachite green which was then heated using a burner (Cuppuccino and Sherman, 1992). As the stain dried up more stain was added and continued up to 5- 10 min followed by washing with tap water. It was then flooded with counter stain – safranin for 1 min and washed. The dried slide was observed under oil immersion.

2.4.2 Motility test

The bacterial cultures were tested for motility using semi-solid agar tube method. (Cuppuccino and Sherman, 1992). For this, semi- solid media (nutrient broth with 0.7% agar) was prepared and dispensed in tubes and autoclaved. 48 hr old endophytic bacterial cultures were stabbed into the media and incubated at 28°C for 24-48 hrs.

2.4.3 Biochemical tests

2.4.3.1 Catalase test

A drop of 3% H_2O_2 was taken on glass slide. Small amount of endophytic bacterial culture was mixed with inoculating needle (Cuppuccino and Sherman, 1992). Rapid and sustained production of gas bubbles or effervescence indicated positive result.

2.4.3.2 Citrate utilization test

A well isolated colony was streaked on the surface of sterilized Simmon's citrate agar slants and incubated at 28°C for 24-48 hours (Cuppuccino and Sherman, 1992). A positive result was indicated by a change in the colour of media from green to pressian blue.

2.4.3.3 Nitrate reduction test

Nitrate broth was prepared and autoclaved. 5 ml of nitrate broth was inoculated with 48 hr old culture and incubated at 28°C for 24-48 hr. After incubation 0.5 ml of reagent A (sulfanilic acid) and reagent B (alpha naphthylamine) was added. (Cuppuccino and Sherman, 1992). The devepment of red colour was considered as positive. Sometimes the red colour was formed after adding a pinch of zinc dust followed by reagent A and B.

2.4.3.4 Hydrogen sulphide production

For this test, triple sugar iron agar was prepared and agar slants are made. The 48 hr old cultures was inoculated into agar slants and incubated for 24-48 hr at 28°C (Cuppuccino and Sherman, 1992). The presence of black precipitate at the bottom of the tube was considered as positive.

2.4.3.5 Starch hydrolysis

Endophytic bacterial culture was streaked on Petri plates containing Starch agar (nutrient agar with 0.3% starch) and incubated at 28°C for 2-3 days (Cuppuccino and Sherman, 1992). The formation of clear zone around the colony after the addition of iodine solution indicates the positive result.

2.5. 16S rRNA SEQUENCE ANALYSIS OF THE MOST PROMISING ENDOPHYTIC BACTERIAL ISOLATES FOR IDENTIFICATION

After the evaluation of endophytic bacteria for plant growth promotion and antagonistic activity, two best isolates (KPR and CPL) were selected and 16S rRNA sequence analysis was carried out to identify. A rapid method for identifying bacteria was based on the amplification of 16S rRNA gene using Polymerase Chain Reaction.

2.5.1. Amplification of 16S rRNA gene

A single colony was taken by inoculation loop, mixed with 10 µl sterile water and kept at 94°C for two minutes. After a brief centrifugation to sediment the bacterial cell constituents, 1 µl of supernatant was taken and used as a template for amplification of 16S rRNA gene. The details of the primer used are given below.

Primer details	Sequence 5' – 3'	Base pair
8F	AGA GTT TGA TCC TGG CTC AG	20
152R	AAG GAG GTG ATC CAG CCG CA	20

Polymerase chain reaction was carried out in Eppendorf Master Cycler (Gradient, Germany). The composition of the reaction mixture for PCR is as follows:

Component	Per reaction volume required
Template	1.0 µl
10X Taq buffer A	2.5 µl
d NTP mix (10 mM)	1.0 µl
Forward primer	1.0 µl
Reverse primer	1.0 µl
Taq DNA polymerase (0.3 U)	2.0 µl
Distilled water	16.5 µl
Total	25 µl

The reaction was set in 200 µl microfuge tube chilled over ice flakes. A momentary spin was given to mix completely all reagents and set in thermal cycler for amplification. The details of thermal cycler programme are as follows:

No.	Step	Temperature (^{0}C)	Time (min)
1.	Initial denaturation	95	3.00
2.	Denaturation	94	1.30
3.	Annealing	55	0.40
4.	Primer extension	72	1.30
5.	Steps 2-4	34 cycles	-
6.	Final extension	72	20.00

2.5.2 Agarose gel electrophoresis

The quality of isolated DNA was evaluated through agarose gel electrophoresis (Sanbrook *et al*., 1989). 1X TAE buffer was prepared from the 50X TAE (pH 8.0) stock solutions. Agarose (Genei, Low EEO) (0.9 per cent) was weighed and dissolved in TAE buffer by boiling. Ethidum bromide prepared from a stock of 10 mg/ml was added to it at a concentration of 0.5 µg/ml and mixed well. The open end of gel casting tray was sealed with cellotape, the comb was placed properly and dissolved agarose was poured into tray. The gel was placed in the electrophoresis unit after 30 min with the well side directed towards the cathode. 1X TAE buffer was added to the buffer tank so as to cover the well with a few mm of buffer. 5 µl DNA sample was mixed with 1 µl tracking dye (6X) and carefully loaded into the wells using a micropipette. The λ DNA/ *Eco RI/ Hind III* Double Digest were used as the molecular weight marker. The cathode and the anode of the electrophoresis unit were connected to the power pack (Hoefer, USA) and the gel was run at constant voltage of 100 V. The power was turned off when the tracking dye reached at about 3 cm from the anode end.

2.5.3 Gel documentation

The gel documentation was done using GeNei UVITEC Cambridge gel documentation system. The agarose gel was visualized in the presence of UV light to illuminate the DNA bands and images of the gel was capyured using the software system attached to it and the band size was compared to the ladder.

2.5.4 Direct purification of PCR product

20 µl of the PCR product was purified using PCR purification kit following the procedure as per the manufactures guidelines.

3.5.5 Sequencing of the purified product

The purified product was sequenced at Seigenome Pvt. Ltd. Cochin using the primers 8F and 152R.

3.5.6 Nucleotide sequence analysis

The Blastn programme (http://www.ncbi.nlm.nih.gov/blast/) was used to find out the homology of the nucleotide sequence

III. RESULTS

A total of 67 endophytic bacterial isolates were isolated from five chilli plant samples. Among 67 bacterial isolates, 22, 24, and 21 isolates were obtained from root, stem and leaf respectively. Maximum numbers of isolates were obtained on nutreient agar medium (25). Two types of bacterial isolates were obtained in nutrient agar medium from root, stem, and leaf of each sample while only one type of bacterial isolate was obtained in all other media. Jensen's agar medium, Pikovskaya's agar medium and Aleksandrow's agar medium had same number of isolates (14). Stem part had the maximum number of isolates (24), followed by root (22) and leaf (21).

3.3 Screening of endophytic bacterial isolates for plant growth promoting activities.

3.3.1 Nitrogen fixation

The organisms which have a transparent, glittering appearance on Jensen's agar medium were considered as nitrogen fixing bacteria. Fourteen nitrogen fixing endophytic bacteria were obtained from root (5), stem (4) and leaf (5) respectively among the five samples collected. These bacterial isolates showed single isolated colonies when streaked on Jensen's agar media with the same colony appearance as obtained during isolation.

3.3.2 Phosphate solubilisation

Fourteen endophytic bacterial isolates were obtained in Pikovskaya's agar medium during isolation. Among them, only 6 isolates (KPR, PPS, PPL, MPR, CPR, CPL) showed clear halo zone around colony when spot inoculated on Pikovskaya's agar medium indicating positive test for phosphate solubilisation. All other bacterial isolates were also tested for phosphate solubilisation and 4 (KJR, PJS, CJL, VNS2) of them showed clear halo zone around the colony. Hence, a total of 10 bacterial isolates have phosphate solubilisation ability among 67 endophytic bacterial isolates. The diameter of the halo zone measured ranges from 10 mm to 3 mm. The highest zone was obtained from isolates KPR and KJR (10 mm) both from Kollengode sample followed by CJL (8 mm) from Cherpulasseri sample. The lowest diameter was obtained from PPS, MPR, CPR, CPL and VNS2 (3 mm).

3.3.3 Potassium solubilisation

Fourteen endophytic bacterial isolates were obtained in Aleksandrow's agar medium during isolation. None of them

showed clear halo zone around bacterial colony when inoculated on Aleksandrow's medium. Hence, among the 67 endophytic bacterial isolates obtained, none of them showed the ability of potassium solubilisation.

3.3.4 Indole acetic acid production

A total of 67 endophytic bacterial isolates were tested for IAA production. Isolates producing pink colour with Salkowaski's reagent indicated IAA production. All the endophytic bacterial isolates produced pink colour in Luria Bertani broth with 0.1% tryptophan after 7 days of incubation. These isolates were tested for quantitative estimation of IAA. The amount of IAA produced ranged from 0.32 to 1.42µg/ml. Out of 67 endophytic bacterial isolates, PJS had maximum IAA production followed by CJS, VPS, CPS, PPS, and MAR. The lowest IAA production was shown by isolate MAS.

3.4 Screening of endophytic bacterial isolates for antagonistic activities.

3.4.1 HCN production

All the 67 endophytic bacterial isolates were screened for their ability to produce HCN in nutrient agar supplemented with glycine (4.4 g/L) and the change in colour of filter paper to brownish orange placed was considered positive. The bacterial colonies produced HCN from medium to low (++ to +). Out of 67 bacterial isolates, ten isolates produced HCN. Only KNS2 showed medium HCN production (++) and remaining 9 showed low level of HCN production (KNR1, KJR, KJS, KPR, PJS, MJL, MPR, CPR, CPL).

3.4.2 Ammonia production

A total of 67 endophytic bacterial isolates tested for NH_3 production, thirty isolates produced ammonia when incubated in peptone broth for 7 days and produced a brownish red colour with Nessler's reagent. The ammonia production was in the range of high (+++), medium (++) and low (+). Four isolates CJR, CJS, CPR, and CPL showed high ammonia production and 6 isolates MPR, PAR, PPS, PJS, PJR and KPR showed medium ammonia production while the remaining ten showed less ammonia production

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3.4.3 Siderophore production

Out of 67 endophytic bacterial isolates, 8 were able to develop orange – yellow hallow zone in chrome azurol S (CAS) medium after 7 days of incubation, which is a positive test for siderophore production. Maximum halo zone was shown by isolates MAS followed by KPR. However, minimum zone was shown by the isolate PPS.

By screening 67 isolates, three isolates were selected as best (Table 1). These isolates namely KPR, PJS and CPL were further characterized morphologically, biochemically and at molecular level.

Table1: Screening of most promising endophytic bacterial isolates for growth promotion and antagonistic properties under *in vitro.*

Isolates	Parameters				
	P solubilisation (cm)	IAA (OD at 540 nm)	Ammonia production	HCN production	Siderophore production
KPR	10	0.23	+	++	+
PJS	5	1.33	+	++	+
CPL	4	0.22	+	+++	+

Note: Endophytic bacterial isolates are denoted as follow:
First letter stands for location, second letter stands for media used and third letter stands for plant part. For e.g., KNR: K – Kollengode, N – nutrient agar, R – root.

3.5 Morphological, cultural and biochemical characteristics of the most promising endophytic bacterial isolates from chilli (*Capsicum annuum*).

3.5.1 Morphological and cultural characters of selected endophytic bacterial isolates

The isolates KPR and PJS were Gram negative and isolate CPL was gram positive bacteria (Table 2. All the three were motile in semi solid media. On nutrient agar medium, the three isolates were circular with entire margin and convex opacity. PJS had a raised colony the other two being flat. PJS and CPL were in creamy colour while KPR was white in colour. All the isolates were negative for endospore staining except CPL.

3.5.2 Biochemical characters of selected endophytic bacterial isolates

On Gram's staining, isolates KPR and CPL were found to be gram negative while CPL was gram positive (Table 2). Three of them were actively motile on semi solid agar media. All the three isolates were positive for catalase test. After 24 hr of incubation, all three isolates changed the colour of Simmon's citrate agar media from green to prussian blue. The isolates KPR and CPL were able to produce red colour in nitrate broth after incubation for 48 hrs followed by addition of reagent A and B indicating positive results for nitrate reduction test. Only CPL was able to form clear zone

on starch medium when flooded with iodine solution. All the three isolates were negative for hydrogen sulphide production.

Based on morphological, cultural and biochemical characters, the isolate PJS was tentatively identified as *Acinetobacter* sp. The colony was circular, entire, convex, smooth opaque and small sized. Cells were rods or cocci, in chain, gram-negative, motile, catalase and citrate positive. However, spores were absent.

TABLE: 2 Morphological, cultural and biochemical characters of the most promising endophytic bacterial isolates from chilli (*Capsicum annuum).*

Characters	Isolate Code		
	KPR	CPL	PJS
Morphological and cultural characters			
Form	Circular	Circular	Circular
Elevation	Flat	Flat	Raised
Margin	Entire	Entire	Entire
Size (diameter) (72 hrs)	6 mm	4 mm	3 mm
Colour of colony	White	Creamy	Creamy
Opacity	Convex	Opaque	Opaque
Gram's reaction	Gram negative	Gram positive	Gram negative
Shape of cell	Rod	Rod	Coccobacilli
Endospore staining	Negative	Positive	Negative
Motility	Motile	Motile	Motile
Biochemical characters			
Catalase	Positive	Positive	Positive
Citrate	Positive	Positive	Positive
Nitrate reduction	Positive	Positive	Negative
Hydrogen sulphide	Negative	Negative	Negative
Starch hydrolysis	Negative	Positive	Negative

3.6 MOLECULAR CHARACTERIZATION OF ISOLATES KPR AND CPL

The endophytic bacterial isolates, KPR and CPL were identified using 16S rRNA sequencing. The isolate KPR showed 95% sequence similarity with *Enterobacter hormaechei* D15 strain and CPL showed 96% sequence similarity with *Bacillus subtilis* LLP-4 strain (Table 3).

The isolate, KPR (*Enterobacter hormaechei*) was obtained from root, on Pikovskaya's agar medium of Kollengode region. It was more efficient than CPL (*Bacillus subtilis*). Isolate CPL was also obtained on Pikovskaya's agar medium from leaf, of Cherpulasseri region.

Table: 3 Identification of the two most efficient endophytic bacteria from chilli.

Isolate	NCBI accession showing maximum homology		Maximum score	Query coverage %	Identity %	e-value
	Accession number.	Name				
KPR	KJ863539	*Enterobacter hormaechei*	2241	98	95	0.0
	CP024908	*Enterobacter xianfangensis*	17782	98	95	0.0
	CP021167	*Enterobacter cloacae*	17821	98	95	0.0
CPL	KU821697	*Bacillus subtilis*	2337	96	97	0.0
	KF158233	*Bacillus subtilis*	2337	96	98	0.0
	KX817931	*Bacillus* sp	2335	96	98	0.0

IV. CONCLUSION

Out of 67 endophytic bacterial isolates obtained from chilli, three of them was selected as most promising isolates namely, KPR, PJS and CPL. PJS was tentatively identified as *Acenetobacter sp*. KPR and PJS was identified as *Enterobacter hormaechei* and *Bacillus subtilis* respectively using 16S rRNA sequencing.

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